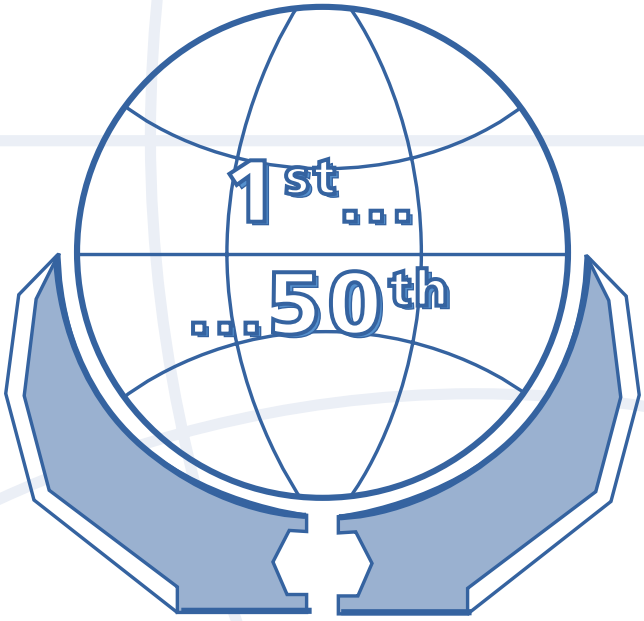
50th International Congress of Meat Science and Technology

August 8th – 13th 2004

Helsinki, Finland



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FROM EMMRW TO ICoMST - SOME FEATURES OF THE BIRTH AND DEVELOPMENT OF INTERNATIONAL COOPERATION

Professor emeritus F.P. Niinivaara, Finland.

The organizing committee has asked me as promoter of the cooperation and as the organizer of the first congress 1955 to tell you about the birth and development of this congress.

The first congress was held in Hämeenlinna in August 1955. It was attended by a group of 22 meat researchers from seven European countries. As this 50th congress is being held again in Finland, it could be said that, the congress has returned to its roots.

This congress was first called "The European Meeting of Meat Research Workers", EMMRW.
It soon expanded, as participants from other continents attended it, too. In 1987 the name was changed to "International Congress of Meat Science and Technology", ICoMST. We, the "founding fathers", could not even imagine that a small group of European meat researchers would grow into a large international congress.

Meat is connecting people over the world.

I am very happy to have the opportunity to tell the prominence of the meat scientists of the world about some memories of the birth of the first meeting.

My own career as meat researcher started after the World War II in 1946. European cities had been bombed to ruins. Finland had its share of bombs. Helsinki was badly ruined. The main building of this University was burned. This festival hall we are sitting now looked like a black ghostly skeleton after the fire. After spending five years in grisly war, I could continue my studies. We did not win the war, but we reached our goal: Finland kept her freedom and was never occupied.

The University was rebuilt, opened its doors again, and I was able to continue my studies of biochemistry. As a mentor and teacher I had the famous biochemist Professor Virtanen, who just had received the Nobel-prize in chemistry. This acknowledgment – a Nobel-prize to a Finnish scientist- was like a shot of stimulant to the depressed people of a ruined country. I had been participating in the research work which he presented in his Nobel-speech in Stockholm.

When I passed my final examinations in December 1946, Profesor Virtanen came to me and asked if I would be interested in starting to work as head of a meat research institute. He said: "I have been asked for a suitable person, and I consider you suitable for this mission". And he continued and said unforgettable words:" I think meat research is an important task. Valuable research has been done with dairy science, which I also have taken part in. Meat is economically and nutritionally just as important as milk. That is why I wish that you would take on this task and start developing meat science in our country."

This was the testament of the Nobel laureate to a young biochemist. I promised to take on this task and I think I have fulfilled it. Meat research and teaching meat technology has become my life work. For this task I have given my best years. It has been 57 years since I took on this task, and here I stand – still lecturing about meat. When I started in 1947, I was perplexed. Meat science. What ever is it? Dairy science and professor Virtanen were well known, but what would meat science be like?

I decided to leave for an excursion abroad in hope for finding ideas from the world. In 1952 I visited Sweden, Norway and Denmark without finding any research work being done on meat. In Germany, I found

one very modest meat research institute. It was founded in Berlin, but was destroyed in the bombings. The rest of it had evacuated to a small South-German town called Kulmbach. With this institute I had many years of cooperation. As an acknowledgment for this, I was first foreigner to receive the Rievel-medal, which is given by this institute to scientists for outstanding work in meat research.

Next year, in 1953, I had the opportunity to visit the American Meat Institute Foundation in Chicago. This Institute had somewhat more capacity. A discussion with Dr. Niven gave me the idea of studying useful bacteria in fermented sausages. After returning I concentrated in this research and in 1955 was able to publish my thesis about this subject. This was the foundation both for the research of Starter cultures and their use in producing dry sausage.

During my excursions I met many distinguished and sympathetic meat scientists. We discussed the possible cooperation between research institutes and scientists. I noticed that the scientists did not know each other personally, but were only reading the publications of one another. It would be very much more useful if information about the work and goals of the institutes would be better available. It also would be more economic and efficient.

Everybody seemed very interested in developing cooperation. It was suggested that all of us scientists should meet to discuss how to start and how to carry out the cooperation. I promised to organize the meeting if it were held in Finland. Everybody was ready for this. So in the spring of 1955 I sent invitations to institutes and persons I knew would be interested in this meeting. This was to be the first meeting of the "European Meeting of Meat Research Workers". There were representatives from seven countries attending this meeting.

They were:

Prof. Reinhold Grau, Germany, Dipl. ing. Mogens Jul, Denmark Dr. Hans Baretta, Holland
Dr. Olle Dahl, Scans laboratorium, Sweden, Spice manufacturer and butcher J.-P. Daul, France Dr. Hans Wyttenbach, Switzerland Director Hermann Herzer, Switzerland
Dipl.ing. Martti Knuuttila, congress secretary, Finland
Dr. F.P. Niinivaara, congress chairman, Finland

The congress was also attended by a number of representatives of the Finnish food industry, Altogether, 22 persons were attending the meeting. This committee worked out the guidelines, which have been followed for 50 years. The most important issue of the first congress was how to create the cooperation. These individuals created the main lines and the content of the future conferences.

The program of this first meeting consisted of the same topics, which have for half a century remained as part of the meeting. So, in addition to being an occasion for discussing the scope of cooperation, the meeting was also the first congress of meat researchers. There were eight lectures: Professor Grau talked about the meaning of chemistry in meat research; Mogens Jul talked about gas stunning of pigs, Martti Knuuttila talked about microwave heating in meat industry, Hans Baretta about the importance of education; I talked about using starter cultures in producing dry sausage; Olle Dahl about the influence of the race on quality of beef. Eino Niemistö talked about a modern solution in handling slaughter by-products, and Eini Laakkonen talked about the meat products in domestic economy. J.P. Daul suggested an alternative solution for the cooperation in form of a subcommittee in the Council of Europe. This solution was considered inefficient and complicated.

The congresses have been held fifty times without a fixed secretariat. It has only had to be cancelled once. This was in the year 1968 in Czechoslovakia. The "Proceedings of the Congress" was already printed, but instead of congress participants the country the country was occupied. Part of the congress participants had already arrived and were caught in the middle of a riot. This would have been the end of the congress series, as the next host country was not elected yet. An emergency meeting was held in Vienna, and it was settled that I should host the 15th congress in Finland. There was only eight months time for the organization, but the congress series was saved. The spirit of meat was stronger than the tanks.

This congress series is a miracle. From a small European beginning it has grown into a world congress, which today is held for the 50th time and with 330 participants. It has always acted on a voluntarily basis, without an elected board or fixed secretariat. In 1955, with high border walls between countries and obligatory visas, we could not as far as imagine this amount of popularity. East and West were divided by the iron curtain, Europe was in ruin, and above these ruins political storms were raging. In these conditions we were building up a co-operation between the meat researchers of different countries. The whole thing seemed utopic. But we made it.

Kenneth Johnson writes that the driving power has been the "spirit of meat" -which he thinks was born in the finish sauna. *Meat is tender, but the spirit of meat is always strong.* I hope the spirit of meat will be the driving force for Meat Congresses in the future, too.

R. B. Sleeth said in the 41st congress in Texas:
"Friendships that last a lifetime are one of the greatest conference benefits."

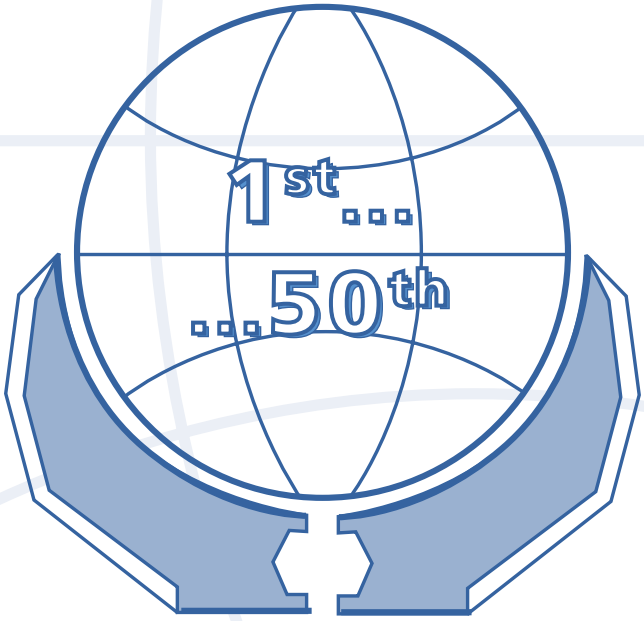
I wish to all of you pleasant days in Finland, in the birth country of ICoMST.

50th International Congress of Meat Science and Technology

August 8th – 13th 2004

Helsinki, Finland

Session 1
Biochemistry and biology



QUALITY OF PORK GENES AND MEAT PRODUCTION

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Abstract

Functional genomics, including analysis of the transcriptome and proteome, provides new opportunities for understanding the molecular processes in muscle and how these influence its conversion to meat. The Quality Pork Genes project was established to identify genes associated with variation in different aspects of raw material (muscle) quality and to then develop genetic tools that could be utilized to improve this quality. DNA polymorphisms identified in the porcine *PRKAG3* and *CAST* genes illustrate the impact that such tools can have in improving meat quality. The resources developed in Quality Pork Genes provide the basis for identifying more of these tools.

Keywords: Pigs; Pork; Meat quality; Muscle genes; Functional genomics

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(Note! The Figures 1–5 on Pages 14–18.)

Introduction

Pig breeding companies are now paying more attention to meat quality and are including quality traits as an integral part of selection programmes. This has been in part driven by consolidation and integration in the meat processing industry and the retail sector together with an increasing consumer awareness of food quality. In addition, the development of the field of genomics has also stimulated interest in breeding for meat quality as this "trait" constitutes the classic case where DNA marker based selection is at its most efficient: where the trait cannot be measured on the selection candidate and can only be measured at high costs on its relatives post-mortem. Once a DNA marker (a polymorphism, see below) has been shown to be associated with variation in the target trait, then it can be used to genetically type young animals for pre-selection before performance testing. This is a distinct advantage over sib slaughter schemes which are increasingly difficult and expensive to implement (see Knap, Sosnicki, Klont, & Lacoste, 2002). Even so sib slaughter schemes will continue to play a role, both for the identification of new markers and for monitoring breeding lines in order to optimize the breeding direction. The advantage of incorporating markers into selection programmes can be sustained when new markers are identified to replace older markers that begin to reach fixation. The database builds up over time to provide a very useful resource for this purpose or further validation of DNA markers identified in experimental populations or to test candidate genes.

In the last decade DNA tests have been developed that allow much more effective eradication of undesirable alleles of the major genes negatively impacting pork quality, such as the *Hal* gene (HAL–1843™, as licensed from the Innovations Foundation, Toronto, Canada, owner of the trademark. Fujii *et al*., 1991) and the *RN* gene (Milan *et al*., 2000) which are associated with pale, soft and exudative meat and processed ham yield respectively.

Breed differences in meat quality traits are large (Sellier, 1998) and commercially relevant. Duroc, Hampshire and Berkshire lines are commonly marketed as "meat quality lines" and several industry lines have been based on these breeds. However, significant variation in meat quality related traits is also present within breeds (e.g. Gil *et al.*, 2003). The discovery of the *Hal* and *RN* genes also encouraged researchers to consider single gene effects as an alternative to breed effects. As indicated above, once DNA markers for meat quality are identified they can be used in a wide range of breeds by changing allele frequencies through selection and/or introgression.

Recent examples of these marker effects include polymorphism in the genes for calpastatin (*CAST*) and *PRKAG3* that are associated with quantitative variation in tenderness (*CAST*) and pH and colour (*PRKAG3*) (Ciobanu et al., 2001, 2002, 2004). Future success for the industry will require the production of consistent and predictable high product quality to ensure customer satisfaction. The target should be to combine efficient growth with the best possible meat quality or alternatively the aim can be described as optimizing meat quality with the lowest cost production.

To date DNA markers have been identified using two basic approaches: quantitative trait loci (QTL) mapping or the "candidate gene approach". The first utilizes specific genetic designs (for example three generation families based on divergent breed crosses such as Chinese Meishan or Wild Boar and Large White) to find the location of QTL on the genetic map. Several QTL studies have addressed pork quality traits (summarized in Bidanel and Rothschild 2002) and they provide the starting point for the identification of individual genes (or markers) influencing these traits (the positional candidate gene approach). Indeed, the RN^- mutation, identified initially by a mapping approach was then elucidated using positional cloning and a "BAC contig" constructed for that region of the genome (a physical representation of the DNA sequence from the QTL region) (Milan et al 2000).

It is now possible to add functional genomics to the range of options available for understanding the molecular basis of pork quality. The objective of this study was to develop the resources required to do undertake this approach. The QualityPorkGENES project (http://www.qualityporkgenes.com) was initiated in order to create a unique phenotypic resource that could be exploited through the application of new

functional genomics tools that determine differences in the transcriptome and proteome of muscle and relate this to the different aspects of meat quality. This approach has the potential advantage of generating information, in parallel, on multiple genes and gene products which, in turn provides the opportunity to identify pathways and interacting genes (see Maltin & Plastow, 2004). In this way the efficiency of marker detection is increased as well as providing insight into epistatic effects that can further the understanding of the genetic component of meat quality.

Materials and Methods

Animals and samples
Five closed populations (breeding lines) were chosen for the project. These were based on Large White, Landrace, Duroc, Piétrain and Meishan breeds (although in the latter case a Meishan/Large White crossbred line was used) in order to represent a significant proportion of European pig production. The Piétrain line was normal for the "Halothane" gene. In addition, initial characterization of lines (at IRTA Monells, Spain) indicated that these lines would provide significant variation both within and between populations. Animals were reared under the same environment and production regime in a breeding nucleus farm in France. Data was collected on farm for growth, ultrasonic backfat and basal stress hormone levels. At approximately 140 days of age the animals were transported in batches of around 25 to overnight lairage at a research abbatoir in Spain (IRTA Monells). Animals were harvested after CO2 stunning and carcass, meat quality, biochemical and sensory data were collected for 100 animals per line (in 22 batches). Urine samples were taken after transport on arrival at lairage and the next morning before they were moved to the CO2 chamber. Immediately after harvest loin (*Longissimus thoracis*) and ham (*Semimembranosus* - SM) muscles were collected and samples prepared for fibre type and proteome analysis (after mounting and freezing in liquid nitrogen) and RNA isolation (RNAlater).

Phenotypic traits
Measurements of fat depths at 45 minutes postmortem (p.m.) were made using the Fat-O-Meat'er equipment at 60mm from the mid-line at the level of the last rib (LRFOM). At 24 hours p.m., the surface area of the eye of the *Longissimus thoracis* (AREALT) was measured between the 3rd and 4th last rib level. This was done by making a transversal cut at this point and taking a digital image. This image was used to calculate the AREALT using a specific program. Each left half carcass was cut and dissected following the method of Walstra and Merkus (1995). In order to have more commercial cuts some parts were jointed as hind hand plus leg (ham), loin minus subcutaneous fat of the loin (loin) and belly plus to ventral part of the belly (belly). Estimated carcass lean content (PLEAN) was calculated using the Spanish official equation (Gispert and Diestre, 1994).

The left side of each carcass was used to assess meat quality. Muscle pH was measured using a Crison portable meter equipped with a xerolyt electrode in the *Longissimus thoracis* muscle (LT) at 45 min. (pH_{45}LT) and 24 h. (pH_u LT) p.m. Colour measurements were taken 24 h. p.m. on the exposed cut surface of the LT at the last rib level, using a Spectrophotometer Minolta C2002 in the CIELAB space (CIE, 1976). Drip loss was determined in the muscle LT according to the method of Honikel (1998).

The metabolic traits of the muscle were determined by measuring the lactate dehydrogenase (LDH) activity according to Ansay (1974) and the isocitrate dehydrogenase (ICDH) activity according to Briand, Talmant, Briand, Monin and Durand (1981). Enzyme activities are expressed as µmol NADH per minute per g of muscle (LDH) and as nmol NADPH per minute per g of muscle (ICDH). The percentage of slow myosin heavy chain (MHC-I) in the muscle was determined with a specific MHC-I monoclonal antibody by the ELISA technique (Picard et al., 1994).

Cathepsin B activity was assayed with N-CBZ-L-arginyl-L-arginine 7-amido-4-methylcoumarin (Z-Arg-Arg-NHMec) (Etherington and Wardale, 1982). One unit of activity was defined as the amount of enzyme hydrolysing 1 nmol of substrate per min at 37°C. Activities were given in enzyme units per mg of extracted protein. The protein concentration of the enzyme extracts was measured according to Lowry, Rosebrough, Farr and Randall (1951), using bovine serum albumin as standard.

Intramuscular fat (IMF) content was determined by a NIT (Near Infrared Transmittance) apparatus. Fat was extracted by the method of Folch, Lees and Stanley (1957) and aliquots of the extracts were collected to perform the analysis of fatty acids and triglycerides. Fatty acids were determined by GC-FID as methyl esters (FAMES) by the method described by AOCS. A capillary column coated with cyanopropylpolysiloxane was used (SGE, BPX-70) 30 m x 250 µm, width film 0.25 µm. Triglycerides were determined by HPLC with RI detector.

Sensory characterization of the samples was performed through a Qualitative Descriptive Analysis (QDA) using 6 selected trained assessors including texture descriptors in cooked meat. Samples of LT (1.5 cm thick) were stored at 4 ºC during 24 h and afterwards frozen. Twenty-four hours before the evaluation, the samples were thawed at 4 ºC. They were cooked in an electric oven at 110 ºC until reaching an internal temperature of 65 ºC and then cut and evaluated. The samples were quantified for hardness and juiciness using a 10 point scoring scale where 0 means 'absence or minimum intensity' and 10 means 'very strong or maximum intensity'

Texture Profile Analysis (TPA) was undertaken in sample cubes of 1.5 cm x 2 cm x 2 cm obtained from an adjacent slice of that used for sensory analysis. Samples were cooked as described in sensory analysis. All the measurements were carried out using a Texture Analyser TA.TX2 (Stable Micro systems Ltd) with a 25 kg load cell and using a crosshead speed of 5 mm/second.

The statistical analysis of the phenotypic traits was performed using the GLM procedure of SAS (1999). The effect of the genetic type was included in the model as a fixed effect. The slaughter day was included as a block effect and the carcass weight as a covariable if they were significant. Family relationships were not accounted for as the dataset contains too few offspring within both full- or half-sib families. Significant differences ($P<0.05$) between means were obtained with the Tukey test. For the sensory analysis, GLM procedure was also used. Genetic line and session were in the model as fixed effects. Significant differences between means were obtained with the Tukey test.

Proteomics

Loin muscle samples from each animal for the five breeds were subjected to two dimensional gel electrophoresis (2DGE) over the p*I* range of 3 to 10 and molecular weight range of 10 to 200 kDa, enabling the separation of more than 750 spots per gel. Image analysis (Phoretix Evolution) was used to generate a master proteome map representing the proteomes of the five divergent porcine breeds. Areas of saturation and poor resolution were removed digitally prior to analysis.

Preparation of cDNA libraries and Real-time PCR

Total RNA and mRNA were prepared from muscle samples preserved in RNAlater (Ambion Inc.). RNA was analysed for quality by Northern blot analysis and visualization using the Agilent BioAnalyser. The RNA was then used for the construction of suppression subtractive hybridisation (SSH) porcine muscle cDNA libraries from animals within and between divergent genotypes and for the construction of a full-length representative pig muscle cDNA library.

Suppression subtractive hybridisation was performed using the PCR-select cDNA Subtraction kit (BD Biosciences Clontech UK). Eight SSH libraries were constructed based on specific contrasts available in the project (breed, muscle type, meat quality and stress). A standard full-length pig muscle cDNA library was prepared to facilitate the isolation of full-length cDNA for candidate genes to be identified from the SSH libraries, microarray screening and proteomics analysis. The full-length cDNA library was constructed using the λ Zap Express cDNA synthesis/Gigapack cloning kit (Stratagene Cloning Systems) using 5 µg of mRNA pooled from LT and SM muscle from all five pig lines, and different slaughter days. The quality of this library was assessed by titration of library size and cDNA fragment length analysis using PCR with the M13 forward and reverse primers.

The expression pattern of the candidate gene *PRKAG3* was analysed by quantitative real-time polymerase chain reaction (PCR) using either the lightcycler system (Roche) or the MX3000P 96 well real-time PCR

System (Stratagene), and the Quantitect SYBR green one-step RT-PCR kit (Qiagen). A PCR fragment of 118bp was generated using primers located at 310-428bp in the gene sequence (Genbank AF214520),

sense: PRKAG3LCF2: (5')CAGTCCAGGCCAGTTGCTGAGTC
antisense: PRKAG3LCR2: (5')GCTCTGTTGGGGGGTTGTCCAC

Total RNAs were selected from LT and SM muscle from Landrace and Meishan animals that had been selected as showing variation in meat quality on the basis of pH_u and LMinolta colour values. A typical experiment analysed expression of *PRKAG3* in RNA from at least eight different animals (up to 14). Samples were analysed in triplicate and each experiment was repeated on two or three separate occasions. Expression level was inferred from the cycle threshold (Ct) value, or Ct values were converted to copy number per µl using a standard curve of cRNA standards *in vitro* transcribed from cloned cDNA products. Results were analysed statistically using ANOVA with Tukey post hoc analysis.

Microarray construction
Microarrays have been constructed using purified PCR products generated from cDNA clones. These clones were derived from the SSH libraries plus additional muscle expressed candidate genes. All PCRs were prepared in a sodium phosphate/ SDS spotting buffer using a 384 well format prior to printing. The products were spotted onto Corning GAPS 2™ slides along with Cy3 and Cy5 orientation spots and control elements, using a 48 x 100 µm pin tool and a BioRobotics Microspot 2500 microarrayer. For the prototype array every element was spotted in duplicate using a 17 x 18 subarray, giving a total of 14688 elements per slide. The second generation slides have been spotted in triplicate using a 21 x 21 subarray allowing a total of 21168 elements per slide.

Post spotting, slides were baked for 2 hrs at 80°C. They were then blocked in 1% BSA (Molecular Biology grade, Sigma Aldrich B 2518), 0.1% SDS in 3x SSC at 55 °C for 1 hour. This was immediately followed by denaturation in a 95 °C water bath for 2 min, rinsing in isopropanol, spinning dry, and storage in dark, dry conditions at RT prior to hybridization.

Microarray hybridization
20 µg of each RNA sample selected for hybridization to the slides was labelled with either Cy3 or Cy5 using an indirect method. RT-PCR (SuperScript II, Invitrogen) with a polyT primer was used to generate complex cDNA in the presence of aminoallyl dUTP (Sigma). The reaction was followed by a post-PCR coupling to the reactive CyDye (Amersham Pharmacia) in the presence of sodium bicarbonate/ hydroxylamine buffer. Samples were then purified using a Qiagen PCR Purification Kit column prior to precipitation in the presence of 10 µg of pig genomic DNA plus 500 ng of cloned pig SINE repeat element. The resulting pellet was resuspended in 100 µl of formamide hybridization buffer (Sigma) along with 1 µl of yeast tRNA (Sigma) and 1 µl of poly dA (Amersham Pharmacia Biotech). Hybridization mixes were applied to the slides, and incubated at 42 °C for 18 hours before washing in an SSC series to a final concentration of 0.1 X. Slides were scanned on an Axon 4100 scanner, at 10 µm resolution, and images captured with the GenePix software.

Image analysis
All images were analysed for the pixel intensities in each of the two channels (Cy 3 and Cy5) using BlueFuse (BlueGnome Ltd.). Background signal was removed from the spot signals, and replicate data fused to produce single values, with a high confidence probability, for each clone represented on the array.

Microarray experimental design and analysis
Animals for the first experiments (investigating water holding capacity and intramuscular fat content) were selected on the basis of a factor analysis, where the first four factors were descriptive of (1) fatness versus leanness (2) water holding capacity (pH, LMinolta, driploss) (3) intramuscular fat and (4) percentage loin. The lines with the greatest variance for each factor were identified: the Meishan – factor (1), Large White and Piétrain – factor (2), Duroc and Meishan – factor (3) and Landrace – factor (4). Animals were ranked on their individual factor scores and the top ten and bottom ten animals for each factor were selected within the lines of interest. Outliers, animals with residual values more than three standard deviations from their respective within-line means, were removed.

For each factor comparison, cDNA samples prepared from animals with high or low scores from at least two different breeds were hybridized to the prototype array using a loop design. At each point within the loop one high scoring sample is compared against one low. Each sample occurs twice within the loop with an intrinsic dye swap. (Figure 1a, Page 14) (Kerr and Churchill, 2001a, 2001b).

A second experiment will be undertaken to enable the relationship between gene expression and tenderness and sensorial traits to be established. One hundred individual animals, twenty animals from each line, will be arrayed. Samples will be compared against a common pooled control sample (reference design: Figure 1b, Page 14) for this second expression experiment.

For both microarray experiments the data will be analysed using microarray ANOVA (Kerr, Martin and Churchill, 2000). ANOVA methods can be used to normalize the microarray data, for example by taking account of variation between slides, and also to provide estimates of changes in gene expression that are corrected for potential confounding effects, such as slaughter batch.

3. Results and Discussion

Carcass, biochemical and meat quality traits
More than 150,000 datapoints have been collected to describe the phenotypic characteristics of the animals in this study. These can be assigned to 358 measured and 62 derived traits for the following groups; performance (7), carcass (20), cutting (84), meat quality (20), biochemical (e.g. enzyme activity) (16) endocrine (18), sensory (13), texture (12), fatty acid content (198) and fibre type (32).

Table 1 (Page 19) shows the mean values and standard deviation of the parameters of carcass quality and also the differences between and within line. The Meishan based line showed lowest value for carcass weight and the Duroc based line the highest value, although not significantly different from the Landrace and Large White based lines. It is important to note that the variation within line (STD) is relatively large and similar in all the lines. In relation to the carcass quality, the Piétrain line had the leanest carcass, showing – with significant differences - the least fat depth and, accordingly, the highest muscular area (57.4 cm^2) and lean content in the carcass (60.3%). The fattest line was the Meishan line, showing 20.9 mm of LRFOM and 48.8% of lean content. The other lines were intermediate. These results were in accordance with Gil *et al.* (2003).

Meat quality parameters are shown in Table 2 (Page 19). It can be seen that the Landrace based line presented the lowest pH_{45} and the highest L* value, indicating lighter meat. None of the lines presented PSE meat. Ultimate pH was significantly higher in the Large White, Duroc and Piétrain lines. Drip losses were always lower than 3% with the exception of the Landrace line (3.3%).

The activities of the enzymes LDH and ICDH and the glycolytic ratio LDH/ICDH were determined to assess the metabolic traits of the muscles (Table 3, Page 20). The within line variability for the LDH was between 19.1 and 25.5%. This variable showed no significant differences between the five lines studied. The within line variability for ICDH activity and for the ratio LDH/ICDH were in the range 26.3 – 39.5%. ICDH differed significantly between lines: The Duroc and Piétrain lines presented higher activity than the other lines. In accordance with this, the glycolytic ratio for the Meishan line was higher than for the Duroc and Piétrain lines, with the Landrace and Large White lines having intermediate values. The percentage of MHCI differed between lines and the Duroc line had the highest value (8.65%) whereas the Meishan line had the lowest (6.96 %). The other three lines presented intermediate values. The within line variability of this variable was between 32.9% and 41.3 %. The values obtained for these variables were similar to those reported before for different porcine genetic lines or crosses (Henckel et al., 1997; Petersen Henckel, Oksbjerg and Sorensen, 1998, Gil et al., 2003). Cathepsin B activity also showed a high variability in all the lines (between 40.9 % in the Large White line and 48.1% in the Piétrain line). The Duroc based line had higher proteolytic activity than the Meishan line, and the other lines showed values in between. Hernández, Zomeño, Ariño and Blasco (2004) also report differences in cathepsin B activity between different porcine lines.

Table 4 (Pade20) shows the means and standard deviations for IMF, fatty acids and triglycerides in LT muscle. IMF showed a high within line variability. The highest values of IMF were found in the Duroc and Meishan lines. The other lines showed similar values, with the Piétrain line having a higher marbling content than the other two lines. Regarding the fatty acids, the percentage of stearic was higher in the Duroc line – that also presented a high IMF content – than in the other four lines. The Meishan line although having a high percentage of IMF, did not show a percentage of stearic acid as high as the Duroc line. It is interesting to note that the within line variability for this acid was quite low (5.8-7.7%). The percentage of linoleic acid was significantly higher in the Landrace, Large White and Piétrain lines than in the Duroc and Meishan lines. These data, together with the values of stearic acid, would suggest that the metabolism for the C18 acids was different between lines, which could be related with the genetics and, thus, with specific genes directly or indirectly associated with lipid metabolism. With respect to the minority triglycerides, dioleyl-steaoryl-glycerol (SOO) and disteaoryl-oleyl-glycerol (SSO), that are important because they are related with the consistency of the fat, the Duroc and Piétrain lines showed higher values for SOO than the other lines; the lowest percentage was for the Large White line, which contained high linoleic acid % but not a very high stearic acid %. For the SSO, no significant differences between lines were observed - possibly due to the high within line variability - but the Landrace and Duroc lines showed the highest percentages. This result could be related to the higher fat firmness in these two lines.

Table 5 (Page 21)shows the least square means and the significant differences per genetic line for hardness and juiciness sensory attributes. Hardness was lower in Meishan, Duroc and Piétrain lines and higher in Landrace and Large White lines. Regarding juiciness it seems that the lean lines were those with the lowest score for juiciness.

Table 6 (Page 21) shows the average values (means) and the significant differences per genetic line (Tukey test) for hardness from instrumental texture analysis (TPA test). Regarding this parameter obtained from the TPA curve, it seemed that the Piétrain line was different from the rest with the exception of the Meishan line. Hardness was evaluated differently by the instrumental test than by the sensory panel. The conditions in the mouth during chewing possibly could explain the different assessment of the sensorial hardness compared with the instrumental evaluation. Further analysis is needed regarding relations or correlations with other variables in order to interpret these results.

Stress hormone analysis

Urine was sampled when spontaneously voided in the farm (basal conditions), when the animals arrived in the lairage area (after approximately 10 hours of transportation in a lorry) and the next morning before slaughter. Levels of cortisol and catecholamines (adrenaline and noradrenaline) were measured in these urine samples as described (Hay and Mormède, 1997a&b). Blood was sampled during exsanguination, and creatine kinase activity, cortisol, glucose, lactate and free fatty acid (FFA) levels were measured in serum. A strong relationship between the urinary concentrations of cortisol in basal conditions and after transportation stress, were found indicating that genetic factors primarily influence the intrinsic activity / reactivity of the corticotropic axis. Across genetic types, concentrations of cortisol in urine were also correlated with carcass fat content, except for Piétrain pigs that combine a very low adiposity together with high urinary cortisol levels. This result confirms previous data demonstrating the genetic linkage between the hypothalamic-pituitary adrenal axis activity and fat accumulation, with *Cbg*, encoding corticosteroid-binding globulin, the carrier of cortisol in plasma, as a strong candidate gene (Désautés et al., 2002; Ousova et al., 2004). A correlation between cortisol levels measured in urine collected after slaughter and adiposity was also demonstrated in a segregating F2 intercross between Large White and Duroc pigs (Foury et al., 2002). Since urinary cortisol levels are directly related to cortisol secretion by the adrenal gland, the present data, together with available literature, suggest that adrenal secretory activity is another candidate for genetic polymorphism that could influence carcass composition. It will be interesting to see if this pathway is identified in the transcriptome analysis to be undertaken in the next stage of the project.

Secretion of catabolic hormones (cortisol and catecholamines) was the lowest in Large White pigs that also showed the lowest levels of energy mobilisation under slaughter stress (low levels of plasma lactate and FFA). Low levels of plasma glucose in Meishan and Duroc pigs suggest that their metabolism is more oriented towards energy storage, in accordance with the higher carcass fat content. Altogether these measures also showed that in the present experiments animals experienced very low levels of stress before and at the time of slaughter, so that the phenotypic and molecular data can be considered as representing 'basal' values with a minimal influence of pre-slaughter stress, that is a main component of meat quality, in interaction with genetic factors (Sellier and Monin, 1994, Mormède et al., 2004).

Longissimus thoracis *proteome*

Proteomics technology is becoming increasingly popular in the identification of gene products to aid in determining meat quality. Recent studies have utilised two dimensional gel electrophoresis (2DGE) to investigate changes in the *Longissimus thoracis* (LT) muscle proteome over 72 hours post-mortem in relation to meat quality (Lametsch and Bendixen, 2001, Lametsch, Roepstorff and Bendixen, 2002 and Lametsch *et al.*, 2003; Morzel *et al.*, 2004). The degradation of specific structural and metabolic proteins was demonstrated that varied significantly with aging time. In contrast, the current study presents the first major investigation comparing the proteome of the LT muscle for five divergent porcine breeds at the time of slaughter. This will enable us to detect breed differences in perimortem muscle metabolism in relation with organoleptic characteristics and phenotype.

A master proteome map was constructed for conditions yielding nearly 800 spots (a typical gel is shown in Figure 2, Page 15). The identity of a number of key muscle proteins was confirmed after excision and analysis by MALDI-TOF mass spectrometry and their expression is comparable with previous studies (Lametsch and Bendixen, 2001, Lametsch, Roepstorff and Bendixen, 2002, Yan *et al.*, 2001). Initial comparison of the proteome of the five breeds has identified 21 spots that significantly differ ($p<0.01$; data not shown). These proteins are now being identified using MALDI-TOF mass spectrometry. Any novel proteins will be characterised by Electrospray mass spectrometry and comparison with translated sequences from the cDNA libraries generated within the project.

cDNA libraries

One of the main aims of the project is the analysis of the transcriptome of muscle at the time of slaughter and to relate this to differences observed in the phenotypic characteristics of the muscle. In order to do this, specific cDNA libraries were prepared from both loin and ham muscle to provide the basis for the construction of microarray tools that could be used for this analysis. The quality of the SSH cDNA libraries was assessed by careful analysis of each step during the subtraction process and by hybridisation of subtracted cDNAs to known housekeeping genes that should decrease in representation during the subtraction process (see Figure 3 for examples, Page 16). Furthermore approx 500 clones from each of the eight SSH libraries have now been sequenced using the M13 reverse primer, and this analysis has revealed a low-level of sequence redundancy, another indicator of the quality of the SSH libraries. This analysis also suggests that these libraries may prove a useful resource for the isolation and characterization of candidate genes associated with meat quality as each library forms a unique population of cDNAs. A standard full-length pig muscle cDNA library was prepared to facilitate the isolation of full-length cDNA for candidate genes to be identified from the SSH libraries, microarray screening and proteomics analysis. The quality of this library was assessed by titration of library size and cDNA fragment length analysis. The results obtained showed that the size of the cDNA library (4.8×10^5 pfu) was above the bench-mark figure of 10^5 cDNA clones required to have a good probability that all rare mRNA transcripts would be represented in the library. Analysis of the size of cDNA inserts from random clones showed the largest cDNA insert to be 3.3 kb in length, with the majority of clones > 0.7 kb and an average insert size of approximately 1.4 kb. The quality of the libraries has also been investigated

by DNA sequence analysis. Using contig assembly followed by BLAST to NR and EST databases to assign tentative gene identities to the SSH library clones, over 900 separate genes are detected, with many genes represented by different non-overlapping sequences.

Microarray development

The SSH libraries have formed the basis of the first microarrays constructed for initial transcriptional profiling using muscle mRNA from animals selected on the basis of variation in key aspects of quality such as pH and colour, drip loss, loin area or marbling. The rationale behind using these clones is that they have already been taken through a pre-selection process, and therefore should be enriched for those transcripts with differential expression profiles at the extremities of commercially important phenotypic traits. Additional clones for candidate genes were selected from the literature or from prior knowledge, and genes defined as muscle specific or muscle variant (based on the human HUGe index analysis of skeletal muscle: Hsiao *et al.*, 2001) were also included on the array. Every element occurs more than once within the microarray grid design.

This prototype array has been verified by hybridization with samples from pooled LT against pooled SM (Figure 4, Page 17). Genes that showed up or down regulation in LT were verified from the known characteristics of the muscle types. In addition, genes enriched in LT by SSH were up-regulated in LT on the array, giving greater confidence that the SSH libraries will provide a valuable resource of potential candidate genes for the phenotypes compared in the subtraction process. This will be particularly important where changes at the transcriptional level are more subtle than can be detected with an array-based approach. The first large-scale microarray experiments are now being undertaken using this array focusing on the relationship between variation in gene expression and the water holding capacity of meat (measured by drip loss, pH and colour) and its intramuscular fat content.

The second-generation microarray now under construction will contain fewer individual elements, with some of the redundancy removed from the gene set, but additional clones from the most unique SSH libraries and further candidates supplementing it, giving approximately 7000 different elements. The reduction in clone number will allow a triplicate printing of all the elements: this means that the internal validation of data by comparison of pixel values from replicate elements on a slide will not be compromised by loss of information from one replicate. This can occur owing to background or hybridization anomalies, and with a duplicate system, singleton data is often discarded owing to lack of confirmation of the intensity ratios.

This second-generation array will be used to assess the correlation of gene expression results with phenotypic measurements of tenderness and sensorial traits, together with other detailed measurements such as fatty acid composition. The five batches of animals selected for this experiment have detailed phenotypic measurements and also proteomics results. One hundred individual animals, twenty animals from each line, will be arrayed and compared against a common pooled control sample in a reference design (Figure 1b, Page14). By using a common reference, data from each animal can be compared against any other animal in the experiment. In addition, some samples from the first series of loop designs occur within the second experiment. As both arrays contain a core common set of elements, these animals are potentially useful for the comparison of data between the experimental designs. In the first experiment genes which show significant differential expression between high and low scoring animals will be identified. In the second experiment the objective will be to find significant correlations between phenotypic trait values and the normalized residual gene expression values, after correction for dye, array and batch effects.

New tools for understanding variation in muscle phenotype

The project was established in order to exploit new technology developments that allow parallel analysis of gene expression (the transcriptome and proteome). The ultimate aim being to identify variation in the genes which contribute to variation in meat quality. The assumption was that some of these genes will be expressed in muscle at the time of slaughter and that variation in expression levels would be associated with variation in quality parameters of meat derived from this muscle. When these genes are identified the resources developed in the project (e.g. cDNA libraries) will enable further analysis of them and also the search for

variation in the sequence of the genes. Once sequence polymorphism is identified this can then be tested for association with variation in the traits of interest. The project database allows for simultaneous analysis of effects on related or secondary traits including aspects of performance and also the yield of some cuts.

This approach is illustrated by the results obtained for the "RN" gene, *PRKAG3* (see Milan *et al.*, 2000, Ciobanu *et al*., 2001), and the protease inhibitor calpastatin, *CAST* (see Ciobanu *et al*., 2002, 2004). Variation in the gene sequence has been identified that explains significant variation in the pH and colour or the tenderness of meat. Simple DNA screening tests can then be developed to select for animals having the preferred genotype. One interesting observation made with respect to the effect of variation in *PRKAG3* was that the impact on pH and colour seemed to be more pronounced in ham than loin (Ciobanu *et al*., 2001). One explanation may be that this difference relates to a difference in the levels of expression in these two muscles. The resources developed in this project allowed this hypothesis to be tested. Initial real time PCR analysis of samples from Landrace and Meishan lines revealed a significant line difference in the expression level of *PRKAG3*, with expression been higher in Landrace (results not shown). Furthermore the expression level of *PRKAG3* in LT muscle was greater than SM ($p = 0.06$)) and this disparity was more evident in the Meishan line than the Landrace line. In a second analysis RNA was selected from LT and SM muscle from randomly chosen Piétrain animals. The expression level of *PRKAG3* in this line was again greater in loin samples than in ham (mean LT versus SM copy number $p=0.041$) although there was also substantial individual variation in expression of this gene (Figure 5, Page 18). The next step will be to see if *PRKAG3* genotype can explain some of this individual variation. The expression results suggest that the *PRKAG3* gene may be expressed differentially between muscles and this may play a role in the variation of metabolism (and ultimately meat quality) between muscles.

Conclusions

DNA markers for meat quality traits have already been identified and are being used by pig breeding companies as part of genetic improvement programmes (see Knap *et al*., 2002). The Quality Pork Genes project has established a phenotypic database and collection of RNA/DNA samples that will enable the search for new genes responsible for variation in pork meat quality, including the interaction with stress. It is anticipated that this unique resource will result in the identification of markers (which may be metabolic, biochemical, and neuroendocrine as well as genetic markers) associated with variation in quality. These markers will then be delivered for further research and/or validation of their utility in breeding and selection or screening, in order to provide the basis for the development of new tools to improve raw material quality.

The project database and samples together with the new research tools (e.g. muscle specific cDNA microarrays), represent important resources that can be used to advance research in muscle physiology and meat quality. Ultimately, these tools will play a role in delivering consistently better product quality to the consumer.

Acknowledgements

The authors thank their QualityPorkGENES colleagues in their respective organisations. The QPG project (http://www.qualityporkgenes.com) is supported by the European Commission under the Fifth Framework Programme (http://www.cordis.lu/life), contract number QLK5-CT-2001-01888. The paper is the work of the authors and does not necessarily represent the position of the Commission.

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Figures 1–5, see below pages 14–18!

Figure 1A

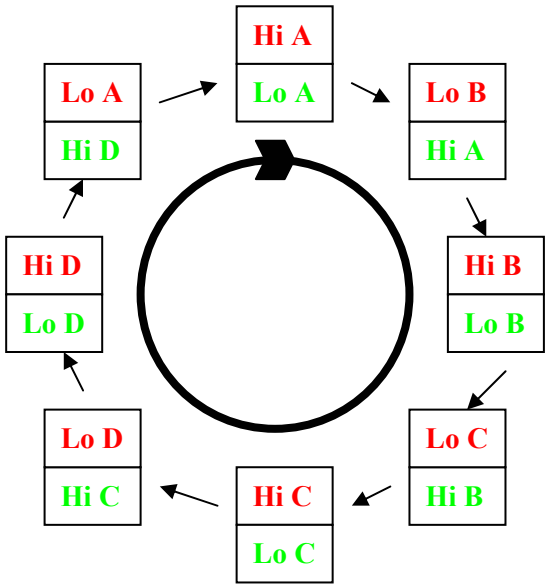


Figure 1B

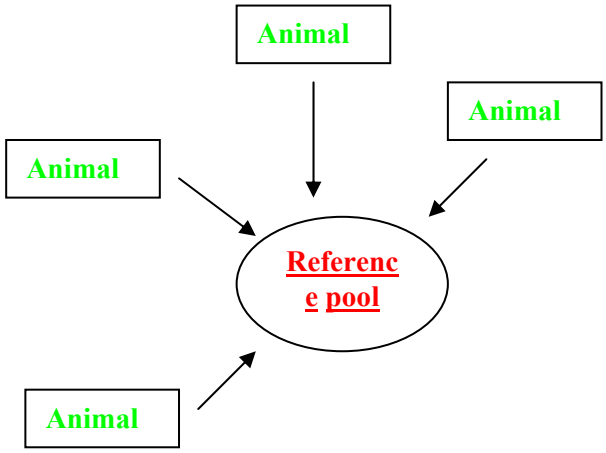


Figure 1. **a) Loop design**: all samples appear twice within the loop, with an intrinsic dye swap. Samples are labelled red (Cy5) or green (Cy3) and each pairwise comparison includes samples from one high and one low scoring animal . **b) Reference design**: each test sample is compared against a common reference. For QPG the reference will be a pool of RNA from LT muscles, taken from approximately 100 animals and equally representing all five breeds. The reference will be labelled uniformly for all slides.

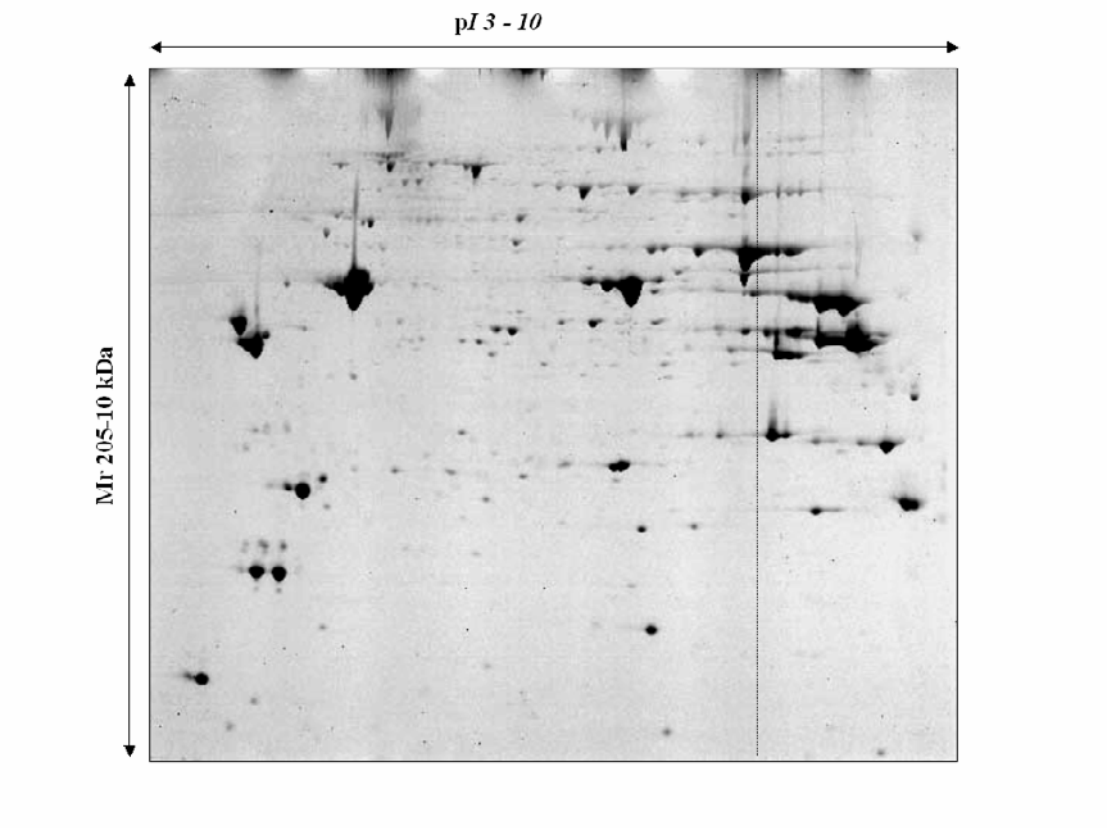
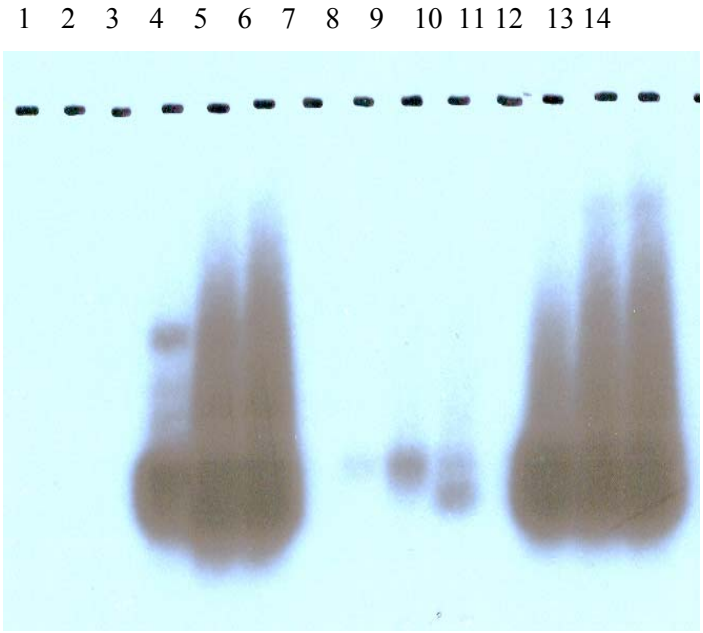


Figure 2. A typical two dimensional gel electropherogram used for proteomic analysis. The p*I* range (3-8.5) used for analysis, which excludes areas of saturation and poor resolution, is shown to the left of the dotted line.

a)



b)

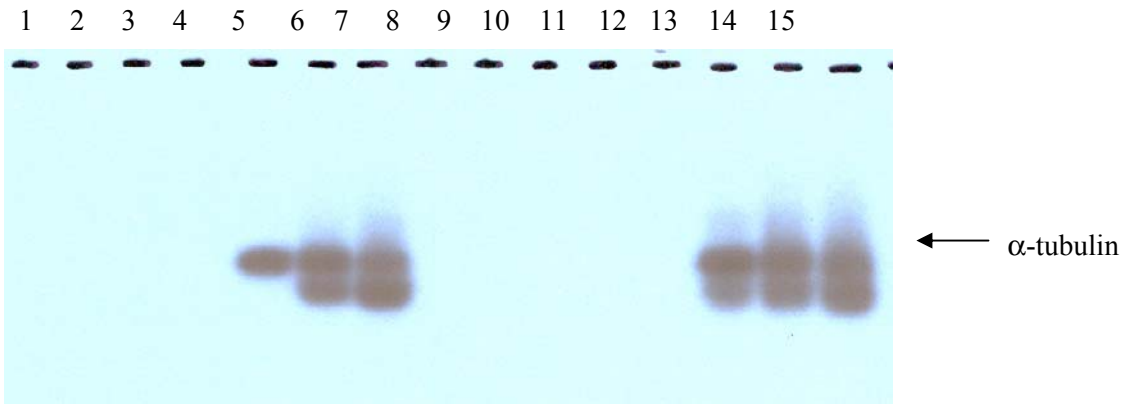


Figure 3. Analysis of the quality of SSH libraries – reduction of the abundance of housekeeping genes in subtracted cDNA libraries

A) SSH 3 and 4 hybridisation to actin
B) SSH 3 and 4 hybridisation to α-tubulin

Lane 1, 2, 3 SSH3 secondary PCR subtracted cDNA cycle 10, 12 and 14 respectively.
Lane 4, 5, 6 secondary PCR unsubtracted cDNA cycle 10, 12 and 14 respectively
Lane 7, 11 blank lanes
Lane 8, 9, 10 SSH4 secondary PCR subtracted cDNA cycle 10, 12 and 14 respectively
Lane 12, 13, 14 secondary PCR unsubtracted cDNA cycle 10, 12 and 14 respectively

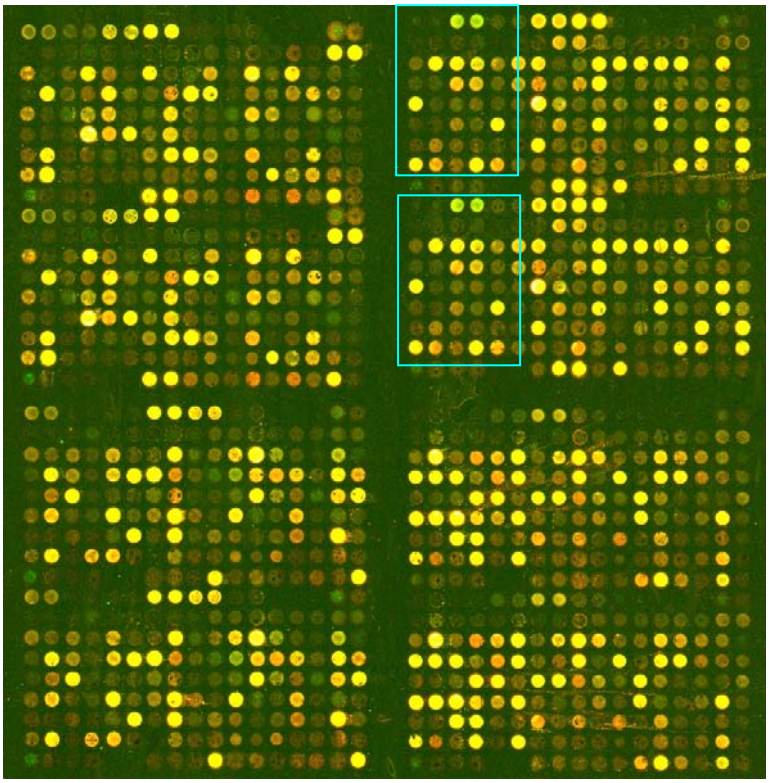


Figure 4. a) Example of a section of a microarray hybridized with LT (Cy3) and SM (Cy5). The boxes highlight duplication of elements within the subarray. Most elements are expressed equally, giving shades of yellow, but a few genes are upregulated in LT (green) or SM (red).

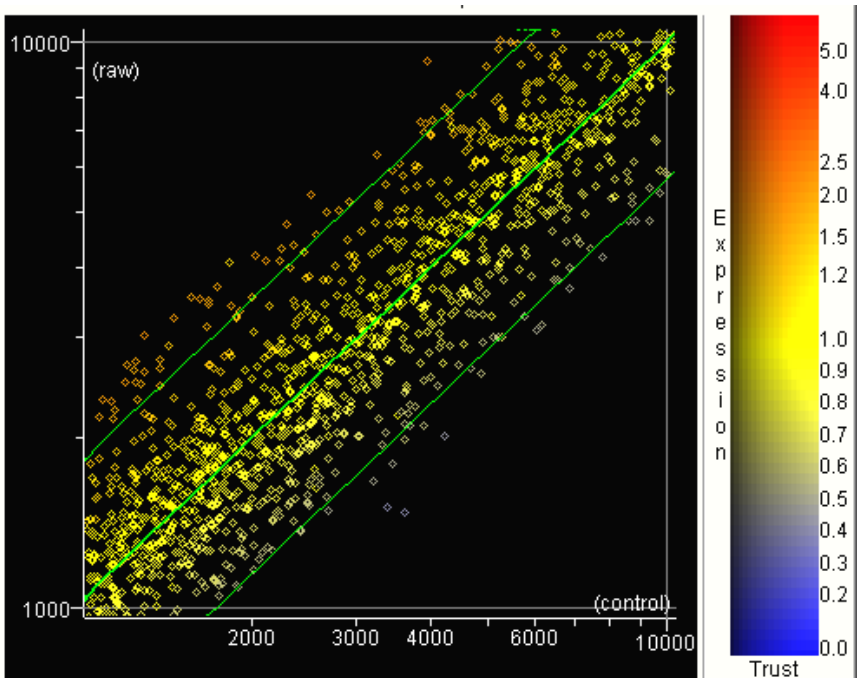


Figure 4 b) Following extraction of pixel intensity data from LT v SM experiments, ratio information has been entered into GeneSpring (Silicon Genetics). This shows a section of the output with the 1:1 ratio line and flanking 1.75 fold up or down regulated.

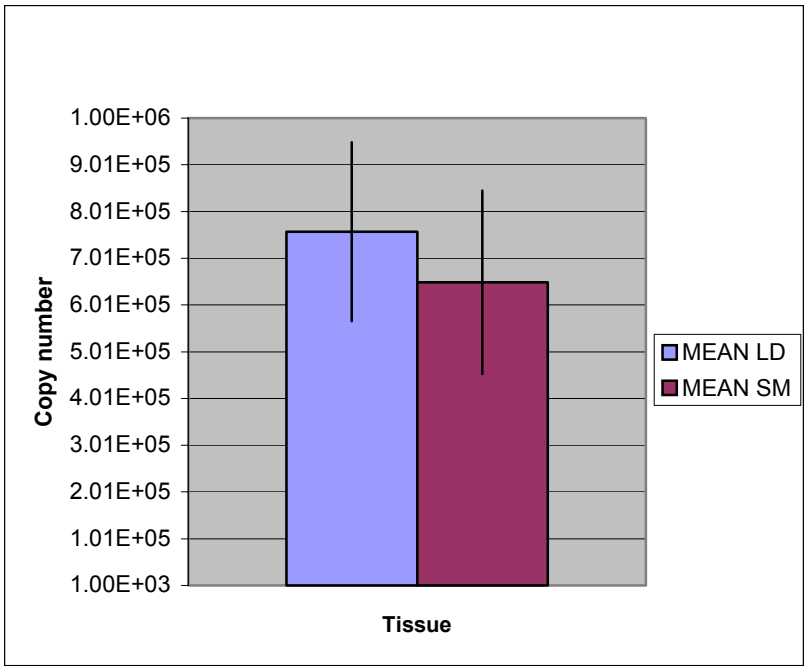


Figure 5. Differential expression of *PRKAG3* in Loin (LT) and Ham (SM) muscle samples from Piétrain animals as determined by real-time PCR.

Table 1: Means and standard deviations (std) of carcass characteristics depending on the genetic line*.

		Landrace		Large White		Duroc		Pietrain		Meishan	
	n	mean	std	mean	std	mean	std	mean	std	mean	std
Carcass weight (kg)	500	89.62 ^{ab}	6.62	90.83 ^{ab}	5.90	91.90 ^{a}	6.81	88.76 ^{b}	6.19	85.73 ^{c}	6.53
Fat depth at the last rib measured with FOM (mm)	499	13.81 ^{c}	2.91	13.38 ^{c}	2.75	16.36 ^{b}	3.60	11.54 ^{d}	2.67	20.94 ^{a}	4.54
Area of LT muscle (cm^2)	500	47.85 ^{cb}	5.85	47.38 ^{c}	5.39	49.54 ^{b}	6.38	57.39 ^{a}	6.65	39.95 ^{d}	5.43
Estimated lean with FOM (%)	500	57.11 ^{b}	2.63	57.30 ^{b}	2.97	55.12 ^{c}	2.75	60.34 ^{a}	2.34	48.79 ^{d}	4.75

*Values in a row with a common superscript are not significantly different (P>0.05)

Table 2: Means and standard deviations (std) of meat quality parameters measured in the Longissimus thoracis muscle depending on the genetic line*

		Landrace		Large White		Duroc		Pietrain		Meishan	
	n	mean	std	mean	std	mean	std	mean	std	mean	std
pH45	500	6.48 ^{b}	0.23	6.62 ^{a}	0.22	6.57 ^{a}	0.19	6.56 ^{a}	0.18	6.58 ^{a}	0.17
pHu i	500	5.58 ^{b}	0.13	5.63 ^{a}	0.13	5.64 ^{a}	0.11	5.64 ^{a}	0.14	5.59 ^{b}	0.11
L* (lightness)	500	48.49 ^{a}	2.73	46.41 ^{bc}	3.43	46.14 ^{c}	2.48	46.69 ^{bc}	3.22	47.22 ^{b}	2.38
A* (redness)	500	2.72 ^{c}	1.10	2.90 ^{c}	0.85	2.95 ^{bc}	0.95	3.32 ^{ab}	1.17	3.38 ^{a}	1.16
B* (yellowness)	500	4.29 ^{a}	1.51	3.63 ^{b}	1.86	3.40 ^{b}	1.36	3.87 ^{ab}	1.67	4.21 ^{a}	1.27
Drip loss	461	3.31 ^{a}	1.43	2.86 ^{ab}	1.20	2.44 ^{b}	1.09	2.84 ^{b}	1.25	2.81 ^{b}	1.44

*Values in a row with a common superscript are not significantly different (P>0.05)

Table 3: Means and standard deviations (std) of biochemical parameters measured in the Longissimus thoracis muscle depending on the genetic line*

		Landrace		Large White		Duroc		Pietrain		Meishan	
	n	mean	std	mean	std	mean	std	mean	std	mean	std
Lactate dehydrogenase (LDH) (μmol NADH·min^{-1}·g^{-1})	249	3133.03	717.62	3047.38	777.03	3102.32	593.91	3295.29	787.66	3143.02	675.77
Isocitrate dehydrogenase (ICDH) (nmol NADPH·min^{-1}·g^{-1})	249	1.33^{b}	0.40	1.33^{b}	0.47	1.57^{a}	0.53	1.57^{a}	0.56	1.23^{b}	0.39
ratio (LDH/ICDH)·10^{-3} (μmol ·nmol^{-1})	249	2.49^{ab}	0.69	2.45^{ab}	0.65	2.18^{b}	0.81	2.32^{b}	0.83	2.80^{a}	1.11
Myosin Heavy Chain-I (%)	248	7.43^{ab}	2.69	7.49^{ab}	2.82	8.65^{a}	2.85	8.46^{ab}	3.49	6.96^{b}	2.71
Cathepsin B (units · mg protein^{-1})	244	0.034^{ab}	0.015	0.031^{bc}	0.0125	0.039^{a}	0.0172	0.032^{bc}	0.0153	0.025^{c}	0.011

*Values in a row with a common superscript are not significantly different (P>0.05)

Table 4: Means and standard deviations (std) of intramuscular fat, fatty acids and tryglicerides of the Longissimus thoracis muscle depending on the genetic line*

		Landrace		Large White		Duroc		Pietrain		Meishan	
	n	mean	std	mean	std	mean	std	mean	std	mean	std
Intramuscular fat (%)	498	1.11^{bc}	0.34	1.00^{c}	0.44	1.82^{a}	0.54	1.24^{b}	0.37	1.96^{a}	1.00
Stearic acid (%)	247	12.31^{b}	0.78	12.39^{b}	0.85	13.45^{a}	1.03	11.95^{b}	0.69	12.19^{b}	0.83
Linoleic acid (%)	247	13.72^{a}	2.98	13.80^{a}	3.00	10.61^{b}	2.14	13.05^{a}	2.67	9.49^{b}	1.93
Dioleyl-steaoryl-glycerol (%)	93	5.33^{ab}	1.03	5.09^{b}	1.02	5.83^{a}	0.90	5.82^{ab}	0.56	5.61^{ab}	1.12
Disteaoryl-oleyl-glycerol (%)	93	1.58	0.77	1.16	0.52	1.46	0.48	1.18	0.35	1.12	0.47

*Values in a row with a common superscript are not significantly different (P>0.05)

Table 5: Least squares means (lsmeans) of the sensory characteristics of the Longissimus thoracis muscle*

		Landrace	Large White	Duroc	Pietrain	Meishan	
	n	lsmeans	lsmeans	lsmeans	lsmeans	lsmeans	RMSE+
Hardness	250	5.3 a	5.1 a	4.6 b	4.6 b	4.2 b	0.8508
Juiciness	250	2.5 b	2.6 b	3.1 a	3.1 a	3 a	0.6222

*Values in a row with a common superscript are not significantly different (P>0.05)
+ RMSE: Root mean standard error

Table 6: Means and standard deviations (std) of the instrumental texture analysis of the Longissimus thoracis muscle*

		Landrace		Large White		Duroc		Pietrain		Meishan	
	n	mean	std	mean	std	mean	std	mean	std	mean	std
Hardness	247	15.36 a	2.57	15.36 a	3.13	15.12 a	2.59	13.86 b	2.51	15.09 ab	2.08

*Values in a row with a common superscript are not significantly different (P>0.05)

CARBOHYDRATE METABOLISM IN MEAT ANIMALS

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Introduction

This review deals with the perimortem oxidative/glycolytic capacity of meat animals, especially in muscle tissue. We discuss carbohydrate metabolism focusing on the relationships between the pre-mortem and post-mortem phenomena. The nutritional and hormonal regulation of energy metabolism in skeletal muscles of meat animals has recently been thoroughly reviewed e.g. by Hocquette, Ortigues-Marty, Pethick, Herpin & Fernanadez (1998) and will not be treated here. The subject discussed here has also been presented by the authors of the current review also elsewhere, at Reciprocal Meat Conference (Puolanne, Pösö, Ruusunen, Sepponen & Kylä-Puhju, 2002)

Carbohydrates are essential in the conversion of muscle to high quality meat, but in live animals they are stored in the muscles to provide the animal with a rapidly available source of energy. In "fight-or-flight" situations the muscles have to produce energy at the highest possible rate and even in animals with high aerobic capacity part of this energy production will involve anaerobic pathways and accumulation lactic acid, which is the end product of anaerobic glycogen breakdown. Within the animal kingdom horses have a high oxidative capacity, as indicated by maximal oxygen uptake up to 160 ml/kg body weight per min (Tyler, Golland, Evans, Hodgson & Rose, 1996; Langsetmo, Weigle, Fedde, Erickson, Barstow & Poole, 1997). This is more than twice the value measured in human athletes and about three times that in cattle (Kayar *et al*., 1994) and almost 6 times the value measured in pigs (Perez-de-Sá, Cunha-Goncalves, Schou, Jonmarker & Werner, 2003). Theoretically, if it is assumed that during intense exercise 90% of oxygen is consumed by exercising muscles and that approximately 40% of the body weight is muscle, it can be calculated that with the reported maximal oxygen uptake of horses, aerobic ATP production in the equine muscle may approach 2 µmol/g per s and a similar calculation for pigs gives a value of 0.35 µmol/g per s. Although this calculation underestimates the true production of ATP in working muscles, because all muscles are not equally active as assumed in the calculation above, the values are well below the approximated maximal ATP demand, the calculated value of which for human muscle is about 3 to 5 µmol/g per s (Newsholme & Leech, review 1986). The difference between the demand and aerobic production has to be met by anaerobic glycolysis, the end product of which is lactic acid. This is possible, because the capacity of glycolytic enzymes, at least in equine and porcine muscles, is high enough to support the ATP production that allows maximal rate of ATP consumption (Essén-Gustavsson, Karlström & Lindholm, 1984; Newsholme & Leech, review 1986; Cutmore, Snow & Newsholme, 1993; Karlström, 1995).

The aerobic capacity is determined by the capacity of cardiovascular system and muscle related factors, such as fibre composition and mitochondrial density. On the cardiovascular side, the cardiac output and capillary density in muscles determine the availability of oxygen and inside the muscle the mitochondrial density is a key factor in determining the rate of oxygen utilization. In meat animals the aerobic capacity decreases in the following order cattle > pig > poultry. The size of the heart in beef cattle has been reported to be approximately 0.44% of live weight (Sainz & Bentley, 1997) and in pigs 0.33% (Ruusunen & Puolanne, unpublished), and even greater difference is seen in the capillary density. In the longissimus muscle of cattle the capillary density is approximately 450/mm^2 (Karlström, Essén-Gustavsson & Lindholm, 1994), but in pigs the value is only 150/mm^2 (Ruusunen & Puolanne, 2004). It is generally accepted that the blood flow into skeletal muscle at rest is about 20% of cardiac output, but it may increase to up to 80% of cardiac output

during intense exercise. When this is combined with the increase in cardiac output, the actual increase in blood flow may be 15-fold (or in the case of athletic animals such as horses even more). In such situations the capillary density of the muscles may play an important role. Higher capillary density in the muscles of cattle may result in a better supply of oxygen and efflux of end products of metabolism than is possible in porcine muscles. Thus it can be speculated that during stress both the production of lactic acid as well as accumulation of lactate will be greater in the muscles of pigs than of cattle.

The amount of glycogen in muscles varies from species to species. In the longissimus muscle of cattle the measured concentrations vary between 60-100 mmol/kg (wet) tissue (Immonen, Kauffman, Schaefer & Puolanne, 2000a), in pigs values are around 85 mmol/kg (Rosenvold, Laerke, Jensen, Karlsson, Lundström & Andersen, 2001), but in horses glycogen concentration is usually 1.5 times the concentration measured in cattle (Lindholm, Bjerneld & Saltin, 1974; Hyyppä, Räsänen & Pösö, 1995).

The Glycogen Molecule Complex

The glycogen molecule contains a core protein, glycogenin (MW 37,300 Da), which has enzyme-like properties and supports and catalyses the synthesis of an 8 glucosyl-unit-long primer, necessary for the activity of glycogen synthase (Nelson & Cox, review 2005). As glycogen synthase takes over, the chain grows and forms a branched structure. Each linear glucose chain contains 13 units, bound together with α-1,4-glycosyl bonds. At the fourth and eighth glucosyl units, each branched chain (B-chain) has a 1,6-bond which gives rise to new unbranched chains of 13 units (A-chains) (Figure 1). Consequently, after twelve branchings (13th tier) the number of chains is $1+2^{12}$, or about 4,100. A glycogen molecule with the molecular weight of nine to ten million Da contains about 55,000 glucose residues, the diameter of the molecule is close to 40 nm and the number of available non-reducing ends is approximately 2,100. The full outermost tier contains always roughly 50% of the total carbohydrate of the molecule. Each glycogen particle is covered with up to 40-50 phosphorylase dimers (or 20-25 tetramers). The structure of the glycogen molecule optimizes its usefulness: it packs glucose into the smallest possible volume, maximizes the number of non-reducing ends, and thus maximizes the proportion of glucose units to be removed by phosphorylase before the proximity of a branching point stops its activity (Meléndes-Hevia,Waddel & Shelton, 1993).

Proglycogen and Macroglycogen

The glycogen molecules exist in two forms: proglycogen (PG) and macroglycogen (MG) (Adamo and Graham, 1998). In resting human muscle, in which glycogen stores are full, the acid insoluble proglycogen (protein content about 10 %, MW 400,000 Da) represents approximately 60% of the total glycogen, while in stressed muscle with less glycogen, its proportion is about 90% (Adamo & Graham, 1993). The relative amount of the acid soluble macroglycogen (0.4% protein, MW 10^7 Da) accounts for the rest of the total glucose content, 10-40%. In pigs, similar PG/MG ratios, approximately 68-82% to 18-32%, have been reported, the variation depending on the level of stress and diet (Rosenvold, Essén-Gustafsson & Andersen, 2002). It remains to be seen, how much of glycogen in the form of acid-insoluble PG has not been taken into account in the earlier published studies in which the glycogen has been extracted by acid.

As each chain, also in PG, has 13 glucosyl residues (MW: 13*162 = 2106/chain) it can be calculated that the PG molecules have 6 (MW ca 300,000 including glycogenin) to 7 tiers (MW ca 575,000). In theory, this provides 64 to 128 non-reducing ends and thus sites for phosphorylase action. This also means that the number of phosphorylase units bound to each PG molecule and the number of non-reducing ends are practically the same. In comparison, in a MG molecule, the number of tiers with a molecular weight of 10^7 Da is 13 and the number of non-reducing ends approximately 2,100.

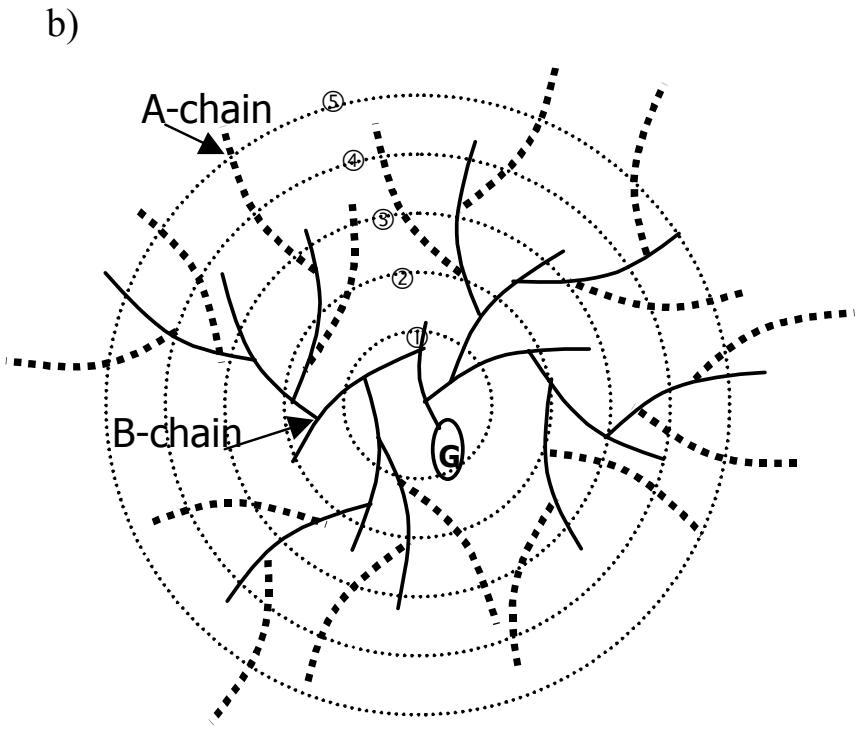

Figure 1. Scheme showing the structure of the glycogen molecule as stated in Whelan's model (Adopted from Immonen, 2000)

Glycogen breakdown: phosphorylase

In muscle, the main sources of glucose are blood glucose (from liver glycogen) and glycogen stored in muscles (Hocquette *et al*., review 1998). During stress, hormonal mechanisms are important for the mobilization and utilization of carbohydrates. In muscle, catecholamines, released from sympathetic neurons and adrenal medulla, induce rapid degradation of glycogen through the activation of glycogen phosphorylase and the inhibition of glycogen synthesis. In addition phosphorylase is under allosteric (AMP and/or Ca^{++} activated) control. Activated phosphorylase cleaves glucosyl units as glucose-1-P from a glycogen molecule at an extremely high speed. The reaction cascade is explicitly described in every textbook of biochemistry and therefore, will not be presented here. The sequence is blocked when the influx of blood glucose meets the demand of energy, and when the enzymes of glycogen synthesis are activated (Nelson & Cox, review 2005).

The catalytic subunit of a glycogen phosphorylase dimer (or tetrame) is bound to glycogen, whereas the other subunit regulates the function of the enzyme (Goldsmith, Sprang & Fletterick, 1982). The phosphorylase content is very high, about 2% of the total protein in muscles (Ryman & Whelan, 1971). Glucose residues from the non-reducing end of the uppermost, unbranched A-chains, are cleaved until the fourth glucosyl unit from the branching point. Phosphorylase cannot cleave glucosyl units from the branched B-chain that is too short. Thus, theoretically 34.6% of the units of the outermost tier are available to phosphorylase, i.e. in the case of 100 mmol/kg glycogen (as glucosyl units) in muscle about 35 mmol/kg glucose-1-P will be produced. This results in the formation of 70 mmol/kg lactate (and 105 mmol/kg ATP), if all energy were produced anaerobically (which is not the case *in vivo*). It has been shown that an increase in the number of non-reducing ends enhances, for spatial reasons, the binding of phosphorylase to the glycogen molecule and therefore, enhances the phosphorylase activity as well. The same spatial factor may limit the release of glucosyl units of the lowermost tiers, when the glycogen content is low (Meléndes-Hevia *et al.*, 1993).

Recently it has been shown in humans and horses that MG is preferentially used in long-lasting aerobic stress (Asp *et al*., 1999; Essén-Gustavsson & Jensen-Waern 2002), but PG is utilized more during anaerobic stress (Graham, Adamo, Shearer, Marchand & Saltin, 2001; Bröjer, Jonasson, Schuback & Essén-Gustavsson 2002) as well as in pigs post mortem (Rosenvold *et al.*, 2002). It can be speculated that the mechanism of phosphorylase activation is different in these cases. Although exact mechanisms are not known, these may involve the allosteric activation of phosphorylase b kinase by calcium ions and/or by AMP (Nelson & Cox, review 2005).

Glycogen breakdown: Debranching Enzyme

When phosporylase has cleaved the glucosyl units to the level of four units from the 1,6-branching point (limit dextrin), the bifunctional glycogen debranching enzyme (GDE) transfers maltotriosyl groups from the 1,6-branching point (A-chain) (transferase function) and then cleaves the remaining 1,6-glucosyl unit as free glucose (1,6-glucosidase function). This enables the continuation of the action of phosphorylase (Nelson & Cox, review 2005). Taking both activities of the debranching enzyme into account the total activity is less than 10% of that of phosphorylase. While phosphorylase is able to cleave 12 glucose residues as glucose-1-P, the debranching enzyme cleaves only one as glucose (Meléndes-Hevia *et al.*, 1993). There is some evidence that the activity of the debranching enzyme limits the rate of glycogen breakdown in situations where more than the uppermost layer is to be used (Nelson, White & Gillard, 1969). It can also be anticipated that because of the activity of phosphorylase on glycogen molecules with low number of non-reducing ends is reduced due to spatial reasons, the glycogen molecule may not be fully utilized. Experimental results show that at least 10 to 20 mmol/kg glycogen is always left in muscles, even post mortem and in well-fed, non-stressed animals the residual glycogen may be much higher, up to 80 mmol/kg, even when the ultimate pH is 5.5 (Immonen & Puolanne, 2000).

Kylä-Puhju, Ruusunen & Puolanne (2004b, c) were able to show that the activity of GDE is not particularly affected by the pH in the pH range 5.5–7. But, on the contrary, temperature fall had a very strong effect on the activity, which was close to zero at temperatures below 15 ºC. This may indicate that the positive effects of fast chilling of porcine PSE carcasses (Honikel, review 1984; Offer & Knight, review 1988; Schäfer, Rosenvold, Purslow, Andersen & Henckel, 2002) may be partly based on the elimination of a source of rapidly fermentable glucose due to inactivation of GDE. In the case of beef, where the rate of glycogenolysis as well as carcass chilling are much slower, the lowering of the temperature may block the GDE activity. However, it is not known, how long the A-chains are at death or how long it takes before 15 ºC is reached. Thus there could be variable amounts of glucose-1-P available. The lactate derived from glucose is effective at lowering the pH. Although the buffering capacity, depending on animal species and type of muscle, is from 40 to 60 mmol/(kg∗pH), already small decreases in pH, e.g. 0.2–0.3 pH units, that can be a result from the accumulation of 10 to 20 mmol/kg of lactic acid derived from 5 to 10 mmol of glucose equivalents, are relevant and have a marked effect on water-holding, colour and tenderness. In addition, small differences in the rate of post mortem pH decline have huge effects on the traits mentioned above.

A limited meta analysis of the data EU Concerted Action CT94-1881 coordinated by Joseph and Troy (1998, see the references in the Proceedings; numbers refer to the papers in the Tomes 1, 2 and 3) revealed that in most cases fast chilling produced beef where the pH_{24} was ca. 0.1–3 units higher than by conventional cooling (e.g. Sheridan, McGeehin & Butler, 1.10; Ronchales & Beltram, 2.23; Beltram & Ronchales, 2.24; Beltran, Jaime & Ronchales, 2.5; Ronchales, Jaime & Beltràn, 2.8; Beltran, Tomas & Ronchales, 3.14; Tomas II176, Steen, Claeys & Demeyer, 3.6, Claeys, Demeyer & Van de Voorde, 3.12; Richardson & Perry, 3.15), although in several studies this was not found, or it was even the opposite (e.g. Klont & Eikelenboom, 1.11; Montanac-Bissay & Lepetit, 2.10; Troy & Vidal, 3.4; Taylor, Richardson & Perry, 3.10; O'Mahony, Joseph, McKenna, 3.17; Trevisani, Loshi & Severini, 3.19; Smulders, van Laack & Hofbauer, 3.20). Sometimes there was only an initial reduction in pH fall suggesting that the remaining activity of phosphorylase and GDE could be enough when the time available is long enough (White, Troy & McKenna, 1.13; Steen, Claeys & Demeyer, 3.6; Trevisani, Loshi & Severini, 3.11). This, however, needs further research, and a similar study on electrically stimulated carcasses should be done. It should also be pointed out that pH and temperature change rates differ much in carcasses, which makes the scrutiny of these aspect difficult for practical considerations (Puolanne & Ruusunen, 1998).

Lactate Dehydrogenases

Pyruvate is the junction point in carbohydrate metabolism. When oxygen is available and the density of mitochondria is not a limiting factor, the citric acid cycle and oxidative phosphorylation are the preferred pathways of oxidation and energy production. When the demand for ATP exceeds the capacity of aerobic metabolism, ATP will be produced anerobically, which, as previously explained, involves the reduction of pyruvate to lactate. The reaction is catalyzed by lactate dehydrogenases in cell cytosol (Nelson & Cox, review 2005). In muscles, as in other tissues, lactate dehydogenases occur as five tetrameric isoenzymes (LDH-1 ... LDH-5). The four units in each LDH molecule are composed of muscle-type (M) or heart-type (H) chains. The extreme combinations are H_4 (LDH-1) and M_4 (LDH-5) (Dawson, Goodfriend & Kaplan, 1964; Fieldhouser & Masters, 1966). LDH-5 has a high K_m for pyruvate, thus favoring the formation of lactic acid when the rate of glycolysis is high. Furthermore it is not inhibited by an excess of pyruvate as is LDH-1. Although the M-type dominates in dark and white muscles, the dark muscles contain more H-type activity when compared to light muscles. For example in infraspinatus muscle about 30% of the chains in LDH isoenzymes are H-type while the percentage in longissimus muscle is only 4 (Ruusunen, Sepponen, Puolanne & Pösö, unpublished). Porcine muscles have an exceptionally high LDH activity (Hamm & El-Badawi, 1991; Suuronen, 1995). Acute stress, coupled with a low capillary and mitochondrial density, will favor the formation of lactic acid, which as it is a utilizable source of energy, is later converted back to pyruvate. This takes place more efficiently in the heart and dark (oxidative) muscles than in light (glycolytic) muscles.

The Formation of Lactic Acid

In the muscle fibres of a living animal, small amounts of lactic acid is produced almost constantly. The activation of glycogen phosphorylase during stress increases the accumulation of lactic acid and consequently that of protons causing fatigue, and ultimately, pain and distress (Gregory, review 1998). The acidification of the muscles leads to attenuation of the rate of glycolysis, because protons inhibit the activities of glycogen phosphorylase and phosphofructokinase and thus energy production from carbohydrates (Fitts, review 1994). Protons may also inhibit the function of myosin ATPase (Fitts, review 1994; Schiaffino & Reggiani, review 1996) and thus impair muscle contractions. Furthermore, both the uptake and release of calcium ions from the sarcoplasmic reticulum are disturbed due to acidification (Westerblad, Lee, Lännergren, & Allen, review 1991; Williams & Klug, review 1995). In fatigued muscle the pH may be as low as 6.5 to 6.3 (Lowell, Reid & Rose, 1987; Juel, review 1996), but this value is significantly higher than the ultimate pH in muscles after slaughter that is usually around 5.4–5.8

When a glucose molecule is broken down into two molecules of pyruvate, two to three molecules of ATP are generated. In addition, two molecules of NAD^+ are reduced to NADH, which must be reoxidized in order to maintain the rate of glycolysis. When oxygen is available, electrons from NADH are transferred to molecular oxygen via the mitochondrial electron transport chain. However, when oxygen is lacking, oxidation of NADH is coupled with the reduction of pyruvate to lactate, i.e. lactate formation is a prerequisite for anaerobic energy production (Nelson & Cox, review 2005). Lactic acid formed is either converted back to pyruvic acid to be used oxidatively via the tricarboxylate acid cycle, or when there is a lack of oxygen and/or mitochondria, transported out of the fibre.

When pH in muscles is 6.2, the concentration of lactic acid in the muscle fibres must be about 50 to 60 mmol/kg. In a 100-kg live weight modern lean pig (47 kg muscle tissue) this would mean 47 kg*55 mmol/kg*90 mg/mmol lactic acid, i.e. ca 230 g. Whether or not this overestimates the amount of lactic acid formed (all the muscles do not produce simultaneously this maximal amount of lactic acid), it means that after a stress situation this amount should be utilised in the adjacent fibres or released to blood. For aerobic tissues lactate is an excellent source of energy, because it contains most of the energy of glucose. In live animals all the lactate formed during stressful situations is either oxidized in heart, liver and aerobic muscles or converted back to glucose in liver (Pösö, 2002). It is also generally accepted that, within the muscle tissue, some lactate may be transported via the interstitial fluid from white, glycolytic, fibres to red fibres (type 1 and IIA) which have higher mitochondrial density. It can be speculated that in domestic pigs this

phenomenon is of minor importance, because the percentage of red fibres is small in muscles such as *M. longissimus, gluteus* and *semimembranosus* (Ruusunen 1994; Karlström, 1995; Ruusunen & Puolanne 2004). Without uptake of lactic acid into tissues for oxidation, only small amounts can be released into blood. Lactic acid concentration in blood may vary between 5–25 mmol/l, which in a 100-kg pig corresponds to about 10 g. The imbalance between formation and transport capacity of blood is non-physiological, and problems caused by the low oxidative capacity of modern pigs and poultry can be expected to rise if the increase in percentage of glycolytic muscle tissue will continue, due to the better feed conversion rate of glycolytic fibres (Hocquette *et al*., review 1998). The problem is not as severe in ruminants, which are lethargic and have higher oxidative capacity than pigs. When they are stressed and catecholamines evoke glycogenolysis, a large part of pyruvic acid will be utilised aerobically, and therefore large variation in pH in living muscle will not be probable.

The Buffering Capacity

Muscles are well adapted to their function. The buffering capacity keeps their pH at a level that allows an effective function of vital enzymes. The buffering capacity is greater in muscles needed for fast, bursts of short duration, and lower in muscles prone to long-lasting activity at relatively low intensity. Therefore, as a general rule, buffering capacity is greater in large glycolytic muscles with large-diameter muscle fibres and sparse capillarization. This suggests primarily anaerobic metabolism. Oxidative muscles with a small fibre diameter and high capillary density have a lower buffering capacity.

At rest the pH of porcine longissimus muscle varies from 7 to 7.2 (Tarrant, McLoughlin & Harrington, 1972; Kivikari, 1996; Kylä-Puhju, Ruusunen, Kivikari & Puolanne, 2004a). Because oxidative pathways produce carbon dioxide, protons are continuously formed in muscle cells according to the following equation:

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightarrow H^+ + HCO^-_3.$$

Large part of the carbon dioxide diffuses from the muscle into red blood cells and protons that are formed there are buffered by hemoglobin. In the muscle cells the formation of protons activates Na^+/H^+ exchange protein that carries one H^+ out from the cell and simultaneously one Na^+ into the cell (Madshus, 1988). This carrier is driven by the Na+ gradient (Madshus, 1988). There are no studies on the activity of Na^+/H^+ exchange in the muscles of meat animals, but in human muscle this carrier plays the key role in the regulation of cell pH at rest (Juel, Lundby, Sander, Calbet & van Hall, 2003).

A major difference in buffers between muscle and meat is the bicarbonate system that is very efficient in the live animals, but does not have any major role post mortem. Low pH acts as a signal to peripheral chemoreceptors and causes an increase in the rate and intensity of respiration. In lungs the reactions that in the tissues lead to formation of protons proceed in the opposite direction and the carbon dioxide is subsequently exhaled:

$$H^+ + HCO^-_3 \rightarrow H_2CO_3 \rightarrow CO_2 + H_2O\ .$$

Together these two reactions show that for each mole of CO_2 exhaled through the lungs one mole of protons is neutralized. Furthermore, the kidneys participate in the compensation by increasing the reabsorption of bicarbonate and also synthesizing it. In live animals lactate is also used in tissues, such as heart and liver, where metabolism is aerobic. Bicarbonate buffering system and the use of lactic acid in oxidative tissues prevent the drop of pH in live animals to such a low values seen post mortem, but after slaughter, the two mechanisms above have no bearing.

Most studies have found the buffering maximum at pH 6.8 and the minimum at pH 5.0. If pH is further lowered to values below 5, there is again a large increase in buffering capacity (Kivikari, review 1996). The latter increase is due to accumulation of lactic acid (Kivikari, review 1996). In addition to pH, the buffering capacity of post mortem muscles also varies according to the method used for its determination. Especially important is the amount of water used in the titration method determination. Kivikari (1996) and Puolanne & Kivikari (2000) found that with high water additions, the buffering capacity $\beta = dA/dpH$ (A= the amount of

added acid or base) has the maximum of ca 70 mmol H^+/(pH*kg) at the pH of ca 7.0, and the minimum of ca 30 mmol H^+/(pH*kg) at the pH of ca 5.5. This is in accordance with many other studies. With lower water additions, the minimum and maximum tend to level off. Kivikari (1996) also suggests that the buffering capacity in an intact muscle is somewhat lower, ca 40 mmol H^+/(pH*kg).

In the physiological pH range myofibrillar proteins have a buffering capacity of about 20 to 25 mmol H^+/(pH*kg). The soluble components influence the buffering capacity by about 10 (pH 5.5) to 30 mmol H^+/(pH*kg) (pH 6.8), carnosine and inorganic phosphate being the most important compounds (Kivikari, review 1996). Because post mortem reactions cause and increase in buffering capacity of about 8 to 10%, the buffering capacity in most post mortem muscles is between 40 to 60 mmol H^+/(pH*kg), but both lower and higher values have been reported. The reported values in beef are slightly lower and less variable than in pork (can be compared only if determined in the same study). In practical terms and within the pH limits from 7 to 5.5, lactic acid is by far the most important free variable in muscle to determine the actual pH value at any given time. The low pKa value of lactic acid (3.86) means that at the physiological pH range it is almost completely dissociated to a lactate anion and a proton. Without buffers, accumulation of lactic acid would mean a very rapid drop of pH. For example, Lindinger, McKelvie and Heigenhauser (review 1995) have estimated that without buffers, human arterial blood lactate concentrations would be 162 mmol/l and that plasma pH would drop almost to 1 after four 30-second bouts of high-intensity exercise. Kivikari (1996) concludes that the buffering capacity value of '50 mmol H^+/(pH*kg)' can be used as a "rule of thumb" in calculations to estimate the relationship of lactic acid content and pH value within the physiological range.

We have done a meta-analysis on literature data on relationships between glycogen contents, lactic acid contents, pH-values and buffering capacities in *M. longissimus dorsi* (Puolanne, Yli-Hemminki & Kylä-Puhju, 2004). The pH value of 7.2 was used as the zero lactate value. Based on the meta-analysis we found that the best fit between lactic acid, pH value and buffering capacity can be found when using buffering capacity value of 62 mmol H^+/(pH*kg). This was markedly higher than the 49 mmol H^+/(pH*kg) found by Kylä-Puhju *et al.* (2004a), who calculated the values on the basis of pH-lactic acid data of muscle analyses. These findings show once again that the buffering capacities depend on the methods by which they are determined, and much still remains to be studied.

Monocarboxylate transporters

When the rate of the formation of protons is high in living animals, buffering capacity alone is not sufficient to maintain pH homeostasis. In addition to Na^+/H^+ exchange, low pH activates also another proton carrier, the monocarboxylate transporter (MCT). MCTs form a transmembrane protein family, which cotransport a proton together with a lactate anion. In addition to lactate, MCTs also facilitate the transport of other monocarboxylate anions, such as short chain fatty acids (SCFA) and ketone bodies, through cell membranes (Halestrap & Meredith, review 2004). MCT-proteins are also involved in the absorption and delivery of monocarboxylic drugs, such as salicylic acid and simvastatin (Enerson & Drewes, review 2003). Recently isoform 8 (MCT8) was shown to transport thyroid hormones into cells (Friesema, Ganguly, Abdalla, Manning Fox, Halestrap & Visser, 2003).

Monocarboxylate transporter family is comprised of at least 14 members, which differ in their tissue and species specificity as well as in substrate affinity (Halestrap & Price, review 1999; Halestrap & Meredith review 2004). Among the best characterized are isoforms 1-4, but the role and characteristics of several isoforms are not known. Very little is also known about the regulation of MCT genes. Leptin has been shown to upregulate MCT1 mediated butyrate uptake in colonocytes (Buyse, Sitaraman, Liu, Bado, & Merlin, 2002) and erythropoietin increases the amount of MCT1 protein in erythrocyte membranes (Connes , Caillaud, Mercier, Bouix & Casties, 2004).

The topology of MCTs shows that they have 12 transmembrane domains with both C- and N-terminae in the cell (Halestrap & Price, review 1999; Halestrap & Meredith, review 2004). At least 3 isoforms, MCT1, MCT2 and MCT4, are expressed in skeletal muscle tissue (Juel & Halestrap, review 1999). MCT1 is a 53 kDa protein which, when expressed in *Xenopus* oocytes, has a K_m for lactate of 3-5 mM. Similarly expressed MCT2 has low K_m for lactate, 0.7 mM, while MCT4 expressed especially in glycolytic tissues has a much

lower affinity for lactate (K_m 28 mM) (Halestrap & Price , review 1999; Halestrap & Meredith, review 2004). The apparent V_{max} values for the influx of lactate into *Xenopus* oocytes are rather similar in MCT1 and MCT4, which is about 17-18 times that of MCT2 (Bröer, Bröer, Schneider, Stegen & Halestrap, 1999; Dimmer, Friedrich, Lang, Deitmer & Bröer, 2000). MCT1 and MCT4, but not MCT2, need a chaperone protein CD147 (basigin, EMMPRIN, neurothelin) to translocate and obtain active conformation in the cell membrane where they form a complex that consists of two MCT molecules and two CD147 molecules (Halestrap & Meredith, review 2004).

In most species including beef cattle (Koho & Pösö, unpublished results), MCT isoforms 1 and 4 are expressed in muscle tissue (Halestrap & Meredith, review 2004). In glycolytic muscle fibres both MCT1 and MCT4 facilitate the efflux of lactate when the rate of its production is high, whereas in oxidative fibres (type I) the dominant isoform is MCT1 which facilitates either influx or efflux of lactate depending on the prevailing electrochemical gradient of lactate (Halestrap & Meredith, review 2004).

In the muscles of domestic pig (Sepponen, Koho, Puolanne, Ruusunen & Pösö, 2003) and also those of wild boar (Sepponen, Ruusunen, Puolanne & Pösö, unpublished) only traces of MCT1 can be found. The low amount of MCT1 is not due to the lack of the MCT1 gene, since in porcine heart and intestines it is expressed (Sepponen, Ruusunen & Pösö, manuscript in preparation). Instead, the pig muscles express MCT2, which has high affinity for lactate (Halestrap & Meredith, review 2004). We have speculated that MCT2 is needed, because of the anaerobic nature of the porcine muscle fibres, which may involve continuous production of small amounts of lactate (Sepponen *et al*., 2003). The role of MCT2 would thus be to facilitate the efflux of lactate from cells where mitochondrial density is low. Although our knowledge on monocarboxylate transporters is increasing, there is no data available on their role post mortem.

The Rate of ATP Consumption and Production

ATP is used for contraction of sarcomeres and other processes in muscle, maximally 3 to 5 μmol/(g*s) (Lister, 1988). In a resting muscle, the consumption is about 1/100 of the maximum (0.03–0.05 μmol/(g*s)) which is needed to maintain the ionic balance and provide the basic heat of the body (69% of the energy produced in the reaction ATP → ADP + P + heat). It can be calculated that during maximal exercise an animal can increase its oxygen muscle consumption by more than 20 to 40 fold from the resting level. The increase is based on increase in cardiac output, increased relative amount of circulation through muscles, and increased release of oxygen from blood to muscles. Because the energy consumption may increase 100 fold, the difference must come from some other source, which is the anaerobic energy production via glycogenolysis. It can be estimated that in an extreme case, the pH value of living muscle may fall to 6.2-6.4 in less than 30 seconds. This is the case only in an anaerobic muscle, because in dark muscles do not easily show such an extensive decrease in pH *in vivo*. This pH change would need about 40–60 mmol H^+/kg.

Post-mortem the rate of pH fall may be in beef and sheep 0.005 and in porcine muscle 0.01 pH units/minute (Pearson & Young, 1989) or in PSE pig 0.02 (extreme PSE pig 0.1), in normal turkey 0.03 and in PSE turkey 0.06 pH units/minute (Sosnicki, Creaser, Piertrzak, Pospiech & Santé, review 1998). From that it can be estimated, using the buffering capacity of 60 mmol H^+/(pH*kg) and ATP/lactic acid molar rate of 1.5/1, that the ATP production varies in post mortem muscles of different species from 0.008 (beef and sheep), 0.015–0.03 (pig – PSE pig) (0.15 extreme PSE pig) to 0.05–0.09 μmol/(g*s) (turkey – PSE turkey) which is well in accordance with the values estimated in living resting muscle. It is not fully known, what are all the relevant reactions where ATP is consumed post mortem and why there are differences like between normal muscle – PSE muscle.

Immediately post mortem ATP is used in muscles for involuntary slow contractions, maintenance of membrane potential and especially to keep the calcium ions within the sarcoplasmic reticulum. Bendall (1979) showed that the ATP content remains at the normal level (8–10 mmol/kg) until half of the ultimate amount of lactic acid has been produced. Then the ATP content starts to decline, and when it reaches about 1 mmol/kg, there will be the onset of rigor mortis (which depend on the temperature of the muscle) (Honikel, Ronchales & Hamm, 1983).

ATP, creatine phospate, and oxygen bound to myoglobin contribute to the ATP level during the immediate post mortem phase. When one molecule of lactic acid is produced, 1 or 1.5 molecules of ATP/lactate are also produced. Therefore, all the metabolic factors discussed here are inter-linked and should be handled simultaneously when discussing the pH of muscle and meat and its effects on meat quality.

Post Mortem Oxygen Utilization

In a living animal, the partial pressure of oxygen in venous blood from resting muscles is about 40 torr, with oxygen saturation of haemoglobin of about 75% and in intensively working muscle the respective values are 20 torr or less and about 38%. If the physical stress further increases, myoglobin which is saturated up to 90%, starts to provide oxygen. When oxygen derived from myoglobin has been utilised, the oxygen pressure is approaching zero, and the fibre will be produce energy anaerobically via glycogen breakdown as described above. Consequently, both routes of ATP production are active simultaneously. The literature provides very variable data on the myoglobin contents of various meats. Generally, the content in light muscles ranges from 0.06 to 0.2 and in dark muscles 0.2 to 0.3 mmol/kg. In beef, the myoglobin content is higher and strongly age-dependent ranging from 0.1 to 1 mmol/kg. The full oxygen saturation of heme would then mean equivalent concentration of molecular oxygen. One 1 mmol oxygen enables an aerobic production of 6 mmol ATP. Consequently, the oxygen stored in myoglobin, allows the production of 0.06*6=0.4 to 1*6=6 mmol ATP, depending on animal species, age and muscle type. In addition, meat contains minute amounts of blood. Depending on the degree of bleeding and the oxygen saturation of blood in various muscles, additional oxygen would be available. No published data on this is available, but based on our preliminary data on hemoglobin hemi (not published) we estimate that the amount of available oxygen is no larger than the myoglobin reservoirs. The amount of oxygen in sarcoplasma is also very small.

The amount of ATP produced by the formation of about 80 mmol/kg lactic acid is 120 mmol/kg (the post mortem formation of lactic acid molecule results in 1.5 molecules ATP). Consequently, we suggest that oxygen in total heme content theoretically equals about 5% of the post mortem production of ATP via glycolysis in beef and less than 1% in pork. This difference might be one of the factors causing the lower initial rate of pH decline in beef than in pork. The variation in the oxidative status of muscles at death and amount of residual blood may also have an influence on the onset and rate of glycolysis, respectively.

Creatine Phosphate

The content of creatine phosphate is *in vivo* 20–25 mmol/kg. The production of ATP through the reaction catalyzed by creatine phosphokinase represents roughly 1/5 of the amount of ATP formed via glycogenolysis post mortem. When CP is broken to C and inorganic P, one proton is bound, which means an increase in pH as follows (Hyyppä & Pösö, review 1998).

$$CP^{2-} + ADP^{3-} + H^{+} \rightarrow ATP^{4-} + H_2O + C$$

This might, explain the initial increase in pH that is sometimes seen to take place during the slaughter process (unpublished observations). Taking the direct formation of ATP and also the elimination of protons into the account it can be calculated that this means a transient initial “buffering of the pH fall of almost one pH unit” compared to the situation when all creatine phosphate resources have been exhausted, and ATP will immediately be formed directly via glycolysis. Later ATP will, however, be formed by glycolysis anyway, and only the effect of the utilisation of protons (see equation above) will remain. Therefore, the content of creatine phosphate at the moment of slaughtering could be of importance. This has been recently thoroughly discussed by the similar way by Henkel, Karlsson, Jensen, Oksbjerg & Pedersen (2002).

Concluding Remarks

In living animals, the accumulation of lactic acid causes pain and distress and can result in a reduction in life quality. Our present knowledge of the cardio-respiratory capacity of meat animals, especially modern pigs and poultry is insufficient. This, together with glycogen and the anaerobic end product of its metabolism, lactate, has a strong influence on animal welfare and meat quality, which underlines the importance of further research on this field.

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INVESTIGATION OF PHYSIOLOGICAL PARAMETERS INDICATING STRESS STATUS IN SLAUGHTER PIGS DURING TRANSPORT AND LAIRAGE

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Background

The quality of pork covers several properties, which have to meet the increasing demands of consumers and processors. The main attributes of interest are color, pH and water-holding capacity, fat content and composition, and also the oxidative stability.
The meat is very sensitive to oxidation leading to quality deterioration and loss of value. The tissues contain antioxidant defence systems. Superoxide dismutase, catalase and glutathione peroxidase are antioxidative enzymes contributing to the oxidative defence, as well as the fat-soluble α-tocopherol and ubiquinone, along with the water-soluble ascorbic acid and glutathione. The adequate activity of these defence systems are essential to develop appropriate meat quality.
The perimortal effects on meat quality and animal welfare are widely investigated. It is generally accepted, that the different environmental factors have a stronger impact than the genetic background. On the other hand the decreasing variance of Hal and RN genes, moreover the elimination of these genes makes it necessary to reevaluate the effect of environmental factors. Many studies showed differences in case of these effects when the experiments were carried out with halothane negative pigs.
The optimum lairage time for pigs is a question of compromise. Very short lairage time does not allow sufficient time for recovery leading to a higher incidence of PSE-meat. No prolonged lairage time is recommended after a long-term transportation, because the animals are already exhausted, so the further lairage may increase the incidence of DFD-meat.

Objectives

- study the effect of transportation as a stress factor,
- the effect of transportation and lairage on the antioxidant defence system,
- effect of lairage time on physiological and meat quality traits.

Materials and methods

The present study was conducted at the Gyula Packing Plant Ltd (Hungary). A total of 40 Dumeco hybrid pigs were transported from one of the largest producer of the meat company were transported to the abattoir. The pigs were assumed to be halothane negative (NN), even though they were not tested, as the gene has been practically eliminated from the breeds used by the pig farm.
Animal were fed 4 hours prior to transportation. They were driven out from the piggery to the waiting pen right before the blood samples were taken. For further identification, the animals were marked individually by ear tag. After sampling the animals were driven up to a three-decker lorry. The experimental animals traveled on the lowest level. The distance between the pig farm and the slaughter-house is 70 km, which lasted 1,5 hr for the lorry. After arrival the animals were weighted, which was followed by the second blood sample taking. Then the pigs were divided into two groups: one hour lairage time (Group A), and 16 hours lairage time (Group B) according to the local practice. Showering was used only in the case of the longer lairage.
After lairage the animals were stunned using electrical stunning method and slaughtered. During exsanguination the third series of blood samples were taken. The further processing method was carried out according to the local practice. After evisceration liver samples were taken from the *lobus caudatus*. The

meat quality traits were measured two times: (1) 45 minutes after slaughtering, right after the slaughter value determination by Fat'o'Meater and (2) after chilling, at the 24th hour.

The blood samples were taken from v. jugularis before and after transportation and during exsanguination. The following indicators of stress were determined from the samples: cortisol, NEFA, lactic acid and glucose. Blood sampling tubes contained sodium-fluoride and potassium-oxalate. After centrifugation (10 min, 2500 rpm) the samples taken were stored at -20 °C until further analysis. For the cortisol assay a direct RIA method, developed in the laboratory of Szent István University, Faculty for Veterinary Sciences (Budapest, Hungary) was applied. For determination in blood plasma 1,2,6,7-3H-cortisol (TRK 407; Radiochemical Centre, Amersham, UK) and a highly specific polyclonal antibody raised against cortisol-21-HS-BSA in rabbit was used. The radiactivity was measured by Beckman Instrument Typ LS 1701 liquid scintillation counter.

NEFA, lactic acid and glucose was analysed with enzyme-colorimetric method using standard kits (*L-Lactate (PAP) kit*, Cat. No. LC 2389, Randox Laboratories Ltd., UK; *NEFA kit*, Cat. No. FA 115, Randox Laboratories Ltd. UK; Glucose kit (Cat. No. 40851, Diagnosticum Ltd.., Hungary).

Indicators of lipid peroxidation processes and antioxidant defence system were measured as follows: MDA (malondialdehyde), GSH (reduced glutathione), GSHPx (glutathione-peroxidase activity) and ascorbic acid. The MDA, GSH and GSHPx values were determined from liver, blood plasma and red blood cells (RBC). The concentration of ascorbic acid was analyzed in liver samples and blood plasma. The blood samples were collected into heparine containing tubes, and centrifugated for 10 minutes (2500 rpm). The RBC hemolisate consisted of 1 vol RBC and 9 vol distilled water. Both hemolisate and blood plasma were stored at -20 °C until analyses.

The liver sample taking was followed by marking, packaging and storing at -20 °C. Immediately before analyses a sample of 0.5 g was homogenized in 9 vol of 4 °C physiological saline using Ultra Turrax (Donau Lab AG, Switzerland) homogenizer. For determination of MDA concentration the original homogenate, for the GSHPx, GSH and protein content the supernatant of the homogenate (centrifugation 10000 g, 20 min, 4 °C) were used.

MDA content describing the level of lipid peroxidation was carried out with 2-thiobarbituric acid determination. In case of blood plasma and RBC hemolizate the method of *Placer et al.* (1966) was followed, while the liver samples were analysed by the method described by *Mihara et al.* (1980).

The examination of antioxidant defence system included the determination of GSH concentration and GSHPx activity. The methods of *Sedlak et al.* (1968) and *Matkovics et al.* (1988) were used, respectively.

The protein content of blood plasma and RBC homogenizate was estimated by a modified biuret method (*Weichselbaum, 1948*), using egg protein (Randox Laboratories, UK) as standard. The protein content of liver homogenates was analyzed using Folin-Ciocalteau phenol reagent (*Lowry et al.,* 1951), and bovine serum albumin (Reanal, Hungary) as standard.

For the ascorbic acid determination 1 ml blood plasma was added to 1 ml 10 % TCA (trichloric-acid) and followed by centrifugation (20 min, 3500 rpm). 0,5 ml of the supernatant was stored at -20 °C until the further analyses. A liver sample of 0,5 g was homogenized in 9 vol 5% TCA, which was followed by centrifugation (20 min, 3500 rpm). The analyses were carried out using the method described by *Omaye et al.* (1979).

Meat quality parameters were measured two times: 45 minutes after slaughtering and after chilling at the 24th hour. The first measurement included pH and core temperature determination in the most valuable muscles: *m. longissimus dorsi* and *m. semimembranosus*. We registered the chilling loss, which was computed from the difference of carcass weight before (approx. 50 min after slaughtering) and after chilling. 24 hour after slaughtering we measured the pH and the color properties (L^*, a^*, b^*, Japanese color scale) of the loin. Temperature was measured with common meat industrial core thermometer, the pH measurement with a WTW 330 portable pH meter (WTW Gmbh., Germany) attached with WTW SenTix sp electrode and the color determination with a Minolta Chromameter CR-300 (Minolta Co., Japan) were carried out.

The data were analyzed with SPSS for Windows 10.0 Program Package, using t-test with independent samples and paired samples.

Results and discussion

Transportation as a stress factor is considerable in case of properties measured. The significance values are summarized in *Table 1*.

The activation of hypothalamus-pitiutary-adrenal cortex (HPA) axis results in a higher cortisol level, which is followed by the increase of NEFA, glucose and lactic acid values. The state of antioxidant defence system is affected by transportation, which is demonstrated by reduced GSH level. However, the oxidative load is not so significant to enhance the activity of GSHPx, moreover both in blood plasma and RBC hemolizate decreasing has obsessed. The MDA concentration differs significantly only in case of RBC hemolizate. The transportation affects also the ascorbic acid concentration, although the level of significance is low ($P<0,05$).
The effects of lairage on biochemical parameters measured are summarized in *Table 2*. The data measured at arrival and during exsanguinations and the data of the two different lairage time were compared.
Both 1 hour and 16 hour lairage increases the level of lactic acid, glucose and ascorbic acid. No significant difference is revealed in case of cortisol and NEFA or in the most of the parameters characterizing the antioxidant defence system.
Statistical analysis did not reveal any difference between the two groups. Only the GSHPx activity measured in RBC hemolizate increases at a low significance level ($P<0,05$).
The results of the liver samples analysis are shown in *Table 2*. Significant differences were found in case of GSH and ascorbic acid. The level of lipid peroxidation increases, which is illustrated with the reducing MDA values, although it is not significant in consequence of high individual variance. No difference is experienced in case of GSHPx.
The results of meat quality measurements are summarized in *Table 3*. Significant differences were found in case of pH_{45} and core temperature, along with the chilling loss. No difference was revealed in any of the meat quality traits measured after chilling, at the 24th hour.

Considering the biochemical parameters measured, we can state that the transportation causes high stress for the animals. The activation of HPA-axis results in an increased cortisol level. This hormone has a key-role in the gluconeogenesis. It is conducive to transform some amino acids and other gluconeogenetic substances (lactic acid, proprionic acid, glycerin) to glucose and glycogen. Addition to the stimulation of gluconeogenesis, the cortisol inhibits glycolisis. The enhanced production and decreased metabolism of glucose resulted in approx. 25 % higher blood glucose level, in spite of the short transportation time. The catabolic effect of cortisol on lipid metabolism is also manifested. This physiological process is important to ensure the energy requirement of animals during stress. The effect of lipid mobilization is revealed in level of NEFA, which increased approx. 100 %.
The increase of lactic acid is a bit surprising. As it was mentioned above, one effect of cortisol is to enhance gluconeogenesis, which changes the lactic acid level negatively. This phenomenon can be explained by the high physical exertion caused by transportation and the surrounding animal handling. These physical loads can enhance the depletion of lactic acid from muscles to blood.
The effect of transportation on the antioxidant defence system is revealed. The considerable oxidative load results in lower level of GSH both in blood plasma and RBC hemolizate. These changes are not so significant to enhance the activity of GSHPx, moreover a slight decrease can be seen. The reduced level of ascorbic acid was induced by stress. The change can be explained by its role in antioxidant defence system, and in the synthesis of cortisol in adrenal cortex.
During 1 hour lairage the stress was further enhanced. It is shown by increasing cortisol level, although it was not significant. The lactic acid enhanced three-times higher during lairage, which means that driving to stunning creates a very stressfull situation. The 16 hour lairage has quite similar results. No significant change in cortisol comparing the arrival state with the exsanguinations state is revealed. The level of lactic acid and glucose enhances with a high degree, along with the other group. The NEFA level do not changes either of the two groups, remains at the level measured at time of arrival. Changes of antioxidant defence system are obsessed only in case of blood plasma, the GSH reduces during lairage.
No significant differences were revealed between the two different groups. Two conclusions might be drawn from the results. (1) It is possible, that the lairage conditions are not adequate, and it not ensures enough rest for the animals. (2) It is also possible, that driving to stunning, and the stunning itself causes such a high stress for the animals, which is enough to eliminate the positive effect of lairage. This is suggested by the biochemical parameters.
The results of the meat quality measurements are in agreement with the biochemical measurements. Even though the pH_{45} and temperature values were lower in case of Group B (the lactic acid values were also higher at the time of stunning), these differences were equalized up to the second measurements. Thus, no significant differences are revealed in case of pH_{24} and meat color.
The analyses carried out on liver samples resulted in differences for two parameters. Ascorbic acid reduces drastically, which is caused by the enhanced metabolism, and depletion to blood. The GSH also decreases

significantly. It is presumable that the large-scale changes in both parameters are caused by fasting. At the case of longer lairage the fasting time exceeded 20 hours, so the reduced GSH level is explainable with the lack of supply of amino acids required for GSH synthesis.
Summing up the results, we can conclude that transportation, lairage and driving to stunning cause a heavy distress effect for the animals. On the other hand, these factors do not cause such a high oxidative damage, which appears in the meat quality. Negative changes in the antioxidant defence system are shown, which is traceable in the level of glutathione and ascorbic acid. The lipid peroxidation processes are not influenced significantly by short-term transportation or lairage. The oxidative stability of liver after long lairage was highly reduced, thus, if it is planned to have further processing from the liver, long lairage and fasting is not recommended.

Conclusions

It can be concluded that when using halothane free animals, meat quality is largely independent of lairage time. It is important to underline, that the decision of lairage time is a question of compromise. In addition to meat quality it is influenced by hygiene and the question of work organization. It can be required to have the animal transportation on the day before slaughtering to have a safely continuous working of the abattoir.

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Table 1.: Effect of transport and lairage on biochemical parameters measured

Parameter	Transportation	Arrival- 1 hour lairage	Arrival- 16 hour lairage	1 hour lairage- 16 hour lairage
	P	P	P	P
Lactic acid	0.013^{*}	0.000^{***}	0.000^{***}	0.571^{NS}
NEFA	0.000^{***}	0.071^{NS}	0.378^{NS}	0.918^{NS}
Glucose	0.001^{***}	0.000^{***}	0.000^{***}	0.463^{NS}
Cortisol	0.001^{***}	0.572^{NS}	0.324^{NS}	0.946^{NS}
Ascorbic acid	0.019^{*}	0.007^{**}	0.001^{***}	0.360^{NS}
MDA (blood plasma)	0.482^{NS}	0.000^{***}	0.000^{***}	0.981^{NS}
GSH(blood plasma)	0.026^{*}	0.458^{NS}	0.004^{**}	0.083^{NS}
GSHPx (blood plasma)	0.021^{*}	0.911^{NS}	0.127^{NS}	0.250^{NS}
MDA (RBC)	0.000^{***}	0.358^{NS}	0.360^{NS}	0.032^{*}
GSH (RBC)	0.000^{***}	0.035^{*}	0.859^{NS}	0.279^{NS}
GSHPx (RBC)	0.000^{***}	0.975^{NS}	0.553^{NS}	0.040^{*}

Table 2.: Effect of lairage on parameters measured in liver

Parameter	1 hour lairage			16 hour lairage			P
	Mean	SD	cv %	Mean	SD	cv %	
MDA (µmolg/g)	2.229	1.359	60.96 %	3.558	2.299	64.61 %	0.108^{NS}
GSH (µmol/ g protein)	21.173	3.048	14.39 %	17.338	5.994	34.57 %	0.000^{***}
GSHPx (U/ g protein)	28.240	6.822	24.15 %	28.108	9.614	34.20 %	0.963^{NS}
Ascorbic acid (ug/g)	508.436	95.280	18.73 %	369.444	74.267	20.10 %	0.000^{***}

Table 3.: Effect of lairage on meat quality

Parameter	1 hour lairage		16 hour lairage		P
	Mean	SD	Mean	SD	
pH_{45ham}	6.495	0.160	6.201	0.272	0.000^{***}
pH_{45loin}	6.213	0.221	5.902	0.225	0.000^{***}
T_{loinj}	41.042	0.562	40.650	0.519	0.034^{*}
L^{*}	40.967	13.194	45.622	11.938	0.269^{NS}
a^{*}	5.018	2.566	3.831	1.546	0.099^{NS}
b^{*}	2.777	0.802	1.660	1.359	0.006^{*}
pH_{24}	5.606	0.077	5.651	0.098	0.132^{NS}

BREED AND CASTRATION EFFECT ON FATTY ACID PROFILE OF NORTHERN SPANISH BEEF CATTLE

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Background

There is an increasing concern in developed countries about the consumption of dietary fat due to its association with cardiovascular and other lifestyle diseases, and for many years there has been emphasis on reducing the fat content from our diets. Meat producers have responded by reducing the fat content of beef by selective breeding, feeding practices and also by new butchery techniques (Hornick *et al*., 1998).
Both the amount and the composition of fat depots in beef may be influenced by factors such as breed or genotype, age or live weight, castration and gender, and feeding regime (Rule *et al*., 1995). In this sense, some breed effects on meat quality have been described out of two local breeds from Asturias in Northern Spain: "Asturiana de los Valles" and "Asturiana de la Montaña" (Oliván *et al*., 1999; Gil *et al*., 2001). On the other hand, steer production is gaining renewed interest because its effect on physico-chemical and sensory traits of meat (Steen, 1995; Osoro *et al*., 2001).

Objectives

The purpose of the present study was to examine the differences in fatty acid composition of muscle fat between bulls and steers in two different Spanish beef breeds ("Asturiana de los Valles" & "Asturiana de la Montaña") managed under grazing conditions.

Materials and methods

Animal management

Eight yearling bulls from "Asturiana de los Valles" (AV) (beef breed adapted to extensive production systems) and eight yearling bulls from "Asturiana de la Montaña" (AM) (small to medium-sized hardy animals, adapted to mountain systems) were reared under extensive conditions on ryegrass and clover pastures. Four animals from each breed were castrated between 10-12 months of age. All animals received a finishing concentrate diet during 60 days (84% barley meal, 10% soya meal, 3% fat, 3% minerals, vitamins and oligoelements) and barley straw *ad libitum* at the housing facilities of the Institute (S.E.R.I.D.A.). Animals were slaughtered with an average weight of 504 kg for AV bulls and 534 kg for AV steers, and 461 kg for AM bulls and 481 kg for AV steers. Slaughtering was performed in a commercial abattoir according to a routine procedure, and after dressing the carcasses were chilled at 3ºC for 24h.

Measurements

Twenty four hours *post-slaughter* the left half carcass was quartered and the part of the rib joint comprised between the 6th and 9th ribs extracted and transported to the laboratory. The 6th rib was excised and *Longissimus thoracis* (LT) muscle was separated, aged at 4ºC for 7 days and then minced with an electrical chopper, vacuum packed and kept at –20ºC until determination of intramuscular fat content by near infrared spectroscopy (Oliván *et al*., 2002). The LT of the 8th rib was extracted, vacuum packed and frozen at –80ºC for subsequent fatty acid composition analysis by gas chromatography (GC).

Total fatty acid analysis

The fatty acids were extracted in 5M KOH in methanol/water (50:50) at 60ºC for 1 hour and esterified at 40ºC during 10 min with 2M trimethylsilyl-diazomethane in *n*-hexane according to Elmore *et al*. (1999) with some modifications. Separation of fatty acid methyl esters was performed on a Varian CX3400 GC with a flame ionisation detector (FID) and a split/splitless injection port (50:1). GC analysis was performed using a B-PX 70 for FAME column (120m x 0.25mm i.d., 0.2µm film thickness) with programmed oven temperature. Injector and detector ports were set at 270°C and 300°C respectively. The carrier gas was hydrogen and the flow rate 1.6ml/min. measured at the initial temperature. Esterified fatty acids were

identified according to similar peak retention times using standards and quantified according to internal standard method ($C_{23:0}$ methyl ester) with its addition prior to saponification.

Statistical analysis
The statistical analysis was conducted using the SPSS11.5 program (2002). The effect of breed, castration and their interaction were studied by ANOVA analysis. Significance level of $p \leq 0.1$ was also considered.

Results and discussion
Table 1 shows the effect of breed, separately in bull and steers, and the effect of castration within both breeds on intramuscular fat and total fatty acid contents of *Longissumus thoracis*.

Breed effect on intramuscular fat percentage was seen in steers, but not in bulls, where AM steers showed more (4.74%) IM fat than AV steers (2.59%). Castration effect was significant only for AM breed where steers had (4.74%) a higher IM fat level than bulls (2.80%).

Breed effect on total FA composition was more pronounced in steers where significant ($p \leq 0.1$) differences were observed in 37% of the individual FAs, while in bulls only 18% of the individual FAs where significantly affected. In castrated animals, AV showed significantly higher quantities of *n-3* FAs than AM animals, and $C_{22:1}c13$ and $C_{18:2}$ *n-6* were also significantly higher in AV castrated animals. However, these differences were not observed in entire animals.
Studying FA groups, in general, breed effect was not significant, except for PUFA group because AV steers showed more PUFAs than AM steers. It should also be emphasised the significant difference in *n-6/n-3* and P/S ratio between steers of both breeds; AV steers showed lower *n-6/n-3* ratio (4.07) than AM steers (5.50). Higher P/S ratio was found in AV (0.49) than in AM (0.22) steers. Therefore, AV castrated animals meat would be the best adapted to the nutritional guidelines recommendations (P/S ratio between 0.45 and 0.70 and *n-6/n-3* ratio about 4.0) (Williams, 2000), and could happened due to a lower IM fat level of AV steers in comparison to AM steers (related also with the overall fatness; Osoro *et al*. 2001) probably reflecting the greater contribution of polar lipids located in membranes (phospholipids) and characterised with a high PUFA content in comparison to neutral lipids, storage lipid fraction (triacylglycerides) that are mainly composed of SFAs and MUFAs (Eichhorn *et al*., 1985; Choi *et al*., 2000; Lorenz *et al*., 2003).

Castration effect on total FA composition was low in AV breed, it significantly affected only to 22% of individual FAs where all of them were unsaturated FAs and appeared in higher quantities in steers than in bulls. This could be because of hormonal differences on enzyme activities (elongation & desaturation) (Malau-Aduli *et al*., 1998). However, castration effect was more remarkably for AM breed where significant differences were given for 70% of the individual FAs studied. AM steers showed in general higher quantities in comparison to AM bulls, particularly for saturated, branched and monounsaturated FAs, resulting significantly different also SFA and MUFA groups. But, it has to be pointed out that some FAs ($C_{18:2}$ *n-6*, $C_{20:2}$ *n-6* and $C_{18:3}$ *n-3*) appeared in higher quantities in bulls than in steers. These differences could be explained also with the lower fat quantity of bulls in comparison to steers and the greater contribution of phospholipids (PUFA) in comparison to triacylglycerides (MUFA & SFA) in this animals. In AM steers, they have higher IM fat proportions, individual FA variations may be more related to this fat content instead of to hormonal differences and enzyme activities caused by castration as might have happened in AV steers.
Studying FA ratios, there was not castration effect in *n-6/n-3* ratio for any breed. However, castration effect was observed in P/S ratio for AM breed, where bulls showed (0.48) a higher P/S ratio than steers (0.22).

Conclusions
Data from this preliminary study indicated that breed effect on intramuscular fat quality was more pronounced in steers than in bulls, what meant that breed effect was significant only when comparing animals with high IM fat level. Castration effect was more remarkable in AM breed than in AV breed. In general, AV steers produced the most nutritionally recommendable meat because of the good *n-6/n-3* and P/S ratios presented, and AM bulls produced a healthy meat from the P/S point of view.

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Acknowledgements

N.A. acknowledges a predoctoral fellowship awarded by the Spanish Ministry of Science and Technology (INIA) and thanks Dr. Celaya from S.E.R.I.D.A. for his advice and support on statistical analysis.

Table 1. Mean values of intramuscular fat (% IM fat) and total fatty acid (mg/100g meat) contents of *Longissimus thoracis* muscle. Signification level of differences observed depending on breed and castration.

	AV		AM		Breed		Castration	
	Bull	**Steer**	**Bull**	**Steer**	**Bull**	**Steer**	**AV**	**AM**
% IM fat	1.80	2.59	2.80	4.74	NS	*	NS	*
Fatty acid								
C14:0	19.80	41.08	29.71	92.10	NS	NS	NS	NS
C15:0	3.96	7.56	4.27	12.75	NS	NS	NS	*
C16:0	292.32	492.25	365.46	793.15	NS	NS	NS	*
C17:0	11.73	19.17	12.57	31.28	NS	+	NS	**
C18:0	209.23	278.02	232.63	362.42	NS	NS	NS	+
i-C15:0	0.75	1.70	0.85	1.79	NS	NS	NS	+
ai-C15:0	1.72	3.07	1.77	3.04	NS	NS	NS	+
i-C16:0	1.79	2.89	1.73	3.28	NS	NS	NS	+
i-C17:0	4.01	4.07	1.95	3.27	*	NS	NS	+
							NS	
C14:1*c*9	6.38	11.80	5.49	19.85	NS	NS	NS	+
C16:1*c*9	23.62	59.31	38.85	122.79	*	NS	NS	+
C17:1*c*10	6.93	15.88	8.74	28.37	NS	+	NS	**
C18:1*t*11	38.40	66.26	42.42	89.26	NS	NS	NS	*
C18:1*c*9	254.53	600.60	406.90	1015.89	+	NS	NS	*
C18:1*c*11	21.83	35.93	28.82	57.13	+	NS	+	*
C22:1*c*13	1.65	3.12	1.65	1.57	NS	***	*	NS
C18:2*n-6*	229.26	186.21	187.40	149.33	NS	*	NS	*
C18:3*n-6*	1.34	2.30	1.18	1.89	NS	NS	*	NS
C20:2*n-6*	1.90	1.79	1.87	1.53	NS	+	NS	*
C20:3*n-6*	10.94	14.11	9.43	11.77	NS	NS	*	NS
C20:4*n-6*	54.55	56.82	45.79	45.75	NS	NS	NS	NS
C22:4*n-6*	3.13	4.14	3.64	5.23	NS	+	NS	*
C18:3*n-3*	24.36	20.74	17.23	11.25	NS	***	NS	**
C20:5*n-3*	15.65	16.46	9.08	8.50	*	**	NS	NS
C22:5*n-3*	16.92	24.74	15.40	17.57	NS	**	**	NS
C22:6*n-3*	1.35	3.48	1.49	2.26	NS	*	**	NS
*c*9,*t*11 CLA	2.86	11.18	3.48	6.84	NS	NS	NS	+
Σ SFA	537.05	838.09	644.66	1291.71	NS	NS	NS	+
Σ MUFA	353.33	792.90	532.87	1334.86	+	NS	NS	*
Σ PUFA	362.28	342.00	296.02	261.94	NS	**	NS	NS
n-6/n-3	5.37	4.07	5.76	5.50	NS	**	NS	NS
P/S	0.69	0.49	0.48	0.22	NS	+	NS	*

+: $p \leq 0.1$; *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; NS: $p > 0.1$. AV: Asturiana de los Valles; AM: Asturiana de la Montaña. *c*9,*t*11 CLA: *c*9,*t*11 C18:2; ∑SFA = sum of all saturated fatty acids; ∑MUFA = sum of all monounsaturated fatty acids; ∑PUFA = sum of all polyunsaturated fatty acids; *n-6/n-3* = (C18:2*n-6* + C18:3*n-6* + C20:2*n-6* + C20:3*n-6* + C20:4*n-6* + C22:4*n-6*) / (C18:3*n-3* + C20:5*n-3* + C22:5*n-3* + C22:6*n-3*); P/S = ∑PUFA / ∑SFA.

LARGE WHITE AND DUROC HEAVYWEIGHT CROSSBRED PIGS: INTERACTIVE EFFECTS OF GENETIC TYPE, REARING AND SLAUGHTER CONDITIONS ON STRESS REACTIVITY AND TECHNOLOGICAL AND SENSORY MEAT QUALITY ASPECTS

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Background

There is an increasing demand for alternatively produced pork. Outdoors produced pork has a good image. However, the effect of rearing environment on technological meat quality varies across studies. To our knowledge, no study reports an improvement in meat quality from outdoor pigs. Use of other genetic types of pigs might improve gustatory acceptability of outdoor produced pork. Increased slaughter weight may increase the effects of rearing environment. As stress at slaughter may reduce or cancel potentially improved pork quality, it is necessary to determine effects of genetic background and rearing environment on reactivity to potentially stressful situations, including slaughter.

Objectives

To study 1) interactive effects of genetic background and rearing environment on stress reactivity and 2) interactive effects of genetic background, rearing environment and slaughter conditions on technological and sensory quality of pork slaughtered at 150 kg live weight.

Materials and methods

Twenty-five Large White (LW) and 52 Duroc (D) crossbreds (barrows and females) were reared indoors (slatted floor, 6.3 m^2) or outdoors (850 m^2 fields with huts) over two consecutive years (April-October), in an unequally balanced design. To study cardiac reactivity, each individual has been subjected to a 5 h isolation test at about 70 kg. In order to obtain similar pre-slaughter and carcass weights, in contrast to outdoor pigs, indoor pigs have been slightly food restricted. At 150 kg, half of the pigs were mixed for 1.5 h and transported for 30 min to the abattoir on the day before slaughter. The other half was unmixed, and slaughtered immediately upon arrival. All pigs had been food deprived for 22 h before slaughter. After bleeding, temperature (15 and 45 min and 24 h), glycogen and lactate content and pH (45 min, 24 h), colour (days 1, 4, 8) and drip (days 3 and 5) were measured on the Longissimus lumborum (LL), the Semispinalis capitis (SC) and the Semimembranosus (SM). Glycolytic potential ([lactate]+2x[glycogen]; GP) was calculated for samples obtained 45 minutes post-bleeding as it reflects muscle glycogen content at the moment of slaughter (Monin and Sellier, 1985). Sensory analysis was performed on half of the loins and on a quarter of the hams, after dry curing, by 12 and 8 trained pannellists, respectively. Data were analysed with analysis of (co-)variance and with pooled Pearson correlations which take into account the means of each treatment group, rather than the overall means. Only main significant effects are reported.

Results and discussion

All pigs showed a similar initial heart rate acceleration following start of isolation, but outdoor pigs showed subsequently a faster decrease ($p<0.01$). Thus, rearing environment influenced cardiac reactivity.

Compared to the no mixing/no lairage group, pigs of the mixing/lairage group had generally lower glycogen levels for the three muscles (Table 1). Compared to indoor pigs, outdoor pigs had generally higher glycogen content for the three muscles. GP of the SM and SC of outdoors pigs was also higher. Effects on glycogen content were partly due to a rearing and slaughter conditions interaction as indoor reared pigs of the mixing/lairage group had lower glycogen content (LL: 24 h, Table 3; SC: 45 min, $p<0.05$ and SM: 45 min, $p=0.06$) than the other three groups. Ultimate pH of the LL and SC reflected main differences in glycogen (Table 2). Lactate content at 24 h was negatively correlated with ultimate pH for the SM ($r=-0.30$; $p<0.01$) and LL ($r=-0.45$; $p<0.0001$) muscles. A slaughter treatment effect was found for lactate content (24

h) of the LL (Table 1). Absence of differences in lactate content (24 h) in the SC despite variations in ultimate pH may be explained by the overall lower lactate production in this muscle, due to its lower glycogen content (Bendall, 1973). Despite their effects on pre-slaughter glycogen content, genetic and rearing background did not influence pH values of the SM. Correlations between pre-slaughter glycogen (glycolytic potential, 45 min) and ultimate pH were stronger for the LL (Pearson, pooled over two years and over LW and D: ($r=-0.42$; $p=0.0001$) and SC ($r=-0.53$; $p<0.00001$) than for the SM ($r=-0.30$; $p<0.01$) suggesting that compared to the SC and LL, ultimate pH of the SM was more strongly influenced by other factors.

The effect of gender depended on genetic type and rearing conditions: female LW had a higher LL glycogen content (Table 3) while outdoor reared barrows had a higher SM glycogen content (24 h; 16.3 ± 1.6 µmol/g) than indoor reared barrows (7.9 ± 1.3 µmol/g; $p<0.01$).

The effect of year depended on the parameter and muscle: for example, the first year, higher levels were obtained for GP of the LL ($p<0.001$), while the SC had lower glycogen content (45 min, 24 h; $p<0.0001$) and GP ($p<0.05$). Ultimate pH did not vary between years, but the first year, at 45 min pH of the LL was higher ($p<0.01$) and of the SM was lower ($p<0.05$). Colour was strongly influenced by slaughter, and to a lesser extent by rearing conditions. LL and SM meat produced by the mixing/lairage group showed overall lower b* and L* values (p varying between 0.06 and 0.0001). Lower a* and b* values ($p<0.05$) of this group of the SC muscle were partly due to the LW of the lairage/mixing group that had significantly lower a* (day 4; $p<0.02$) and b* values (days 4 and 8; $p<0.01$) than the other three groups. Outdoor reared pigs produced meat with higher a* (SM, LL; $p<0.01$) and b* values (LL; $p<0.01$).

In contrast to objective colour data, dry-cured hams of the mixing/lairage group were perceived as redder and in addition, with a higher overall intensity of taste (Table 4). Their loins were also perceived as pinker ($p<0.08$), with a higher colour intensity ($p<0.07$). Dry-cured hams from outdoor produced and D pigs had higher marbling scores and were perceived as fattier. Their hams were overall better appreciated: easier to chew, more tender, less dry although pastier. D hams were found less smoky, while outdoor reared pigs gave softer meat, with more piquant and a longer persistence of taste. Loins from D were also more marbled ($p<0.001$) and had less intense colour ($p<0.05$), pig odour ($p<0.05$) and abnormal taste ($p<0.05$). Outdoor produced pigs gave loins with a stronger grilled aspect ($p<0.03$) which was probably due to an increased Maillard reaction due to their lower humid aspect ($p<0.06$; Bejerholm and Aaslyng, 2003) as these two aspects were negatively correlated ($p<0.02$). Loins from barrows were described as more marbled ($p<0.01$) and with more intramuscular fat ($p<0.001$). Their hams were more also marbled ($p<0.05$) and had a thicker fat layer than females ($p<0.01$), were drier ($p<0.05$) and had a saltier taste ($p<0.05$). Interactions between gender and crossbreed showed that hams from female D had less rancid and spicy odour, and less acid, piquant and spicy taste than the other three groups ($p<0.05$). D barrows produced hams with a peppery taste ($p<0.05$).

Technological and sensory meat quality aspects were not correlated with cardiac stress reactivity measured during rearing, suggesting that rearing or genetic effects are not explained by differences in cardiac reactivity to the slaughter procedure.

Conclusions

Overall, data show that in heavy pigs, meat from an outdoor production or a Duroc genetic background is better appreciated, especially dry-cured ham. Present data show no synergism between outdoor production and Duroc genetic background. The increased appreciation may be related to increased fat content of outdoor or Duroc types of pigs which would also support the advantage of using heavier pigs for high quality dry-cured ham production. However, although fatter than females, castrated males of the Duroc cross were slightly less appreciated, probably due to hormonal differences. Despite large effects of year of experimentation, reported effects of outdoor rearing, genetic type and slaughter conditions were consistent.

Acknowledgements

The study is part of the EU-project “Sustainability in the production of pork with improved nutritional and eating quality using strategic feeding in out-door production”. QLK5-2000-00162. Semen has been supplied by Pig Improvement Company. The authors thank E. Haye and J.F. Chazeix (INRA, Theix), B. Aubard, F. Cinquin et C. Pontet (LEGTA, Roanne), JY. Tixier and J. Thevenet (abattoir Charlieu) for their valuable contributions.

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Tables

Table 1a. Main effects of slaughter conditions, housing system and genetic type on glycogen and lactate content of the LL muscle

		Slaughter conditions mixing/lairage no mix/no lair. *F(1,54); p*	housing system in out *F(1,54); p*	genetic type LW D *F(1,54); p*
lactate	24 h	63.79 ± 2.24 **71.55** ± 1.86 *9.18; 0.004*		
glycogen	45 min	36.60 ± 1.91 **43.19** ± 2.00 *4.18; 0.04*		**43.76** ± 2.70 37.93 ± 1.60 *3.11; 0.08*
glycogen	24 h	8.89 ± 0.81 **12.89** ± 0.70 *15.57; 0.0002*	9.41 ± 0.88 **12.44** ± .067 *13.34; 0.0006*	**13.70** ± 1.05 9.46 ± 0.61 *19.51; 0.0001*
GP	45 min	110.4 ± 4.0 **125.8** ± 3.2 *8.74; 0.005*		**131.0** ± 4.96 111.5 ± 2.79 *9.7; 0.0003*

Table 1b. Main effects of slaughter conditions and housing system on glycogen and lactate content of the SC muscle. No effects of genetic type were found.

		Slaughter conditions			housing system		
		mixing/lairage	no mix/no lair	*F(1,54); p*	in	out	*F(1,54); p*
lactate	45 min				**27.90** ± 1.31	23.60 ± 1.37	*15.06; 0.0003*
glycogen	45 min	17.53 ± 2.13	**24.72** ± 1.93	*3.70; 0.06*	15.93 ± 1.44	**26.57** ± 2.35	*15.26; 0.000*
glycogen	24 h				3.30 ± 0.67	**15.72** ± 2.18	*17.16; 0.0001*
GP	45 min	59.78 ± 4.11	**76.32** ± 3.38	*4.70; 0.03*	59.75 ± 3.13	**76.74** ± 4.31	*6.58; 0.01*

Table 1c. Main effects of slaughter conditions, housing system, and genetic type on glycogen and lactate content of the SM muscle

		Slaughter conditions mixing/lairage no mix/no lair. *F(1,54); p*	housing system in out *F(1,54); p*	genetic type LW D *F(1,54); p*
glycogen	45 min	39.66 ± 1.85 **44.88** ± 1.64 *3.04; 0.08*	39.05 ± 1.63 **45.64** ± 1.81 *7.38; 0.009*	**45.99** ± 2.54 40.38 ± 1.35 *4.26; 0.04*
glycogen	24 h	9.87 ± 1.06 **14.46** ± 1.04 *11.78; 0.001*	9.64 ± 0.97 **14.69** ± 1.05 *11.81; 0.001*	**15.82** ± 1.60 10.33 ± 0.70 *12.24; 0.001*
GP	45 min	109.0 ± 3.3 **120.0** ± 2.7 *4.22; 0.04*	108.9 ± 3.2 **120.3** ± 2.8 *6.59; 0.01*	**122.6** ± 4.2 110.4 ± 2.38 *4.11; 0.05*

Table 2. Main effects of slaughter conditions, housing system and genetic type on temperature, pH and drip loss of the LL, SC and SM muscles

			slaughter mixing/lairage	no mix/no lairage	housing system in	out	genetic type LW	D
			F(1,54); p		*F(1,54); p*		*F(1,54); p*	
pH	LL	24 h	**5.59** ± 0.03	5.49 ± 0.01	**5.58** ± 0.03	5.50 ± 0.02		
			8.65; 0.005		*3.65; 0.06*			
drip3	LL	3 days					**5.24** ± 0.40	4.16 ± 0.26
							4.72; 0.03	
T	SC	45 min					**37.2** ± 0.5	35.5 ± 0.6
							3.55; 0.07	
pH	SC	45 min	**6.58** ± 0.02	6.51 ± 0.02				
			7.23; 0.01					
pH	SC	24 h	**5.97** ± 0.05	5.85 ± 0.02	**6.00** ± 0.04	5.82 ± 0.03		
			6.15; 0.02		*6.05; 0.02*			
T	SM	15 min	**40.2** ± 0.1	39.5 ± 0.2	**40.1** ± 0.12	39.6 ± 0.2		
			12.12; 0.001		*9.65; 0.001*			

Table 3. Interactive effects of slaughter conditions, housing system, genetic type and gender on glycogen of the LL muscle

	slaughter * housing				genetic type * gender			
	mixing/lairage		no mixing/no lairage		LW		D	
	in	out	in	out	CM	F	CM	F
	F(1,54); p				*F(1,54); p*			
Glycogen (45 min)					37.4^{a} ± 3.7	49.7^{b} ± 3.4	38.4^{a} ± 2.3	37.4^{a} ± 2.3
					4.62; 0.04			
Glycogen (24 h)	6.4^{a} ± 1.0	11.5^{b} ± 1.2	12.4^{b} ± 1.1	13.4^{b} ± 0.8	11.4^{a} ± 1.3	15.8^{b} ± 1.5	9.5^{a} ± 1.0	9.4^{a} ± 0.7
	10.82; 0.002				*7.73; 0.007*			

	housing * genetic type				
	LW		D		
	in	out	in	out	*F(1,54); p*
Lactate (24 h)	75.60^{a} ± 2.78	$67.07^{a,b}$ ± 4.42	61.22^{b} ± 2.99	70.41^{a} ± 1.69	*16.35; 0.0002*

Table 4. Significant main treatment effects on sensory analysis of dry-cured ham.

	slaughter conditions			housing system			genetic type		
	mix/lairage	no mix./no lair.	*F(1,8); p*	indoors	outdoors	*F(1,8); p*	LW	D	*F(1,8); p*
appearance									
fat betw. muscles							1.10 ± 0.11	**1.42** ± 0.10	*7.92; 0.02*
redness	**3.95** ± 0.15	3.45 ± 0.15	*5.55; 0.05*						
marbling				1.94 ± 0.28	**2.65** ± 0.24	*4.71; 0.06*	1.72 ± 0.18	**2.87** ± 0.26	*19.3; 0.007*
hardness				**1.24** ± 0.13	0.89 ± 0.09	*5.16; 0.05*			
uneven colour	2.10 ± 0.14	**2.83** ± 0.17	*7.86; 0.02*						
texture									
easy to chew				3.57 ± 0.08	**4.05** ± 0.11	*10.65; 0.01*	3.65 ± 0.11	**3.98** ± 0.12	*7.34; 0.03*
tender				3.21 ± 0.10	**3.66** ± 0.16	*5.89; 0.04*	3.21 ± 0.13	**3.66** ± 0.13	*7.30; 0.03*
pasty				0.75 ± 0.16	**1.29** ± 0.21	*4.98; 0.06*	0.64 ± 0.15	**1.41** ± 0.18	*11.9; 0.009*
dry				**3.06** ± 0.11	2.40 ± 0.16	*20.6; 0.002*	**3.01** ± 0.11	2.46 ± 0.17	*20.5; 0.002*
fatty	1.14 ± 0.14	**1.46** ± 0.12	*5.55; 0.05*	1.08 ± 0.10	**1.51** ± 0.14	*8.50; 0.02*	1.17 ± 0.11	**1.41** ± 0.15	*6.65; 0.03*
taste									
overall intensity	**4.20** ± 0.05	3.92 ± 0.08	*5.97; 0.04*	3.97 ± 0.07	**4.15** ± 0.08	*5.18; 0.05*			
smoky							**0.12** ± 0.03	0.03 ± 0.02	*6.23; 0.04*
piquant				0.77 ± 0.09	**0.96** ± 0.13	*5.02; 0.06*			
persistence				3.10 ± 0.07	**3.40** ± 0.09	*6.26; 0.04*			

MORPHOMETRIC CHARACTERISTICS OF PIG CARCASSES: EFFECTS OF GENETIC AND REARING BACKGROUND AND CONSEQUENCES FOR POST-MORTEM METABOLISM

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Background

Pig selection companies aim to improve simultaneously carcass morphology, especially muscularity, defined as muscle thickness relative to skeleton dimensions (Purchas, 1991), and meat leanness. It is likely that post-mortem metabolism and muscle morphology are related. Post-mortem metabolism is strongly influenced by metabolism during the pre-slaughter period (Lawrie, 1966). During this period, muscle morphology, which influences the way muscles are used, probably affects post-mortem metabolism and consequently technological meat qualities. Improvement of muscularity may influence post-mortem metabolism in relation to changes in contractile and metabolic fibre type (Klont et al. 1998, Laville et al., 2002). As alternative pork production (outdoor production, different genetic types) gains an increasingly important place on the market, it is further relevant to evaluate effects of rearing environment and genetic background on carcass composition and meat quality. This study is part of a larger study (see Astruc et al, 2004, for more details).

Objectives

To study 1) effects of rearing environment and genetic background on carcass morphology and on technological meat quality and 2) possible relationships between shape and size of body parts and post-mortem metabolism.

Materials and methods

Twenty-five Large White (LW) and 52 Duroc (D) crosses (pure bred LW and D sires, LW x French Landrace dams) were reared indoors (slatted floor, 6.3 m^2) or in fields (850 m^2) with huts over two consecutive years (April-October), in an unequally balanced design. Pigs were slaughtered at 150 kg body weight; half of each treatment group after mixing and overnight lairage, the other half immediately upon arrival at the abattoir. Various morphometric measurements were taken on carcass images (Fig. 1). Glycogen and lactate content, temperature and pH, at 45 min and 24 h after bleeding, were evaluated in the *Longissimus lumborum* (LL) and the *Semimembranosis* (SM). Treatment effects have been assessed with analyses of variance. For the study of correlations, simple and multiple regression analyses have been associated analyses of covariance to correct for treatment effects and with pooled Pearson correlations, which take into account the means of each treatment group, rather than the overall means.

Results and discussion

The second year, summer temperatures were exceptionally high, repeatedly reaching 35 to 40 °C. Carcass weight was not influenced by genetic background or type of housing, but tended to be lower the second year (128.8 ± 2.5 *vs* 123.1 ± 1.5; p=0.08). Carcass weight was positively correlated with leg and body lengths for the both crosses (Table 1). Due to these combined effect, leg and body lengths were lower the second year. LW crosses or indoor reared pigs had longer legs ($p<0.05$) and this was not caused by differences in carcass weight. The D cross had a larger external angle of the ham ($p<0.05$) while outdoor bred pigs had greater thoracic depth ($p<0.00001$). Compared to the first year, the second year, length of the iliac bone and thoracic depth were reduced and pelvis width, and posterior and internal angles of the ham were increased (p between 0.03 and 0.00001), but these variables were unrelated to carcass weight. For the LW cross, body length was negatively and for the D cross, positively correlated with posterior angle of the ham (Table 2). Internal angle of the ham was correlated with iliac bone length, pelvis width and posterior angle of the ham for the D cross (Table 2). Similar tendencies existed for the LW cross, but Pearson correlations, pooled over the two years did not reach significance, possibly to insufficient animal numbers. For example, unpooled Pearson

correlations found a positive correlation between internal and posterior angle of the ham ($r=0.43$; $p<0.05$) for the LW cross.

Thus, data show only a few direct effects of genetic and rearing background on morphometric characteristics. Year of rearing had more pronounced effects on all parameters. Data on the D cross show that shapes and sizes of different body parts are related to each other: pigs with a rounder interior angle of the ham have a rounder posterior angle of the ham and a larger pelvis with a shorter iliac bone. The different fused bones of the pelvis receive attachments from various muscles of the thigh and from the *Psoas major*, allowing posture maintenance and flexing of the thigh upon the pelvis (Barone, 1980). Muscles and bones act together in growth and movement (Barone, 1980) and the observed relationships show that size and length of the pelvis and shape of the ham are connected. More data are needed to determine exact relationships between shape of the pelvis and of ham muscles in the LW cross. The two genetic types showed opposite correlations between body length and posterior angle of the ham indicating that differences between breeds exist also.

Glycogen content was higher in LW than D crosses at both times in both muscles (p between 0.08 and 0.001) and higher in outdoors than indoors reared pigs ($p<0.001$). Lactate content was higher the first year for both sampling times in both muscles ($p<0.01$). For the LL, lactate content (24 h) was lower and ultimate pH was higher in pigs slaughtered after mixing and lairage ($p<0.01$).

For the D cross, SM glycogen content (both times), and lactate content (24 h), were negatively correlated with external angle of the ham (Table 3; Fig. 2). These correlations were probably due to negative correlations between this angle of the ham and pre-slaughter glycogen of the SM muscle: glycolytic potential (GP; [lactate]+2x[glycogen]) at 45 minutes, reflecting muscle glycogen content at the moment of slaughter (Monin and Sellier, 1985), was also negatively correlated with external angle of the ham. GP was further positively correlated with glycogen content of the SM at both times ($p<0.0001$) and lactate content at 24 h ($p<0.0001$). SM lactate content (24 h) was simultaneously, negatively correlated with iliac bone length (Table 3). For the LW cross, the external angle of the ham was negatively correlated with SM lactate content (24 h) and positively with SM and LL temperature (45 min; Table 3). Lactate content of the LL (24 h) was negatively correlated with the iliac bone length and positively with pelvis width for D and LW crosses, respectively (Table 3). Temperature (both times) of the LL was positively correlated with iliac bone length for the D cross (Table 3).

For both genetic types and for both muscles, at 45 min, pH was positively correlated with glycogen content (e.g. D, SM: $r=0.45$; $p<0.01$) and negatively with lactate content (e.g. D, SM: $r=-0.73$; $p<0.001$). Ultimate pH was negatively correlated with GP (e.g. D, SM: $r=-0.31$; $p<0.05$) and with lactate content at 24 h (e.g. D, SM: $r=-0.28$; $p<0.05$). Despite these correlations, initial and ultimate pH's of the 2 muscles were not correlated with morphometric measurements.

Thus, morphometric characteristics are related to post-mortem metabolism, with similarities and differences between the two genetic types. LL post-mortem metabolism was related to pelvis shape and size, differently according to genetic cross. Pigs of both genetic types with rounder posterior angles of the ham had lower SM lactate content. Data on the D cross suggest that lower pre-mortem glycogen levels explain at least part of this effect. Data show further that D crossbreds with a longer iliac bone produce less post-mortem lactate. Lower pre-mortem glycogen content may be simultaneously related to lower resting glycogen levels (i.e. before mixing/transport) and higher energy expenditure of the muscle during the pre-slaughter period. However, generally, the larger a given muscle is, the more glycolytic fibres it contains (Laville et al., 2002), it is therefore expected that pigs with rounder external angles of the ham had higher muscle glycogen content. Shape of the external angle measures predominantly the thickness of the *Biceps femoris* and may be a poor estimate of SM size, more closely related to the posterior angle of the ham. Possibly, pigs with a rounder external angle or longer iliac bone had higher energy expenditure during the pre-slaughter period, indicating that efficiency of certain muscles in terms of energy use depends on shape or other characteristics of surrounding muscle and bone structures.

Conclusions

Shape and size of bones and muscles influence post-mortem metabolism. While data on the D cross show clearly relationships between the shape of pelvis and ham and post-mortem glycogen metabolism, more data on LW crosses are needed to confirm that at least part of these relationships exists in this breed. The observed variations between genetic types and years suggest that other factors (possibly related to pre-slaughter stress reactions) may intervene and modify or cancel some of the relationships between conformation and post-mortem metabolism.

Ackowledgements

The study is part of the EU-project "Sustainability in the production of pork with improved nutritional and eating quality using strategic feeding in out-door production". QLK5-2000-00162. Semen has been supplied by Pig Improvement Company. The authors thank J.F. Chazeix and E. Haye (INRA, Theix), B. Aubard, F. Cinquin et C. Pontet (LEGTA, Roanne), JY. Tixier and J. Thevenet (abattoir Charlieu) for their valuable contributions.

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Figures and Tables

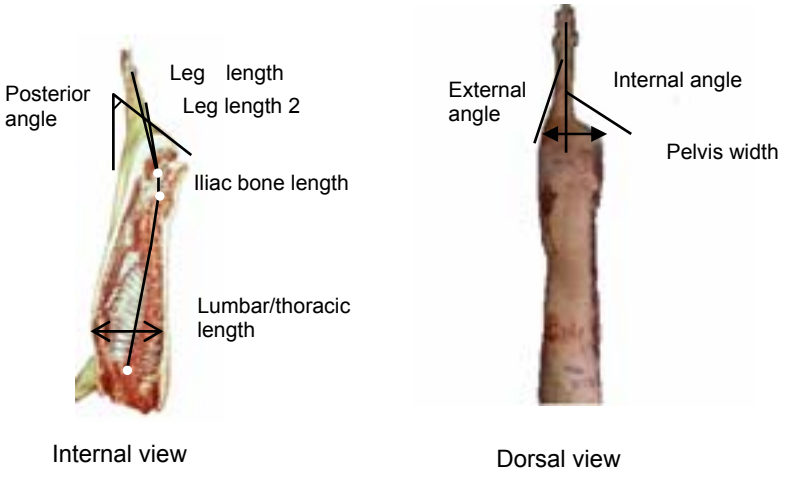

Fig. 1. Morphometric measurements on the carcass images. Iliac bone length showed large variations in sheep in an earlier study (Laville pers. obs.). Measurements of angles reflect muscle plumpness.

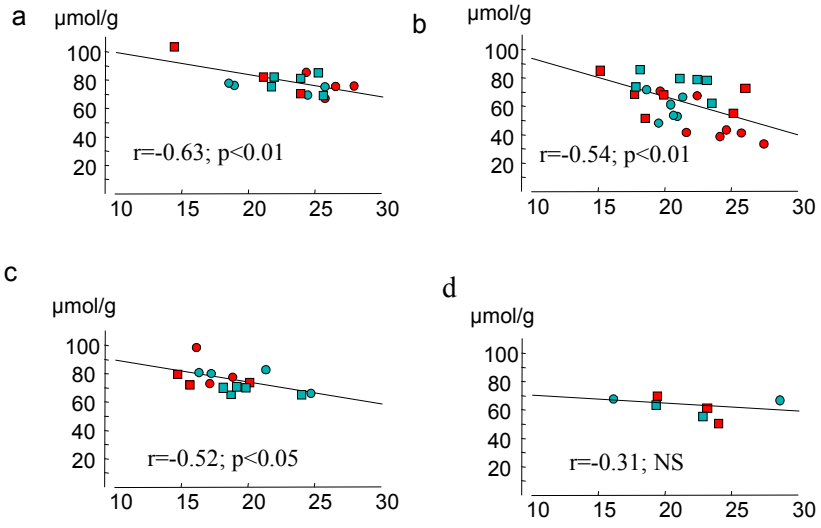

Fig. 2. Negative correlations between external angle of the ham and lactate content at 24 h for the D cross, during the first (a) and second year (b) and LW cross, in the first (c) and second year (d). Circles and square represent indoor and outdoor rearing, respectively. Red and green colours represent slaughter after mixing and lairage, and without mixing and lairage, respectively.

Table 1. Pearson correlations (pooled over the two years) between carcass weight and leg and body length, for the two genetic types separately.

	Large White cross		Duroc cross	
	r	p	r	p
Carcass weight, with				
Leg length 1	0.55	0.005	0.46	0.001
Leg length 2	0.47	0.02	0.33	0.02
Lumbar/thoracic length	0.50	0.01	0.54	0.0001

Table 2. Morphometric characteristics described by r-values for simple (pooled over the two years) and multiple regression analyses for the two genetic types separately.

	Large White cross		Duroc cross	
	Simple regression			
	Pooled r	p	Pooled r	p
Lumbar/thoracic length, with				
Posterior angle of the ham	-0.63	0.001	0.32	0.02
			Multiple regression	
			Semi-partial r	p
Internal angle of the ham, with				
Pelvis width			0.41	0.001
Iliac bone length			-0.42	0.001
Posterior angle of the ham			0.33	0.01

Table 3. Pearson correlations (pooled over the two years) between morphometric characteristics and post-mortem metabolism-related parameters, for the two genetic types separately.

	Large White cross		Duroc cross	
	r	p	r	p
External angle of the ham, with				
SM glycolytic potential, 45 min			-0.41	0.01
SM glycogen content, 45 min			-0.45	0.01
SM glycogen content, 24 h			-0.39	0.01
SM lactate content, 24 h	-0.44	0.05	-0.54	0.001
SM temperature, 45 min	0.53	0.02		
LL temperature 45 min	0.63	0.01		
Iliac bone length, with				
SM lactate content, 24 h			-0.48	0.01
LL lactate content, 24 h			-0.56	0.0001
LL temperature, 45 min			0.30	0.04
LL temperature, 24 h			0.44	0.01
Pelvis width, with				
LL lactate content, 24 h	0.69	0.001		

THE EFFECT OF INTRAMUSCULAR WATER FLUID TRANSPORT ON *RIGOR MORTIS*

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Background

Rigor mortis is one of the main macroscopic events after death in all animals, and it is of importance to understand how this event affects the quality and quantity of meat. For the meat producer it is important to understand *rigor mortis* in order to optimise slaughter and processing procedures for different meats. Our understanding of the biochemical reactions *post mortem* and how these affect the meat quantity and quality from different animals is limited (Devine *et al.*, 1999, Erikson, 2001).

Development of *rigor mortis* has been studied by several methods such as loss of extensibility (Bendall, 1973, Briskey *et al.*, 1962, Honikel *et al.*, 1983, Schmidt *et al.*, 1968) muscle shortening (Currie and Wolfe, 1979), tension development (Nuss and Wolfe, 1980-81), resistance to strain (Lepetit *et al.*, 1998) and by combination of muscle tension and shortening (Hertzman *et al.*, 1993, Olsson *et al.*, 1994, Rosenvold *et al.*, 2003).

After death, catabolic reactions predominate. ATP concentrations decline, and pH in mammalian meat are lowered to around 5.6 as a result of the formation of lactic acid. ATP is continuously used for muscle relaxation and transport of Ca^{2+} across the sarcoplasmatic membrane. Partially due to the interactions between actin and myosin after death, ATP is lowered to around zero and the actomyosin bridges formed are used to explain *rigor mortis*. These bridges are also used to explain the stiffness that characterizes *rigor mortis*. The actomyosin bridges are judged as irreversible. However, it is also agreed that the stiffness is resolved without any changes in the binding between actin and myosin. Thus, another factor has to be introduced to explain the resolution of *rigor mortis*.

All the catabolic reactions after death increase the number of molecules within the muscle. Glycogen is converted to glucose that is further metabolised to lactate. Proteolysis and lipolysis give amino- and fatty acids and ions are released. Together with other enzymatic reactions the increased number of molecules changes the osmotic pressure ($\pi = cRT$) to a higher value. It has been found that the osmotic pressure increased from an at-death level of 379 to 528 mOsmole in lamb *M. longissimus dorsi,* and one-third of the increase occurred after 24 hours (Veiseth *et al.,* 2003). It was also found that the conductivity changed from 11.3 to 5.7 mS/cm during the 12 first hours.

The volume of muscle cells, however, does not change during *rigor mortis* (Kobayashi *et al.*, 2001). In addition, our morphological study of muscle cells, immediately after death and in the *rigor* state, indicates that the shape of cells changes from a circular to a more squared form, and this is calculated to be statistically significant (Slinde *et al.*, 2003). Since the volume of the whole muscle is the same at slaughter and in *rigor* this means that the intracellular volume has increased, and this space filling create stiffness.

Tenderness of meat is a very important quality parameter, but the tenderisation process is very variable in meat. Different apparatus determines the toughness of meat that characterizes the tenderness mechanically, and the Warner-Bratzler (WB) shear force is often used. Unfortunately, most experimental measurements are generally done after the animal has passed through *rigor mortis*. These texture analyses have in general a rather high standard deviation since it is measured in many individuals, but also due to the difference in muscle fibre types and variation in the amount of connective tissue. We have found that when WB shear forces are measured from slaughter and onwards in Atlantic salmon, the WB shear force decline from the first measurement onwards (Roth *et al.*, 2002, Slinde *et al.*, 2003). Partman (1963) showed that texture analysis of fish and other animals showed such high standard deviations after slaughter that the effect of *rigor mortis* was not detected.

Objectives

It is generally agreed that after death, the main catabolic biochemical reactions and formation of the actomyosin bridges leading to *rigor mortis* are the same in muscle of animals, birds, reptiles and fish (Partman, 1963). The purpose of the present study was to strengthen the evidence that changes in osmolarity and the flow of water into the cells are the main cause of the macroscopic felt stiffness of *rigor mortis* (Roth *et al.*, 2002, Slinde *et al.*, 2003),

Materials and methods

Atlantic salmons (*Salmo salar*) were stunned by a blow to the head, and gill arches were cut. Muscle samples were taken from the region behind the dorsal fin. A blow by a nail through the crocodile's (*Crocodylus niloticus*) head followed by decapitation killed the animal. Crocodiles, approximately 150 cm long, were reared at ambient temperature 18 – 20°C. Right and left tail muscles *M. caudal femoral, M. longissimuis caudalis* and *M. ilioishiocaudalis* were used for measurements.

Shear forces were measured on individual fishes and crocodiles using a Warner-Bratzler blade connected to a texture analyser. *Rigor* index was measured as "tail bending" and calculated as $IR=(L_0-L_t)/L_0 \times 100$ where L represents the vertical drop of the tail when half of the length was outside the table. L_0 represents the vertical drop at start and L_t represents measurement during the experiment (Bito *et al.*, 1983).

Results and discussion

We have selected fish muscle as a model to study development of *rigor mortis,* since the large swimming muscle is very homogenous with regard to fibre- and connective tissue composition. Partman (1963) states that penetration measurements show high average deviations in different fish species and an increase in rigidity in each fish during *rigor* is therefore not seen. There are also great deviations in *rigor* development within a single species. When following *rigor* development in each salmon separately as shown in Figure 1, the variation in both *rigor* index and shear force is clearly different. This is due to the biochemical status of each individual animal at slaughter. But it is very clear that the animal's shear force decreases from slaughter through *rigor mortis* while the measured *rigor* index goes through a maximum. Crocodiles are regarded as more developed than fish and have a fibre- and connective tissue structure that is more complex. Figure 2 shows that the shear force spans a much wider range in individual crocodiles reared at similar conditions when chilled at 20 and 30°C when compared to fish. The changes in shear force from slaughter and onwards decrease slightly but the standard deviations are high. We have therefore concluded that another factor besides the actomyosin binding has to contribute to the stiffness observed in *rigor mortis,* and this has to be a property that is of general nature, and must be found in all animals.

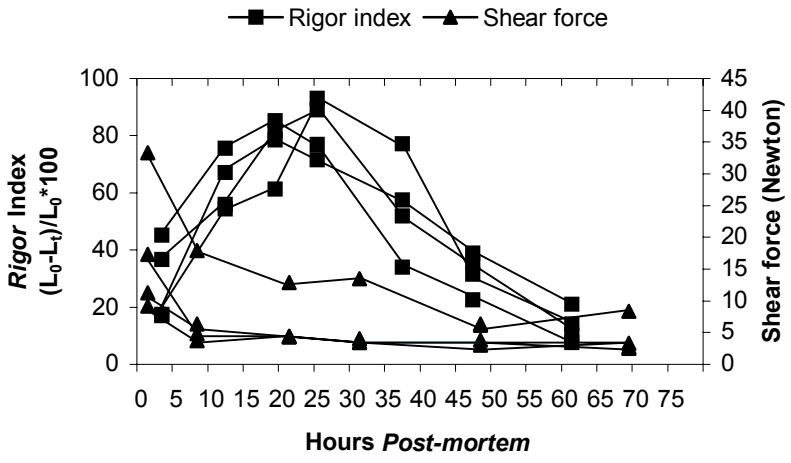

Figure 1. Measurements of Warner-Bratzler (WB) shear force and *rigor* index in four Atlantic salmon (*Salmo salar*).

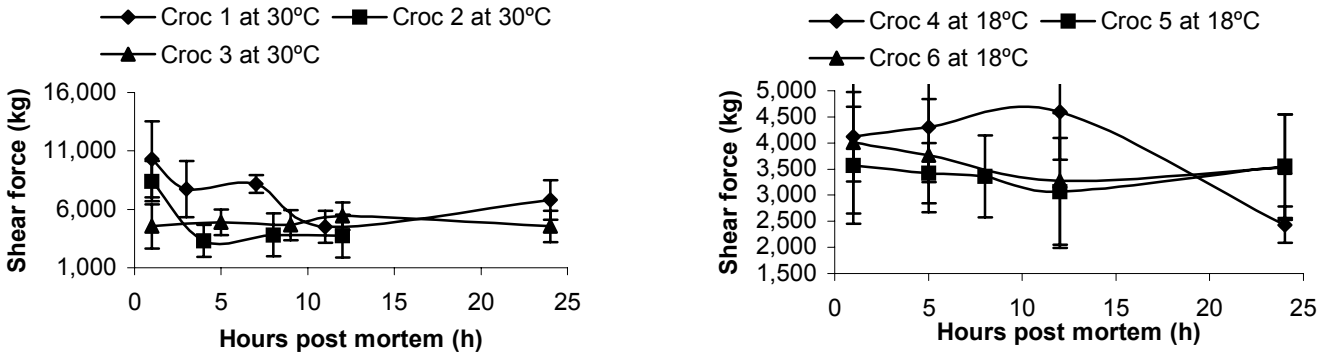

Figure 2. Change in Warner-Bratzler (WB) shear force (kg) in individual crocodile tail muscles during *rigor mortis*.

Catabolic reactions are a general property in all animals after death. Veiseth *et al*. (2004) have performed measurements of osmolar changes. Figure 3 shows the osmolarity within lamb muscle together with the change in pH. We can see that these two properties are mirror images of each other. The rather large change in osmolarity causes water to flow from the extracellular space into the cell. The volume of the cells expands and the cell walls follow the surrounding connective tissue more closely (Slinde *et al*., 2002). In Figure 4, a model of this water transport between intra- and extracellular space within a muscle has been drawn. The increase in cell volume strengthens the structure of the connective tissue and give rise to stiffness. A better understanding of the osmotic behaviour in the muscle after slaughter might increase our understanding of meat texture, as well as its water binding properties. Preliminary studies indicate that the conductivity in cells are low after slaughter, but a sudden raise is observed after some time indicating cellular membrane rupture. The osmotic changes found (Veiseth *et al*., 2004) and shown in Figure 3 may also be studied using NIR (Near Infrared Reflectance), since this method has the property of measuring water in different environments *i.e*. the intra- and extra cellular compartments.

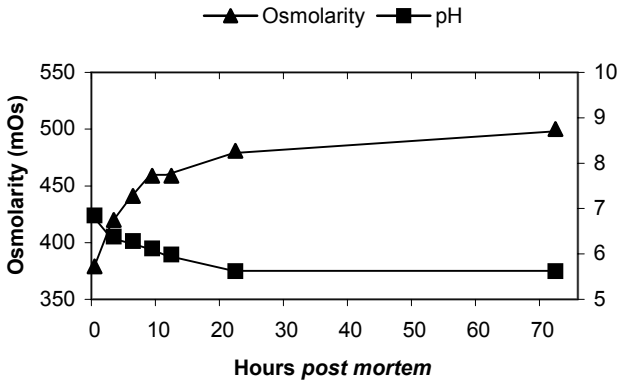

Figure 3. Data from lamb *M. longissimus dorsi* (Veiseth *et al.*, 2004) shows how the osmotic pressure increases after death, Note that the pH is almost a mirror image of the change in osmolarity.

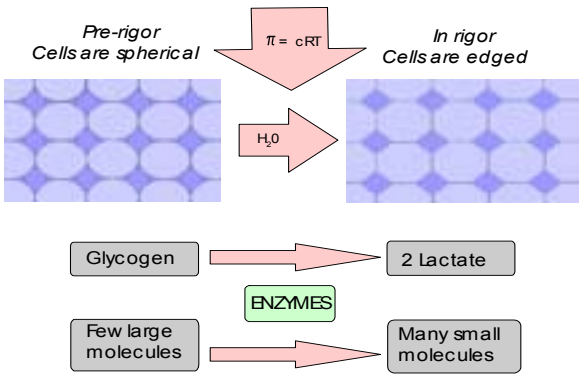

Figure 4. The osmotic change in muscle cells during *rigor mortis*. The enzymatic breakdown increases the number of molecules within the muscle cells. The contour of the cells changes from a spherical to a more edged form due to osmotic influx of water ($\pi = cRT$).

Conclusions

Figure 1 and 2 shows the same tendency, that in individual animals, the water being intra- or extracellular does not affect the WB shear force. The WB shear force is not affected by *rigor mortis* as opposed to the *rigor* index (Figure 1 and 2). The *post mortem* reactions increase the number of molecules within the muscle cells and causes an alternation in the osmotic potential (Veiseth *et al.*, 2004). Since the volume of the muscle is constant (Kobayashi *et al.*, 2001) the increased cell volume makes the muscle stiff. Further investigation of the transport of muscle juice will increase our understanding of *rigor mortis*, the DFD and PSE syndrome as well as the water holding capacity of meat. It will also increase our understanding of tenderisation.

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METMYOGLOBIN REDUCING ACTIVITY IN VEAL AND VENISON MEATS

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Background

The colour of fresh meat is an important quality attribute which influence the consumers purchase decision. The failure to have an attractive colour will affect the dynamics of meat sales and have financial consequences[1,2]. Meat discolouration is caused by the accumulation of metmyoglobin (MetMb) during aerobic retail display[3]. The existence of enzymatic system/s capable of reducing MetMb has been reported[4]. Such system/s can reduce MetMb to myoglobin (Mb), hence potentially decrease the degree of discolouration and increase shelf life. While there is agreement on the presence of MetMb reducing systems in meat, their role in maintaining fresh meat colour is more controversial. Metmyoglobin reducing activities were measured and characterized in beef[5], pork[6] and lamb[7]. To our knowledge such information is not available for other economically important meats, depending on availability and cultural preference, such as veal and venison. In normal fresh meat when colour stability is not altered through special dietary or processing regimes, different species exhibits different colour stabilities and consequently different shelf lives. Information on MetMb reducing activities in meat from these species could advance our knowledge on the relationship between MetMb reducing activity and fresh meat colour stability.

Objectives

The current study was undertaken to characterize and measure MetMb reducing activity in veal and venison and to compare the activity in these meats with the reported MetMb reducing activities in beef, pork and lamb meats with the aim of investigating whether a meaningful relationship could be found between MetMb reducing activity and the known shelf life of these meats.

Materials and methods

Venison: samples from longissimus dorsi (LD) muscles of two years old stags (n=8) were obtained from carcasses (average weight 55.3 ± 4.1 kg) and vacuum packed for 3 weeks (standard aging time according to the New Zealand game industry board specifications).
Veal: samples were obtained at 48 h post mortem from LD muscles of 75 (± 2) days old male calves (n=6, average live wt 88 ± 5 kg).
Metmyoglobin reducing activity. A sarcoplasmic and particulate metmyoglobin reducing activity (SMRA and PMRA, respectively) were obtained from the samples as described ealier[8]. The supernatant and the particulate extracts were oxidised with a slight excess of $K_3Fe(CN)_6$, dialysed (10000 MW cut-off membrane) against 2.0 mM phosphate buffer (pH 7.0) at 4 ^{0}C several times. Metmyoglobin reductase activity was determined as described[6,7] using $VERSA_{max}$ microplate reader (Molecular Devices, Sunnyvale, CA, USA) after scaling down the assay final volume to 200µl. The standard assay mixture contained 15 µl 5 mM EDTA; 15 µl 50 mM phosphate buffer (pH 7.0); 15 µL 3.0 mM K_4 Fe $(CN)_6$; 50 µl 0.75 mM Mb Fe(III) in 2.0 mM phosphate buffer (pH7.0); 10 µl 2.0 mM NADH; muscle extract (50 µl for SMRA and 10 µL, of 5x dilution of dialysed samples, for MMRA) and water to a final volume of 20 µl assay mixture. The standard assay mixture pH was 6.8 and the assay was carried out at 25 °C. The reaction was initiated by adding NADH and followed by the change in absorbance at 580 nm. Blanks contained all the additions except NADH, which was replaced by water. The activity was calculated as the mean of three replicates and expressed as nanomoles of MetMb reduced per min per gram of meat.

Results and discussion

High propotions (82- 88 %) of the total MetMb reducing activity, were present in the pellet fraction of LD muscles in veal and venison meat (Table 1). SMRA in veal LD muscle was higher than those reported for pork[6,9] and less than that in beef or lamb meats[7,10]. PMRA in veal LD was less than that in beef, but higher than that in lamb LD muscle. Venison on the other hand, had comparable SMRA and PMRA to those reported in beef [10].
The effects of different assay pH and NADH concentrations on MetMb reducing activities in the different fractions of the muscle is presented in Figure 1. The pellet fractions from veal and venison seems to be more

sensitive to changes in pH and NADH which indicate the possibility of different MetMb reducing system/s from that present in the supernatant. Earlier[10], it was suggested that this activity could be activity remained in the pellet fraction due to the hydrophobic segment that binds NADH cytochrome b_5 metmyoglobin reductase to membranes[11], because the current methodology do not employ a detergent to solubilize the enzyme or unsufficient mechanical release of microsomes and mitochondria by homogennization. However, the behaviour of sarcoplasmic and pellet MetMb reducing activities in regard to the effects of assay pH and NADH concentration indicate to a possible different MetMb reducing systems in these two fractions. The capacity of SMRA, beef= venison > lamb > veal > pork, do not correspond with the expected display shelf-life of these meats, beef > veal > lamb > pork > venison. Moreover, given that beef and venison exhibit similar SMRA and PMRA and the shelf-life of beef >> venison, it is unlikely that MetMb reducing activity is contributing to the colour stability of fresh meat during display storage.

Conclusions

MetMb reducing activity is higher in venison than in veal LD muscles. Since the order of the MetMb reducing activity in beef, lamb, veal , pork and venison do not generate a meaninful relationship with their expected shelf-life display, MetMb reducing activity is unlikely to contribute to fresh meat colour stability. However, from a physiological point of view, differences in SMRA and PMRA presented in this study could be of interest.

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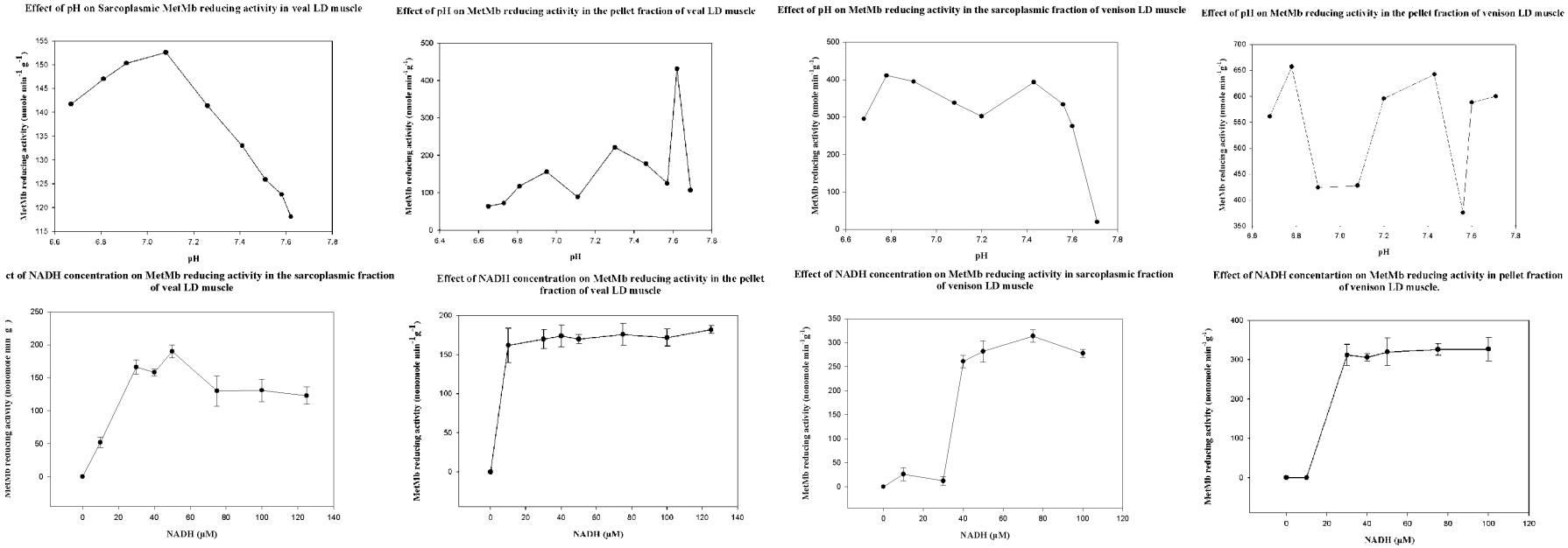
Table 1. Metmyoglobin reducing activity in veal and venison LD muscle extracts (SN and Pellet) under different assay conditions.

	Veal						Venison					
	EDTA	$K_4Fe(CN)_6$	NADH	Muscle extract	Equine MbFe(III)	Activity (nmol min^{-1} g^{-1})	EDTA	$K_4Fe(CN)_6$	NADH	Muscle extract	Equine MbFe(III)	Activity (nmol min^{-1} g^{-1})
Supernatant extract	+	+	+	+	+	162 ± 7	+	+	+	+	+	312 ± 5
	+	-	+	+	+	56 ± 3	+	-	+	+	+	135 ± 4
	-	+	+	+	+	164 ± 5	-	+	+	+	+	274 ± 8
Pellet extract	+	+	+	+	+	775 ± 95	+	+	+	+	+	2422 ± 101
	+	-	+	+	+	204 ± 24	+	-	+	+	+	1474 ± 43
	-	+	+	+	+	802 ± 43	-	+	+	+	+	1817 ± 81

(+) = presence (-) = absence

-No MetMb reducing activities were found when NADH, muscle extract or equine MetMb was omitted from the assay mixture. The absence of EDTA from the assay mixture decreased MetMb reducing activities in venison extracts but not veal. The absence of $K_4Fe(CN)_6$ from the assay mixture resulted in decreased MetMb reducing activities and the extend of the effect varied between venison and veal. The present results and earlier reports[5-7,10] indicates that MetMb reduction by various species extracts is influenced by the assay components.

Figure 1. Effects of NADH concentrations and pH on MetMb reducing activity in sarcoplasmic and pellet fraction of veal and venison LD muscles.



PROXIMATE COMPOSITION AND COLLAGEN CONTENT OF BEEF AND PORK MEAT CUTS

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Background

Both physical and chemical composition parameters and pH of beef- and pork-cuts are highly important for the meat processor, since such parameters are closely connected with the ability of the meats to retain water and fat.

One concern in finely comminuted emulsion-like products such as frankfurter and mortadellas is with levels of collagen free proteins (CFP) and fat. These compounds affect directly emulsion stability and fat and water retention during processing. Meat connective tissue is constituted mainly of collagen that is part of muscle and adipose tissue. Collagen is characterized by a high content of glycine, proline and hydroxyproline and total lack of sulfur containing aminoacids and tryptophane.

Meats with high collagen content or containing tough collagen are used in sausage processing, where collagen is comminuted by the action of grinders, cutters or mills (Bailey and Ligth, 1980; Olivo and Shimokomaki, 2002). The addition of cooked pigskin to sausages also increases its collagen content. Sausage manufacturers use meat with high collagen content although they are aware that its amount in the sausages should be limited to prevent defects like gelatin release, difficulty in peeling, shrinkage, and poor texture (Rao and Henrickson, 1983; Kenney et al., 1992; Olivo and Shimokomaki, 2002). It is clear that the determination of collagen content and proximate composition in meats is essential to predict sausage and other meat processed products quality and for the use of least cost formulation formulae. In Brazil there is a lack of available information on the proximate composition of the main beef and pork cuts used to produce sausages. This knowledge would help industry to better formulate meat products and help inspection services to enforce legislation concerning meat products composition.

Objectives

The purpose of this work was to determine moisture, fat, total protein, collagen content, and pH of meat cuts of hybrid pork and bovine (Nelore) used in the processing of sausage and other meat products.

Materials and methods

Three castrated Nelore (Bos indicus) 30 to 36 months of age, pasture fed, with an average weight of 437,5 kg (±16,2) were slaughtered in intervals of 15 days. The half-carcasses were chilled at 0 ± 4°C for 24 hours. The half carcasses were split into forequarters and hindquarters between the 5th and the 6th ribs. The forequarter beef meat cuts chuck, shoulder, brisket, neck, inside skirt (diaphragm), thick skirt, neck trimmings, foreshank, and plate were analyzed.

Three hybrid male pigs cross from Large White x Pietrain males with Large White x Landrace females and with a live weight of approximately 125-130Kg were slaughtered on the same day. After chilling in storage room kept at 0 ± 4°C for 24 hours, the half carcasses were cut into picnic shoulder, fresh ham, loin, tender loin, shoulder butt, belly, spareribs, neck trimmings and skin. The whole cuts of beef were ground three times in a Hermann grinder with 5mm holes plates. The pork cuts were ground two times in the same grinder with holes plates of 3mm. The cuts were not dressed, to simulate industry procedures. Physico-chemical analysis: moisture (oven at 102-105°C), total protein (Kjeldahl, Nx6,25), fat (diethyl ether extractable) and ash contents were determined following Instituto Adolfo Lutz procedures (São Paulo, 1985). pH values were measured with a spear-tip electrode attached to a digital pH-meter (HANNA Instruments – HI9321 microprocessor). The hydroxyproline assay was carried out according to the method described by AOAC (1996). Hydroxyproline was quantitatively determined, in order to measure the collagen content. Samples were hydrolyzed with 6N HCl for 8h at 110°C. After hydrolysis, 4-hydroxyproline was converted to pyrrole with chloramine T in acetate-citrate buffer pH 6.0, and pyrrole was converted to a red-coloured complex (absorption at 558nm) by reaction with Ehrlich reagent [p-(dimethylamino) benzaldehyde in perchloric

acid/2-propanol]. Total collagen protein was determined by multiplying hydroxyproline contents by 8. The physico-chemical analyses were carried out in duplicate for each pork and beef cut, the average being calculated from the three animals. Statistical analysis: all data underwent analysis of variance and Tukey's Test to determine differences ($p<0.05$) between pairs of means, using GraphPad InStat tm, Copyright 1990-1993, V2.01.

Results and discussion

The weights of the hot half carcasses of the three bovines were 124.6, 130.6, and 135.4 Kg. The weights of the chilled forequarters were respectively 41.1, 46.5, and 51.6 Kg. On Table 1 are shown the means for the proximate composition parameters, hydroxyproline, collagen and relative collagen and pH. Significant differences ($p<0,05$) were observed for all parameters among the different meat cuts except for ash content, which was not significantly different between the different meat cuts. For the three forequarter beef meat cuts of the categories chuck, shoulder, brisket, neck, inside skirt (diaphragm), thick skirt, neck trimmings, foreshank and plate, ranges of percent moisture were 66.5 (plate) to 75.4% (neck); fat, 4.0 (neck) to 15.9% (plate); total protein, 16.4 (plate) to 19.9% (foreshank); ash, 0.8 (plate) to 1.0% (diaphragm); hydroxyproline, 0.19 (diaphragm) to 0.61% (foreshank); collagen (COL) from 1.5 to 4.9%; relative collagen from 8.4 (diaphragm) to 24.77% (foreshank); pH varied from 5.73 (brisket) to 6.00 (plate). Largest variation in composition was found for the brisket, which presented the largest coefficient of variation for most of parameters evaluated. The diaphragm meat was the one with the smallest coefficient of variation for most of the products evaluated.

The swines' hot half carcasses weighted 43.4, 46.2, and 49.0 Kg. The proximate composition, hydroxyproline content, collagen content, relative collagen content, and pH are presented on Table 2. There were significant differences among different cuts for all parameters studied. In the evaluations of pork cuts from picnic shoulder, fresh ham, loin, tender loin, shoulder butt, belly, spareribs, neck trimmings and skin, large variation was found for moisture 20.6 (neck trimmings) to 73.2% (tender loin), fat 5.2 (tender loin) to 75.6% (neck trimmings), total protein 5,4 (neck trimmings) to 20,8% (tender loin), ash 0.3 (neck trimmings) to 1.2%(tenderloin), hydroxyproline 0.08 (tenderloin) to 1.28% (skin), collagen (COL) from 0.6 to 10,2%, relative COL 3.0 (tender loin) to 66.1%(skin), and pH 5.79 (belly) to 6.24 (shoulder butt). The largest variances between the animals in the determinations were those for neck trimmings, while the smallest were those for picnic shoulder and loin. It is important to underline that the variations observed reflect variations between samples from the animals and not analytical. The pork-cuts (except the skin) presented the greatest content of fat and the lowest of water, hydroxyproline and COL, if compared with those of beef-cuts meat.

Conclusions

As expected, the moisture, fat, protein, and collagen content and pH of the different meat cuts from pigs and bovines forequarters showed a large variability. The pig cuts comparable to those of beef had lower moisture and hydroxyproline (collagen) content and higher fat content. It is believed that the meats composition mean values determined in this study can be used in formulating meat products to comply with pertinent Brazilian legislation.

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Table 1. Proximate composition, pH and hydroxyproline contents of beef cuts

Beef cuts		Determinations*							
		Moisture (%)	**Fat (%)**	**Total Protein (%)**	**Ash (%)**	**Hydroxy-proline (%)**	**COL [a] (%)**	**COL [b] rel. (%)**	**pH**
Chuck	**Average**	**74.7^{a}**	**5.8^{b}**	**18.4^{ab}**	**0.9^{a}**	**0.3^{bcde}**	**2.4^{bcde}**	**13.3**	**5.9^{ab}**
	SD	*1.9^{a}*	*1.7*	*0.3*	*0.1*	*0.04*	*0.3*		*0.02*
	CV(%)	*2.5*	*29.7*	*1.4*	*6.5*	*14.1*	*13.9*		*0.34*
Shoulder	**Average**	**74.6^{a}**	**4.8^{b}**	**19.6^{a}**	**0.9^{a}**	**0.4^{bc}**	**3.1^{bc}**	**16.0**	**5.83^{ab}**
	SD	*14*	*1.7*	*0.7*	*0.1*	*0.1*	*0.6*		*0.10*
	CV(%)	*1.9*	*34.6*	*3.7*	*10.6*	*20.0*	*20.4*		*1.72*
Brisket	**Average**	**72.6^{ab}**	**7.8^{b}**	**18.3^{ab}**	**1.0^{a}**	**0.3^{cde}**	**2.0^{cde}**	**11.1**	**5.73^{c}**
	SD	*3.8*	*4.4*	*1.3*	*0.2*	*0.1*	*0.5*		*0.06*
	CV(%)	*5.2*	*55.7*	*7.2*	*16.8*	*25.6*	*25.6*		*1.05*
Neck	**Average**	**75.4^{a}**	**4.0^{b}**	**19.3^{a}**	**0.9^{a}**	**0.4^{bcd}**	**2.9^{bcd}**	**14.9**	**588^{ab}**
	SD	*1.5*	*1.3*	*0.2*	*0.04*	*0.1*	*0.5*		*0.06*
	CV(%)	*2.0*	*32.3*	*1.2*	*4.3*	*18.0*	*18.0*		*1.02*
Diaphragm	**Average**	**73.0^{ab}**	**7.2^{b}**	**18.2^{ab}**	**0.98^{a}**	**0.2^{e}**	**1.5^{e}**	**8.4**	**5.87^{ab}**
	SD	*0.2*	*0.3*	*0.2*	*0.02*	*0.02*	*0.1*		*0.04*
	CV(%)	*0.3*	*4.0*	*0.9*	*2.0*	*9.4*	*9.2*		*0.68*
Thick skirt	**Average**	**74.9^{a}**	**6.4^{b}**	**17.1^{b}**	**0.9^{a}**	**0.2^{de}**	**1.9^{de}**	**10.3**	**5.97^{ab}**
	SD	*0.5*	*1.5*	*0.4*	*0.1*	*0.03*	*0.3*		*0.05*
	CV(%)	*0.6*	*23.8*	*2.3*	*9.2*	*15.8*	*16.0*		*0.84*
Neck trimmings	**Average**	**70.9^{ab}**	**9.9^{ab}**	**18.8^{ab}**	**0.9^{a}**	**0.4 ^{b}**	**3.0^{b}**	**18.2**	**5.77^{bc}**
	SD	3.2	4.0	0.9	0.1	0.03	0.2		0.03
	CV(%)	*4.5*	*40.9*	*4.8*	*8.7*	*6.1*	*6.1*		*0.52*
Foreshank	**Average**	**74.5^{a}**	**5.1^{b}**	**19.9^{a}**	**0.9^{a}**	**0.6^{a}**	**4.9^{a}**	**24.8**	**5.88^{ab}**
	SD	*0.9*	*1.3*	*0.8*	*0.1*	*0.06*	*0.5*		*0.16*
	CV(%)	*1.2*	*25.3*	*3.9*	*6.8*	*9.6*	*9.6*		*2.72*
Plate	**Average**	**66.5^{b}**	**15.9^{a}**	**16.4^{b}**	**0.8^{a}**	**0.4^{b}**	**3.3^{b}**	**19.8**	**6.00^{a}**
	SD	*3.7*	*5.4*	*0.8*	*0.06*	*0.03*	*0.2*		*0.06*
	CV(%)	*5.6*	*34.2*	*4.8*	*7.2*	*6.9*	*7.1*		*1.00*
Minimum value		66.5	4.0	16.4	0.8	0.2	1.5	8.4	5.73
Maximum value		75.4	15.9	19.6	0.98	0.6	4.9	24.8	6.00
Median		74.5	6.8	18.2	0.9	0.4	2.9	14.9	5.87
Mean		**73.0**	**7.7**	**18.2**	**0.9**	**0.4**	**2.8**	**15.2**	**5.87**
S.D.		2.8	3.8	1.0	0.05	0.1	1.0	5.2	0.09
C.V. (%)		3.9	48.9	5.7	5.6	36.0	36.1	33.9	1.45

* Means of three forequarters

COL [a] = collagen content estimated from hydroxyproline content X 8 **COL [b] rel**. = (COL/ total protein) x 100

S.D.= Standard deviation **C.V.**= Coefficient of variation

Table 2. Proximate composition, pH and hydroxyproline contents of pork cuts

Pork cuts		Determinations*							
		Moisture (%)	**Fat (%)**	**Total Protein (%)**	**Ash (%)**	**Hydroxy-proline (%)**	**COL [a] (%)**	**COL [b] rel. (%)**	**pH**
Chuck	**Average**	**71.8^{a}**	**7.4^{ef}**	**20.1^{ab}**	**1.1^{b}**	**0.2^{cd}**	**1.2^{cd}**	**5.9**	**5.89^{ab}**
	SD	*0.8*	*1.3*	*0.2*	*0.1*	*0.01*	*0.1*		*0.19*
	CV(%)	*1.1*	*17.5*	*1.1*	*2.8*	*9.5*	*9.3*		*3.23*
Shoulder	**Average**	**70.1^{ab}**	**10.6^{e}**	**18.6^{bc}**	**1.0^{bc}**	**0.2^{b}**	**1.8^{b}**	**9.7**	**6.12^{ab}**
	SD	*0.3*	*0.2*	*0.1*	*0.02*	*0.04*	*0.3*		*0.25*
	CV(%)	*0.4*	*2.0*	*0.7*	*2.0*	*18.7*	*18.9*		*4.08*
Brisket	**Average**	**64.0^{c}**	**18.8^{d}**	**16.9^{cd}**	**1.0^{cd}**	**0.2^{bc}**	**1.4^{bc}**	**8.4**	**6.24^{a}**
	SD	*1.2*	*1.8*	*0.6*	*0.1*	*0.01*	*0.1*		*0.39*
	CV(%)	*1.8*	*9.4*	*3.6*	*5.3*	*6.2*	*5.6*		*6.25*
Neck	**Average**	**68.0^{b}**	**12.3^{e}**	**19.8^{ab}**	**1.0^{bc}**	**0.2^{bc}**	**1.2^{bc}**	**6.3**	**5.80^{b}**
	SD	*0.8*	*0.3*	*0.1*	*0.02*	*0.01*	*0.04*		*0.15*
	CV(%)	*1.2*	*2.6*	*0.5*	*2.0*	*3.2*	*3.2*		*2.59*
Diaphragm	**Average**	**73.1^{a}**	**5.3^{f}**	**20.8^{a}**	**1.2^{a}**	**0.1^{d}**	**0.6^{d}**	**3.0**	**5.80^{b}**
	SD	*0.5*	*0.8*	*0.1*	*0.1*	*0.02*	*0.1*		*0.10*
	CV(%)	*0.7*	*16.0*	*0.3*	*4.3*	*19.5*	*19.4*		*1.72*
Thick skirt	**Average**	**55.2^{d}**	**30.0^{c}**	**14.9^{e}**	**0.8^{e}**	**0.2^{bc}**	**1.6^{bc}**	**10.9**	**5.79^{b}**
	SD	*0.9*	*2.3*	*0.3*	*0.03*	*0.01*	*0.1*		*0.05*
	CV(%)	*1.5*	*7.8*	*1.8*	*3.8*	*4.0*	*4.3*		*0.86*
Neck trimmings	**Average**	**20.6^{f}**	**75.6^{a}**	**5.4^{f}**	**0.3^{g}**	**0.2^{bc}**	**1.7^{bc}**	**31.9**	**6.05^{ab}**
	SD	1.7	2.3	0.8	0.04	0.1	0.4		0.02
	CV(%)	*8.3*	*3.1*	*15.5*	*12.9*	*23.2*	*23.7*		*0.33*
Foreshank	**Average**	**38.0^{e}**	**46.0^{b}**	**15.5^{de}**	**0.4^{f}**	**1.3^{a}**	**10.2^{a}**	**66.1**	**6.13^{ab}**
	SD	*0.5*	*3.0*	*1.7*	*0.03*	*0.3*	*1.9*		*0.06*
	CV(%)	*1.3*	*6.5*	*11.1*	*6.8*	*18.6*	*18.6*		*0.98*
Plate	**Average**	**62.5^{c}**	**19.7^{d}**	**17.1^{cd}**	**0.9^{d}**	**0.2^{bc}**	**1.4^{bc}**	**8.3**	**5.91^{ab}**
	SD	*1.9*	*2.0*	*0.7*	*0.04*	*0.02*	*0.2*		*0.14*
	CV(%)	*3.0*	*10.1*	*4.1*	*4.4*	*10.7*	*11.4*		*2.37*
Minimum value		20.6	5.3	5.4	0.3	0.1	0.6	3.0	5.79
Maximum value		73.1	75.6	20.8	1.2	1.3	10.2	66.1	6.24
Median		64.0	18.8	17.1	1.0	0.2	1.4	8.4	5.91
Mean		**58.1**	**25.1**	**16.6**	**1.0**	**0.3**	**2.4**	**16.7**	**5.97**
S.D.		17.8	22.9	4.7	0.3	0.4	3.0	20.3	0.17
C.V. (%)		30.6	90.9	28.2	34.9	125.9	125.8	121.7	2.83

*Means of three pig forequarters

COL [a] = collagen content **COL [b]** rel. = (COL/ total protein) x 100

SD = standard deviation **C.V.**= coefficient of variation

Acknowledgement

This work was supported by FAPESP projects n° 2000/06438-8 and 01/03499-9. The authors thank ELANCO-Lilly (Animal Health Division) for the donation of the pigs carcasses.

TECHNOLOGICAL QUALITY OF BROILER BREAST MEAT IN RELATION TO MUSCLE HYPERTROPHY

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Background

In broiler, recent studies reported a great variability of the muscle *post mortem* metabolism with both the animal genetic background and the pre-slaughter stresses. Indeed, selection for rapid growth and/or muscle development can produce breast meat with slower pH decline, higher ultimate pH and consequently lower drip loss (Le Bihan-Duval et al., 1999; Berri et al., 2001) and therefore affect processing quality of meat. While some of pre-slaughter conditions which affect *post mortem* pH fall are well identified (Debut et al., 2003), the muscle characteristics responsible of the pH fall variations remains uncertain. In broiler, there is no evidence of changes in breast fibre typing with selection for growth (Rémignon et al., 1995). By contrast, fibre radial and longitudinal growth significantly increased with selection for breast yield (Guernec et al., 2003) but, as far as we know, the impact of such structural changes on further broiler breast meat quality has never been evaluated.

Objectives

The purpose of this study was to relate breast muscle development, including muscle fibre size, to *post mortem* metabolism and further breast meat quality. Phenotypic and genetic relationships between fibre and meat traits were estimated for a total of 600 commercial broilers. For all birds, we measured muscle fibre cross-sectional area, glycolytic potential, lactate content, *post mortem* pH fall and classical meat traits (colour, drip and thawing-cooking loss, Warner-Bratzler shear force). We also determined the proportion of connective tissue and the occurrence of giant fibres in relation to muscle fibre size or *post mortem* metabolism.

Materials and methods

Animals and muscle sampling

A total of 600 broilers (males and females), originating from a commercial grand-parental male line (Hubbard Europe, Chateaubourg, France), were reared under similar conditions in a conventional poultry house at the INRA Avian Research Centre (Nouzilly, France). Birds were reared in 2 successive batches of 300 birds. Feed and water were provided *ad libitum* throughout the growth period. After a 8-h feed withdrawal, six week-old broilers were weighed then slaughtered in the experimental processing plant of the Avian Research Centre. Broilers were electrically stunned in a water bath (60 mA; 125 Hz; 5 s) before bleeding by ventral neck cutting. After scalding (51°C; 3 min), plucking and manual gut removal, whole carcasses were air chilled in a cold room at 2°C for 24 h.

Fifteen minutes after slaughter, two samples of the right *Pectoralis major* (PM) were collected. One sample was rapidly frozen in isopentane cooled with liquid nitrogen and stored at -80°C until histology assays. The other was mixed in 0.55M perchloric acid (1 g / 10 mL) then stored at -20°C until metabolite measurements.

Pectoralis major muscle trait analyses

Lactate, free glucose, glucose from glycogen and glucose-6-phosphate were measured according to Dalrymphe and Hamm (1973). The glycolytic potential was calculated using the equation of Monin and Sellier (1985): glycolytic potential = 2 x [glycogen + glucose-6-phosphate + free glucose] + lactate. The mean cross

sectional area (CSA) of muscle fibres was determined as described by Rémignon et al. (1995) on 12 µm thick-cross sections stained with red azorubin. For 40 birds diverging for fibre CSA, the proportion of connective tissue was quantified by image analysis using the Visilog software (Noesis, France). For 54 animals diverging for fibre CSA and/or pH at 15 min *post mortem*, the occurrence of giant fibres was determined by using a micrometric ocular that comprises 25 intersection points. For each sample, we classified a total of 1000 intersection points in either 'normal fibre', 'giant fibre' or 'connective tissue' to assess the ratio giant fibres/normal fibres.

Meat trait measurements

Fifteen minutes after slaughter, pH of the right PM muscle (pH15) was recorded according to the 'iodoacetate reference method' described by Santé and Fernandez (2000). At this time, the muscle temperature was also checked. Twenty four hours *post mortem*, carcasses were dissected and measured for breast (*pectoralis major and minor*) weight and yield (calculated in relation to body weight). At this time, the PM muscle ultimate pH (pHu) and colour parameters (L*, a*, b*) were measured according to Berri et al. (2001). The upper half part of the PM muscle was hanged in plastic bag to determine drip loss during a 2 day-storage at 2°C (Debut et al., 2003). At 3 day post-mortem, the PM muscle was vacuum packaged, rapidly frozen in ethanol then stored at -20°C until thawing and cooking treatments. Thawing-cooking loss (One night at 4°C and 15 min at 85°C) was determined as: (muscle weight after cooking/muscle weight before freezing) x 100. The Warner-Bratzler maximal shear force (N/cm^2) was measured on cooked muscle samples (1 x 1 x 3 cm). Samples were sheared perpendicular to the longitudinal orientation of fibres using an Instron Universal Testing Machine with a triangular blade (height = 5.2 cm, width = 6.1 cm, thickness = 0.11 cm; speed = 80 mm/min).

Statistics

To assess the relationship between muscle fibre CSA and the other traits, phenotypic correlations were analysed with the CORR procedure of SAS (SAS Institute, 1989). Muscles were also classified in 5 classes of equal numbers according to their mean fibre CSA (RANK procedure of SAS) and a one-way analysis of variance was performed to test the effect of fibre CSA classes on other muscle traits (GLM procedure of SAS). Then, means were compared using a Newman-Keuls test for multiple mean comparisons. A multiple regression test was performed to assess the relative involvement of fibre CSA or muscle pH on the water retention and textural properties of breast meat. Finally, the genetic correlations between fibre CSA and other muscle traits were estimated by the REML (Restricted Maximum Likehood) methodology using the VCE software (Neumaier and Groeneveld, 1998) on a total of 600 birds born of 15 males and 64 females.

Results and discussion

Relationship between the fibre CSA and other muscle traits

The fibre cross sectional area (CSA) was highly phenotypically related to body weight (+0.51) and breast muscle weight (+0.65) and yield (+0.51). The increase in fibre CSA did not affect the proportion of connective tissue (about 21% of the muscle cross section surface) and did not induce fibre necrosis as it has been previously reported in turkey (Sosnicki et al., 1998). According to both the phenotypic and genetic correlations (table 1), as the fibre CSA increased the glycogen reserve of muscle before death (glycolytic potential) decreased. As a consequence, PM muscles with the largest fibres exhibited the highest ultimate pH. Besides, they contained the lowest lactate at 15 minutes *post mortem* and thus exhibited the highest pH15. As a consequence of their *post mortem* metabolism, PM muscles with the largest fibres exhibited the lowest L*, drip and thawing-cooking losses and were more tender after cooking. According to the multiple regression test (table 2), the drip loss and L* of meat appeared chiefly determined by the pHu and at a lower extent by the pH15 of muscle. By contrast, the properties of cooked meat (thawing-cooking loss and shear force) were also partly determined by the fibre CSA and therefore the muscle weight. The analysis of variance by class of fibre CSA confirmed that breast meat traits were greatly affected by muscle hypertrophy (table 3). Indeed, the drip and thawing-cooking losses as well as the maximal shear force of cooked meat were respectively 30% and 16% lower for breast from the highest fibre CSA class (average fibre CSA of 2447 μm^2) than for breast from the lowest fibre CSA class (average fibre CSA of 1257 μm^2).

Occurrence of giant fibres in relation to muscle fibre CSA and rate of pH fall

According to our observations, the occurrence of giant fibres in breast muscle was chiefly determined by the rate of pH fall (Table 4). Whatever the muscle weight and fibre diameter, the occurrence of giant fibres greatly increased when muscle pH15 was below 6.30. However, in muscles with pH15 above 6.30, the proportion of muscles in which giant fibre occurred was dependant of the fibre size. It was greater in muscles exhibiting large fibre CSA than in muscles exhibiting small fibre CSA. Therefore, the occurrence of giant fibres would be more sensitive to pH fall rate in muscles with large fibres than in muscle with small fibres.

Conclusions

According to the present study, the fibre diameter is closely phenotypically and genetically related to the overall and muscle growth of broilers, the increase in breast muscle weight resulting essentially from fibre hypertrophy. We reported that muscle hypertrophy did not alter overall breast muscle structure but induced a significant decrease in muscle glycogen reserve before death and lowered the rate of muscle pH fall *post mortem*. These changes resulted in breast meat with higher pH15 and pHu, and consequently a better water holding ability and a greater tenderness after cooking. In conclusion, this study did not evidence any genetic antagonism between growth rate or muscle development and breast meat quality and suggested that meat of current broiler genotypes selected for growth and breast yield are well adapted to further processing.

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Table 1: Phenotypic and genetic correlation between the fibre CSA and other PM muscle traits (n = 600).

	Correlation with fibre CSA	
	phenotypic	genetic
Glycolytic potential	-0.25	-0.52
Lactate (15 min *post mortem*)	-0.27	-0.41
pH (15 min *post mortem*)	0.38	0.40
pHu	0.22	0.61
L*	-0.27	-0.41
a*	0.08	-0.12
b*	0.002	-0.42
Drip loss	-0.24	-0.44
Thawing-cooking loss	-0.38	-0.63
Maximal shear force	-0.31	-0.64

Table 3: Growth and PM muscle traits in relation to fibre CSA (n = 600).

	Class 1 1257µm^2	Class 2 1582µm^2	Class 3 1812µm^2	Class 4 2048µm^2	Class 5 2447µm^2	*P*
Body weight (g)	1861^{d}	2025^{c}	2208^{b}	2248^{b}	2328^{a}	***
PM weight (g)	118.8^{e}	139.0^{d}	151.2^{c}	159.3^{b}	170.6^{a}	***
Breast yield (%)	16.5^{d}	17.6^{c}	17.7^{c}	18.4^{b}	18.8^{a}	***
Lactate (15 min; µM/g)	36.1^{a}	34.8^{a}	34.1^{a}	31.3^{b}	28.9^{b}	***
Glycolytic potential (µM/g)	111.6^{ab}	113.8^{a}	107.9^{bc}	105.0^{cd}	102.4^{d}	***
T° (15 min)	38.0	38.4	38.3	38.5	38.4	ns
pH (15 min)	6.39^{d}	6.42^{c}	6.44^{c}	6.48^{b}	6.53^{a}	***
pHu	5.62^{b}	5.61^{b}	5.65^{a}	5.66^{a}	5.68^{a}	***
L*	55.9^{a}	55.5^{a}	55.2^{a}	54.3^{b}	53.8^{b}	***
a*	-0.87	-0.89	-0.79	-0.79	-0.70	ns
b*	11.7	11.9	11.8	11.9	11.8	ns
Drip loss (%)	1.89^{a}	1.77^{b}	1.56^{b}	1.36^{c}	1.33^{c}	***
Thawing-cooking loss (%)	17.5^{a}	15.5^{b}	14.9^{b}	12.9^{c}	12.4^{c}	***
Maximal shear force (N/cm^2)	15.7^{a}	15.2^{b}	14.5^{b}	14^{c}	13.2^{d}	***

Means with different letter in the same row differ (P<0.05); ns = non significant; ***P<0.001

Tableau 2: Multiple regression test (n = 600).

Dependant variables	Partial R^2			Total R^2
	pHu	pH 15	AST	
Drip loss	0.168	0.089	0.001	0.293
	***	***	ns	
	pHu	pH 15	AST	
L*	0.374	0.070	0.002	0.446
	***	***	ns	
	AST	pHu	pH 15	
Thawing-cooking loss	0.145	0.081	0.062	0.289
	***	***	***	
	pHu	AST	pH15	
Maximal shear force	0.112	0.058	0.016	0.186
	***	***	***	

ns = non significatif ; ***P < 0.001

Table 4: occurrence of giant fibre (% GF) in relation to muscle fibre CSA and pH15 (n = 54).

Fibre CSA	pH15	% GF	N^{a}
1238 µm^2	pH < 6.30	3.59	80%
	6.30 < pH < 6.40	0.38	11%
	pH > 6.50	0	0%
2147 µm^2	pH < 6.30	3.92	83%
	6.30 < pH < 6.40	1.14	40%
	pH > 6.50	0.52	30%
CSA effect		ns	
pH15 effect		***	
CSA x pH15 effect		ns	

ns = non significant; ***P<0.001
[a]N = percentage of muscles in which giant fibre occurred

SEMIQUANTITATIVE DETERMINATION OF GOAT TISSUE IN MEAT PRODUCTS BY MEANS OF PCR

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Background

The "Quantitative Ingredient Declaration" (QUID) for important components in food products was put into force as EU-regulation. Therefore for meat products reliable methods for species identification and quantification gained importance. In a previous paper a species-specific primer system BC290501 for the identification of goat in meat products was presented (Altmann et al., 2004). Both primers bind in the 5´-flanking promotor region of the nuclear single-copy gene *beta-casein.* Twelve different species were tested, but only goat-DNA leads to amplification of a specific product by means of PCR.

At the moment PCR systems for quantification of animal tissue in food or feed are only available for the commercially most important species cattle and pig (Wolf and Lüthy 2001; Calvo et al., 2002; Palisch et al., 2003; Frezza et al., 2003) but not for minor relevant species like goat and sheep.

Objectives

In this paper we report about a PCR assay using two different detection systems based on the primer system BC29051 for semiquantitative determination of goat meat in meat products.

Methods

DNA-extraction was carried out applying the CTAB method optimised for meat and meat products (Binke et al., 2003). Emulsified type sausages with varying goat meat content (Tab. 1) filled into cans with a volume of 50 ml were heated under different temperature regimes: home made cans "KK" (20 min/82 °C; $F_C < 0.9$), "normal" cans "VK" (33 min/116 °C; $F_C = 3.4$), up to cans for use under extreme conditions "TK" (60 min/116 °C; $F_C = 12.3$).

Tab. 1: Composition of emulsified type sausages

Reference product	Ingredients	Goatmeat / muscle meat [%]
Sausage 1	50 % goat meat; 25 % oil; 23 % ice; 1.5 % salt; 0.25 % spices; 0.25 % additives (phosphate; ascorbate)	100
Sausage 2	50 % goat meat; 25 % fat (pork); 23 % ice; 1.5 % salt; 0.25% spices; 0.25% additives (phosphate; ascorbate)	100
Sausage 3	25 % goat meat; 25 % pork meat; 25 % fat (pork); 23 % ice; 1.5 % salt; 0.25 % spices; 0.25 % additives (phosphate; ascorbate)	50
Sausage 4	10 % goat meat; 40 % pork meat; 25 % fat (pork); 23 % ice; 1.5 % salt; 0.25 % spices; 0.25 % additives (phosphate; ascorbate)	20
Sausage 5	1 % goat meat; 49 % pork; 25 % fat (pork); 23 % ice; 1.5 % salt; 0.25 % spices; 0.2 5% additives (phosphate; ascorbate)	2.0

The goat specific primers BC290501 F (5´ TCTGGTCCAATTGGTGAGAG 3´) and BC290501 R (5´ AGGCCACAGGTGAAAAAGTC 3´) were commercially synthesized by Qiagen (Hilden, Germany). The amplification products were detected by means of a dual labeled probe BC290501 P (FAM-5´AGGGAAATGTTGAATGGGAAGGATATGC 3´-Tamra) as well as by the intercalating dye SYBR-

Green 1. A 97 bp animal specific DNA fragment based on the myostatin gene, designed by Laube et al., (2002) and a dual labeled probe were used as a reference system for relative quantification.
The amplification of the DNA fragments was performed by Real-Time PCR in a Rotor Gene 2000 with vials containing a final volume of 20 µl: 1x reaction-buffer (Qiagen), 4.5 mM magnesium chloride, 0.05 mM of each dNTP, 0.8 µM of each primer, 0.4 µM dual labeled probe (Qiagen) or 1 µL SYBR-Green 1 diluted 3000 fold, 1.25 unit of HotStarTaq™ DNA-polymerase (Qiagen) and 5 µl DNA solution diluted 40 fold.
The applied PCR-program was the following (40-45 cycles): Initial DNA-denaturation at 95 °C for 15 min; 95 °C for 30 s, 58 °C for 30 s, 65 °C for 30 s for the probe detection system and 72°C for 30 s for SYBR-Green 1 system.
The determination of relative amounts of goat meat per total meat in meat products was calculated according to the equation based on the delta-delta C_T method modified by Pfaffl (2001). Sausage 4 containing 20 % goat meat with a F_C- value at < 0.9 was used as calibrator (tab. 1).

Results and Discussion

For relative quantification it is necessary to have a reference gene which has a constant efficiency for each commercially relevant animal species. DNA from muscle tissue of 12 animal species (Fig. 1) was extracted and diluted under the same conditions.
Figure 1 shows that the most aminal species like goat, pig, cattle have a comparable course of amplification using the myostatin gene. For these animal species the 97 bp fragment of the myostatin gene is suitable as reference gene. In contrast to this the course of amplification in the case of duck, ostrich and kangaroo is different.

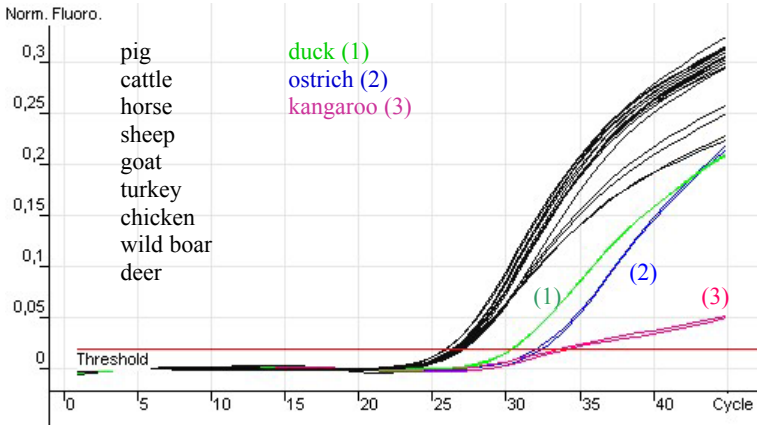

Fig. 1: Course of amplification of the 97 bp myostatin gene fragment of 12 commercially relevant animal muscle tissues

The reaction efficiency is the second critical point for quantification. The efficiency of amplification was calculated for the myostatin and beta-casein systems by means of standard curves (Fig. 2 and 3). A standardized DNA solution (10 µg/ml) obtained from goat meat was 4-fold diluted (2.50, 0.63, 0.16, 0.04 and 0.01 µg/ml). Amplification with a reaction efficiency of 2 means a doubling of amplification product for each cycle resulting in a standard curve graph slope of -3.322. Figure 2 and 3 show a comparable optimum reaction efficiency over 3 magnitudes for the 97 bp myostatin reference gene fragment and the 161 bp goat specific beta-casein gene fragment. A significant difference of efficiency in comparison of the probe and SYBR-Green 1 - systems was not detectable.

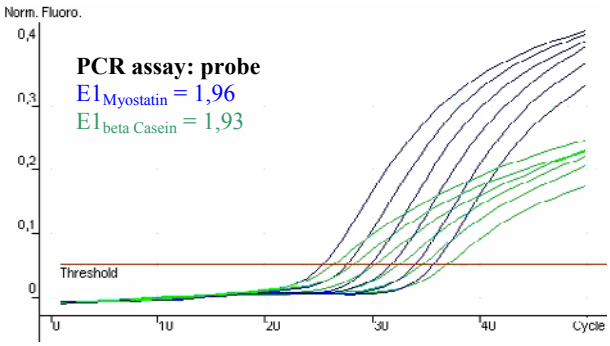

Fig. 2: Determination of reaction efficiencies for the probe assay

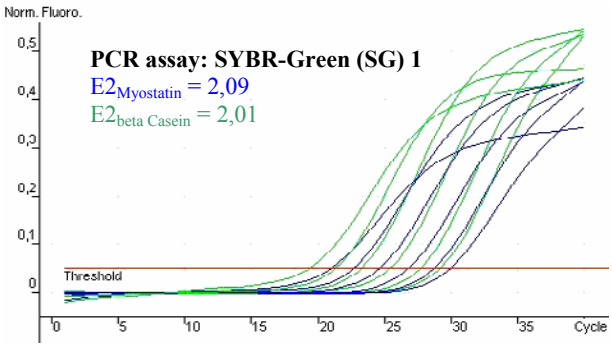

Fig. 3: Determination of reaction efficiencies for SYBR-Green 1 assay

The obtained reaction efficiencies for both assays were applied to determine the content of goat meat in reference products (Tab. 2 and 3). The data show that the probe assay represents a better comparability of the real and calculated content of goat meat than the SYBR-Green 1 assay with a mean coefficient of variation (CV) of 20 %.

Furthermore sausage 2 containing 25 % pork fat shows a lower goat meat content than sausage 1 prepared without pork fat (tab. 1 and 2). Fat tissue contains also DNA like other animal tissues and leads to an increase of total DNA copy number. For this reason the relative content of goat meat is decreasing in comparison to the total meat content. This effect is especially important for the quantification of animal species in liver sausages as liver shows an extremely high DNA content, which is at least tenfold higher than in muscle tissue. In contrast to this the DNA content of fatty tissue is approximately only a quarter in comparison to muscle meat.

With respect to this fact applying this assay a semiquantitative determination of goat meat in meat products is possible for sausages treated at low or medium heating conditions (Tab. 2 and 3).

Tab. 2: Contents of goat meat in reference sausages with standard deviations calculated according to the equation of Pfaffl (2001) in the case of the probe assay (N = 4)

Assay probe	theoretical [% goat]	calculated [%] unheated	calculated [%] KK[1]	calculated [%] VK[2]	calculated [%] TK[3]
Sausage 1	100	103 ± 25	95 ± 25	57 ± 12	29 ± 3
Sausage 2	100	85 ± 7	83 ± 10	60 ± 14	27 ± 7
Sausage 3	50	37 ± 10	43 ± 9	28 ± 6	15 ± 2
Sausage 4	20	20 ± 4	24 ± 2	10 ± 1	8 ± 1
Sausage 5	2	2.3 ± 0.2	2.1 ± 0.1	1.1 ± 0.3	0.3 ± 0.2

Tab. 3: Contents of goat meat in reference sausages with standard deviations calculated according to the equation of Pfaffl (2003) in the case of the SYBR-Green (SG) 1 assay (N = 4)

Assay SG 1	theoretical [% goat]	calculated [%] unheated	calculated [%] KK[1]	calculated [%] VK[2]	calculated [%] TK[3]
Sausage 1	100	129 ± 25	161 ± 22	97 ± 23	63 ± 12
Sausage 2	100	112 ± 27	124 ± 21	98 ± 8	48 ± 4
Sausage 3	50	66 ± 7	73 ± 23	50 ± 12	31± 13
Sausage 4	20	19 ± 2	29 ± 4	14 ± 3	10 ± 2
Sausage 5	2	3.5 ± 1.8	2.6 ± 0.2	1.3 ± 0.2	1.5 ± 1.1

[1]KK = home made cans (F_C-value < 0.9 means 20 min at 82 °C), [2]VK = "normal" cans (F_C-value = 3.4 means 33 min at 116 °C), [3]TK = cans treated for use under extreme conditions (F_C-value = 12.3 means 60 min at 116 °C)

Reference samples heated under extreme conditions are resulting in a significantly reduced content for each sausage. Using the probe assay for samples heated at F_C = 3.4 (VK) a content of about 50 % of the added amounts was determined, whereas samples heated at F_C = 12.3 (TK) showed only about 30 % mean goat meat content (tab. 2). The decreasing content of goat meat is caused by an increase of DNA fragmentation (Binke et al., 2003). Applying a modified myostatin gene fragment with a length of 154 bp (Laube et al., 2002) instead of 97 bp a semiquantitative determination of goat meat in meat products heat treated up to a F_C-value of 3.4 (VK) is also possible. However, the latter system is also not able to quantify the animal species turkey and chicken.

Conclusion

The presented PCR assays are suitable for a semiquantitative determination of goat meat in meat products. A quantitative determination with a small error of determination is only possible if there is sufficient knowledge about the quality and purity of the DNA extracted from processed products. Therefore quantification procedures demand suitable reference standards with a comparable composition and procedure. Further critical points are the specific variations caused by the thermocycler and the DNA extraction as well as the DNA polymerase and the applied dye.
In summary: A quantitative analytical determination of meat (here goat) content in processed food for control of the QUID regulation is not yet possible and needs further studies.

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PARAMETERS MEAT QUALITY OF BROILERS FROM DIFFERENT REARING SYSTEMS[1]

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Addresses

Background

The share of certain tissues in major basic carcass parts is no doubt an important element determining broiler meat quality *(Karan-Djuric et al., 1977).*
The quality trait mentioned and also carcass quality are affected by a number of factors. Of the biological ones, the greatest effect was exerted by genotype, sex and age *(Preston et al., 1973, Antonijevic et al., 1982, Orr and Hund 1984 etc.).* Among numerous non-genetic factors that may considerably influence meat quality, the rearing systems, or broiler keeping systems have been particularly stressed by some authors over the past years *(Bogosavljevic-Boskovic et al., 1999, Pavlovski et al., 2001, Milosevic et al., 2003)*. The authors also maintained that intensive industrial production in poultry farming results, among other things, in lower product quality, due to which there is an increasing number of advocates of non-industrial broiler rearing system among not only researchers and consumers but also among producers, particularly with the aim of producing better quality meat.

Objectives

The aim of this paper was to analyze the meat quality of the broilers reared in two different non-industrial ways of rearing (extensive rearing in a chicken pen and free-range rearing). The basic tissues shares in major broiler carcass parts serving as quality parameters were examined. The aim of the paper was a comparative analysis of the broiler meat quality trait mentioned in terms of the effect of the non-industrial rearing systems used.

Materials and methods

The initial experimental material consisted of 200 one-day-old chicks of the line hybrid Hybro G. The fattening of the broilers lasted 56 days. In the first four weeks the rearing was conducted within the same construction with the deep floor covering. Then, at 28 days, the experimental chicks were divided into two groups. One group was reared within a closed object, in a chicken pen, at population density of 12 broilers per square metre (under the extensive production conditions in the chicken pen) - according to the European Union regulations on non-industrial poultry meat production, mentioned by *Tolimir and Masic 2000, as well as by Ristic 2003*. The second group was provided with the same useful area within the closed construction, but there were outlets overgrown with grass for these chickens, too. The free-range area size was such to provide each chicken with 1 square metre of the area. The trial chickens were fed two appropriate complete feed mixtures: the initial one (up to 28th day) and the final one (from the 28th to the 49th day of fattening). In the last week of the fattening period, the chicks diet consisted of 70 % ground corn mixture and about 30 % of concentrate mixture.
Following the fattening period, with the aim of examining major meat quality characteristics, 12 broilers were chosen at random from each experimental group. The dressed and cooled carcasses were then dissected into basic parts (breasts, thighs, drumsticks, wings, pelvis and back). After the dissection, for the purpose of determining the yield and share of basic tissues (muscles, bones and skin) in major carcass parts, the breast and right thigh dissections were made.
The research data analysis was made by using the usual variation statistics methods. The testing of the significance of differences was conducted through the following mathematical variation analysis model:

$$Y_{ijk} = \mu + S_i + (SR)_{ij} + e_{ijk}$$

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that is, the model corresponding to the two-factor experimental plan 2x2 (2 sexes -S and 2 systems of rearing -SR).

Results and discussion

Table 1 shows values of slaughter yields and shares of basic tissues in both hens and cocks reared in two different non-industrial rearing systems (I group – in the free-range rearing and the II one – in the extensive chicken-pen rearing system).

T1

From table 1 one can see a somewhat higher muscular tissue share in breast mass in both hens and cocks of the first experimental group (free-range rearing) compared to the broilers reared extensively, in the chicken-pen (II experimental group). A somewhat higher bone share was registered with the chick of the other trial group. Skin share in the breast mass ranged from 10.96 (males of the II trial group) to 13. 53 % (females of the same group). However, from the point of view of both meat share and bone and skin share in breast mass, the emerged differences between both sexes and the rearing systems investigated were not significant.
The mentioned study results on the participation of basic tissues in broiler breasts are in between the results for free-range-reared chicks by *Bogosavljevic-Boskovic et al. (1999) and Milosevic et al. (2003).*
Table 2 presents the yields and shares of basic tissues in drumstick mass of the males and females investigated.

T2

Based upon the table 2 data it could be concluded that a somewhat higher muscular tissue share in the drumstick mass was recorded in the I experimental group chicks. Furthermore, a somewhat higher participation of the muscular tissue in the basic carcass part mentioned was registered with the females in both rearing systems. However, the emerged differences were not statistically significant neither from the point of view of the sex influence, nor from the viewpoint of the effect of the rearing systems investigated. Further data in table 2 show that the bone share in the experimental broiler drumsticks ranged from 23.52 (females of the I experimental group) to 28.21 % (males of the I investigated group). The data also indicate that the lowest and highest skin shares in drumstick mass were registered with the males fattened extensively in the chicken-pen (9.30%) and with the free-range-reared males (10. 89 %), respectively. However, the differences in terms of both the bone share and the skin share in the drumstick mass between the trial broilers were also not significant.
The established relative values of the occurrence of muscular tissue in drumstick mass were somewhat higher than the results of *Bogosavljevic-Boskovic et al. (1999)* and were in accordance with the results of *Milosevic et al. (2003).*

Conclusions

Based upon the study results on the basic tissue shares in major carcass parts of broilers reared in two different non-industrial rearing systems (I group – rearing using free-range and II – extensive rearing in the chicken-pen), the following may be inferred:

- The free-range-reared broilers had a somewhat higher meat share in breast mass and drumstick mass compared to the ones reared extensively in the chicken-pen.
- A somewhat higher meat share in drumstick mass was recorded with the females than with the males, whereas the other differences in terms of the sex effect were also slight.
- The analysis of the significance of the emerged differences in terms of the effect of the rearing systems investigated and the sex of broilers on the share of certain tissues in major carcass parts has shown that the differences were not statistically significant.

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Table 1. Percentage of meat, skin and bone in breast(%)

Experimental group	Sex		Breast	Meat		Bone		Skin	
			g	g	%	g	%	g	%
I	Male	$\bar{x}$	818,98	615,20	75,09	99,58	12,32	99,44	12,01
		S_d	65,80	61,03	4,05	23,95	3,67	34,75	3,38
		C_V	8,03	9,92	5,39	24,05	29,79	34,94	28,14
	Female	$\bar{x}$	728,50	560,16	76,78	77,44	10,77	86,28	11,83
		S_d	71,99	67,48	2,97	10,63	1,85	22,54	2,75
		C_V	9,88	12,04	3,87	13,67	17,18	26,12	23,24
II	Male	$\bar{x}$	752,46	563,38	74,58	103,0	13,89	81,74	10,96
		S_d	100,82	99,46	3,72	23,49	3,61	7,32	1,23
		C_V	13,40	17,65	4,99	22,80	25,99	8,95	11,22
	Female	$\bar{x}$	695,52	506,16	72,67	89,90	12,99	94,06	13,53
		S_d	77,21	63,59	1,49	10,23	1,46	17,66	1,94
		C_V	11,10	12,56	2,05	11,38	11,24	18,77	14,34
	Fexp	F_1		2,52	2,58	0,90	2,23	0,23	0,08
		F_2		2,83	0,01	4,54*	0,94	0,00	1,17
		F_{12}		0,00	1,56	0,28	0,07	1,56	1,56

Table 2. Percentage of meat, skin and bone in drumstick(%)

Experimental group	Sex		Drumstick	Meat		Bone		Skin	
			g	g	%	g	%	g	%
I	Male	$\bar{x}$	189,38	119,56	63,14	46,84	24,80	20,76	10,89
		S_d	18,82	13,63	3,61	7,32	3,73	4,96	1,93
		C_V	9,94	11,40	5,72	15,63	15,04	23,89	17,72
	Female	$\bar{x}$	156,00	101,18	64,80	36,50	23,52	16,54	10,52
		S_d	10,02	8,75	2,39	4,09	3,46	4,23	2,16
		C_V	6,42	8,65	3,69	11,20	14,71	25,57	20,53
II	Male	$\bar{x}$	182,78	113,00	62,46	51,20	28,21	17,28	9,30
		S_d	26,84	18,47	3,84	6,58	3,56	5,32	1,66
		C_V	14,68	16,34	6,15	12,85	12,62	30,78	17,85
	Female	$\bar{x}$	156,02	100,46	64,28	37,52	24,04	16,74	10,68
		S_d	9,56	5,89	3,03	4,81	2,68	3,34	1,70
		C_V	6,13	5,86	4,71	12,82	11,15	19,95	15,92
	Fexp	F_1		0,42	0,17	1,06	1,70	0,66	0,74
		F_2		7,49**	1,42	21,11**	3,25	1,38	0,36
		F_{12}		0,27	0,01	0,41	0,91	0,83	1,09

STUDIES ON CHANGES OF SOME SELECTED PORK MEAT QUALITY TRAITS IN RELATION TO THE TIME AFTER SLAUGHTER

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Background

One trait, which can be used to characterise meat technological quality, is meat pH measured 45 minutes after slaughter. This measurement is employed to reveal meat quality defects and is highly correlated with meat colour (Briskey 1964).
For many years two main pork meat quality defects have been discussed in literature, namely watery meat and acid meat. Their occurrence is associated with genetic factors and is attributed to the presence of RYR1 and RN^- genes (Fujii et al. 1991, Monin and Sellier 1985).
Meat colour is an important trait to be taken into account when assessing the commercial meat quality. With regard to the subjective meat colour assessment, the greatest attention is paid to its lightness, which is affected by the optical properties of the surface meat layer.

Objectives

The objective of the performed investigations was to determine changes in colour lightness, pH and electrical conductivity ascertained at different times after slaughter in the *longissimus lumborum* muscle *(m. LL)* in swine carcasses with normal and watery meat.

Materials and methods

The experimental material consisted of 1050 pork carcasses derived from different swine genotypes.
Within 45 minutes after stunning, pH was determined in the LL muscle at the level of the 1^{st} – 2^{nd} lumbar vertebra with the aim to select experimental carcasses. From the population, 25 carcasses with PSE meat (pH_{45} < 6.0) and 25 carcasses with normal meat (pH_{45} 6.50 – 7.00) were selected randomly for further investigations (Kortz 2001, Borzuta et al 1975).
In the LL muscles of both groups of carcasses pH values, electrical conductivity (LF) and colour lightness (L) measurements were performed at the following times after pig stunning: about 45 minutes and 2, 3 and 24 hours.
The values of pH were determined using the Radiometer PHM 80 Portable pH-meter equipped in a complex electrode, whereas electrical conductivity was measured using a pork meat quality tester of MT-03 type manufactured in Poland.
Meat colour lightness was determined in the *LL muscle* after cutting out a slice at the following times *post mortem*: 45 minutes and 2, 3 and 24 hours. The above-mentioned measurements were performed using the Minolta Chroma Metter CR 300 apparatus (Oziembłowski and Grashorn 1997).

Results and discussion

The pH values determined in the group of pigs with normal meat at the examined times after slaughter indicate proper acidification of the muscle tissue (Tab.1). Two and three hours after stunning pH values dropped, on average, by 0.37 unit (pH=6.36), while 24 hours after slaughter – by 0.91 unit. The obtained final pH_{24} – 5.82 value of these muscles indicates the proper process of glycolysis as confirmed by numerous publications (Lambooij et al. 2004, Bertram et al 2003, Kortz 2001).
The examined muscles were characterised by low electrical conductivity ranging from 2.93 to 3.45 mS, which was found not to be dependent on the measurement time after slaughter. The electrical conductivity obtained for this group of carcasses was typical for normal meat (Koćwin-Podsiadła et al 1998).
Furthermore, in this group of carcasses no significant differences were found between the meat colour lightness measured 45 minutes as well as 2 and 3 hours after slaughter. However, a significant brightening of the colour was observed 24 hours after slaughter.
In the group of carcasses with watery meat, the pH value remained on a similar level 2, 3 and 24 hours after slaughter (pH 5.64 to 5.83). The pH value determined 45 minutes after stunning was only slightly higher (by

0.19 unit). These observations are corroborated by data reported by Simek et al. (2004), who also did not record significant differences between the pH_{45} and pH_{24}. On the other hand, significant differences were found between the degree of tissue acidification of normal and PSE meat 45 minutes and 2 and 3 hours after slaughter. However, 24 hours after slaughter pH values of both groups of muscles were quite similar.
The analysis of values of electrical conductivities of the PSE meat revealed its significant increase during the initial hours after slaughter changes. An increase in the electrical conductivity of 3.10 mS was recorded in the period from 45 minutes to 2 hours after slaughter. On the other hand, between the second and third hour after slaughter only a slight, but statistically significant, increase in electrical conductivity (by 0.95 mS) was observed. In the period from 3 hours to 24 hours after slaughter, a significant drop in the LF of 2.61 mS was observed. Nevertheless, the difference in the electrical conductivity between normal and PSE meat became quite conspicuous already 45 minutes after slaughter (3.45 and 7.41 mS, respectively), which indicates that this measurement can be very useful for the identification of PSE meat already 45 minutes after slaughter. Also LF measurements 2, 3 and 24 hours after slaughter were significantly different between the two groups of carcasses.

45 minutes after slaughter the colour of PSE meat was uneven and pale with the colour brightness reaching 43.2%. The colour became much lighter in the following hours. In the period from 45 minutes to two and three hours after slaughter, a significant increase of the light reflection, on average by 8.6%, was recorded and 24 hours after slaughter this value increased by 11.5%, in relation to the colour lightness measured 45 minutes after slaughter. The PSE meat 24 hours after slaughter was usually pale and exudative on the entire slice surface.
During the analysed periods of time the percent of light reflected from the watery meat tissue was higher and differed significantly when compared with normal meat (<0.01). The recorded differences were as follows: after 45 min. – 4.08%, after 2 and 3 hours – 13.35%, after 24 hours – 8.26%. It is evident from the above-presented data that the measurement of colour 45 minutes after slaughter was not a good indicator for the identification of PSE meat as differences in the colour brightness were the smallest at this point in time. However, this indicator can certainly be used effectively already 2 hours post mortem.

Conclusions

- The pH of PSE meat after 45 minutes as well as 2, 3 and 24 hours after slaughter exhibited almost similar values, whereas in the case of normal meat, a gradual acidification of tissue occurred.
- The electrical conductivity of PSE meat increased the 3rd hour after slaughter, while that of normal meat remained at similar level during the entire 24-hour period. It was stated that the measurement of electrical conductivity 45 minute after slaughter could serve as a good parameter identifying PSE meat.
- The colour lightness of PSE meat, from the 2nd hour post mortem, increased progressively, but measured after 45 minutes was not a good indicator of PSE meat. In normal meat, colour lightness remained on a similar level during the period from 45 minutes to 24 hours after slaughter.

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Table 1. Measurement results of pH, LF and colour lightness L (%) in LL muscle

Time after slaughter	Normal meat						PSE meat						Significant differences		
	pH		LF		L		pH		LF		L		pH	LF	L
	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD			
45 min	6,73^{A}	0,17	3,45	0,71	39,12^{A}	1,53	5,83	0,13	7,41^{A}	2,55	43,20^{A}	5,11	**	**	**
2 h	6,35^{B}	0,27	3,18	0,43	38,70^{A}	1,49	5,67	0,14	10,51^{B}	3,59	51,11^{B}	4,99	**	**	**
3 h	6,37^{B}	0,26	2,93	0,61	38,29^{A}	2,57	5,66	0,10	11,46^{C}	3,36	52,59^{B}	4,45	**	**	**
24 h	5,82^{C}	0,18	3,36	0,84	46,74^{B}	4,69	5,64	0,11	8,77^{D}	2,58	54,70^{C}	4,24	ns	**	**

** - significant differences between normal and PSE meat at $P \leq 0,01$
A,B,C,D - significant differences between time of measure at $P \leq 0,01$
ns - non-significant differences

THE EFFECT OF STORAGE ON THE OXYGEN CONSUMPTION RATE OF BOVINE M. *LONGISSIMUS DORSI* AND M. *PSOAS MAJOR*

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Background

Colour is the most important factor determining acceptability of beef steaks. Consumers associate a bright cherry red colour with quality. The *longissimus dorsi* (LD) and *psoas major* (PM) muscles exhibit very different colour stabilities. The LD is considered to be a colour stable muscle with a colour shelf life of 4 - 5 days, while the PM has a colour shelf life of 1 - 1.5 days and is thus regarded as being very colour unstable (O'Keeffe and Hood, 1982; Isdell *et al.,* 1999). The oxygen consumption rate (OCR) influences colour stability by altering the depth at which the metmyoglobin (brown) layer forms. When the OCR is high, oxygen does not penetrate far into the meat, the metmyoglobin layer that forms at the limit of penetration will be near the surface, and colour deteriorates rapidly as this thickens and reaches the surface (Madhavi and Carpenter, 1993).

Objectives

The objective was to determine if time post mortem has an affect on the OCR of bovine LD and PM muscles. A secondary objective was to determine whether the OCR is affected by anatomical location within the muscle and whether there is variability within individual steaks.

Materials and methods

M. *longissimus dorsi* and M. *psoas major* (n=6) (pH 5.4 - 5.8) were excised from steers (≤ 36 months) at 48h post mortem at a commercial meat plant (Kepak Group, Clonee, Ireland). A steak (20 - 25mm thick) was cut from three areas of each muscle – posterior, centre and anterior to account for variability within muscle. The steaks were then placed in laminated retail polystyrene trays (Linpac 2-37 EPS) and overwrapped with a high oxygen permeable film (OTR: 20,000cm^3 m^{-2} $24hr^{-1}$ atm^{-1}). All steaks were stored in a cooled incubator (LMS, Davidson & Hardy Ltd, Ireland) at 4 ± 0.5ºC for 2 hours in the dark to allow them to bloom. The remaining portion of each muscle was vacuum packed (20/70 PA/PE; OTR: 40-50cm^3 m^{-2} $24hr^{-1}$ atm^{-1} at 23ºC, 75% RH) and stored at 0 ± 0.5ºC in a coldroom until 4, 7, 14 and 21 days post mortem. The temperature in the coldroom and incubator was recorded every 5 minutes using a Tinyview temperature logger (Gemini Data Loggers (UK) Ltd, Chichester, UK).

Bloomed steaks were vacuum packed (20/70 PA/PE; OTR: 40-50cm^3 m^{-2} $24hr^{-1}$ atm^{-1} at 23ºC, 75% RH). To account for any variability within the steaks, circles (3 for the LD and 2 for the PM) were marked on each pack and labelled 'a', 'b' and 'c' so that colour readings during storage could be taken at the same places. Reflectance spectra (360-750nm at 10nm intervals) were taken after 0, 10, 20, 30, 40, 50, 60 minutes in a vacuum pack using a HunterLab UltraScan™ XE spectrophotometer (Hunter Associates Laboratory, Inc. Reston, USA). During this time the samples were held in the incubator at 4 ± 1ºC in the dark, except while reflectance measurements were being taken. Reflectance values were used to determine the proportions of the three colour pigments (oxymyoglobin, myoglobin and metmyoglobin) present, using the method described by Kryzwicki (1979). Reflectance values at wavelengths not given by the instrument (473, 525 and 572nm) were calculated using linear interpolation. The OCR was determined by following the pigment changes that occur since oxygen, present as oxymyoglobin would be converted to myoglobin or metmyoglobin after vacuum packaging (Madhavi and Carpenter, 1993). The OCR was expressed as 'oxymyoglobin converted to myoglobin after 10 minutes in vacuum'.

Statistical analysis

At each storage time the effects of anatomical location and within steak location on the percentage of each form of myoglobin and on the OCR were tested using a tow-way ANOVA using SYSTAT (Systat Inc. Illinois, USA). For OCR, the difference between the two muscles was tested in a one-way ANOVA.

Results and discussion

There was no significant difference in oxygen consumption rate as a result of positional effects within the muscle or within the steak for the LD at each time post mortem. This is in agreement with Young, Priolo, Simmons and West (1999) who found no significant positional effects for bovine LD pieces. There was no significant difference within the PM steak ('a' and 'b'). However on days 7, 14 and 21 the posterior section was more susceptible to metmyoglobin formation than the centre and anterior sections ($P<0.01$).

The initial oxymyoglobin concentration increased with time post mortem from 58% to 66% on days 2 and 21 respectively. This trend was reflected in a decrease in the % myoglobin from 20% on day 2 to 14 % on day 21. The initial percentage metmyoglobin was consistent with respect to storage time at 20-22%. This increase in the % oxymyoglobin and decrease in the % myoglobin during storage would be expected to reflect a decline in the OCR of the LD with time post mortem.

The rate of conversion of oxymyoglobin to myoglobin for the LD was highest on day 2 post mortem, intermediate on days 4 and 7 and almost non-existant on days 14 and 21 post mortem (Figure 1). Oxymyoglobin was converted mainly to myoglobin and to a lesser extent to metmyoglobin on days 2, 4 and 7 post mortem. The metmyoglobin concentration remained constant on days 14 and 21, with a slight decline in oxymyoglobin and increase in myoglobin. This suggests that LD steaks are more susceptible to metmyoglobin formation in the first days post mortem.

PM oxymyoglobin concentration varied between 55% (day 2) and 66% (day 21) and decreased rapidly with time in vacuum at all days post mortem, the rate of decline being highest at day 2. As in the LD, storage time had no effect on the initial % metmyoglobin, but the % myoglobin ranged from 12% at days 7, 14 and 21 to 23% at day 2 post mortem. A concurrent increase in the myoglobin concentration occurred at each storage interval. Unlike the LD, the metmyoglobin concentration increased with time in vacuum for all storage times, indicating that the PM is susceptible to metmyoglobin formation up to 21 days post mortem.

The OCR of both muscles decreased with time post mortem (Figure 2), however there were differences between the muscles at each storage time. PM had a higher OCR than the LD at all times post mortem ($P<0.01$). The OCR of the LD was almost completely exhausted by day 7. The OCR has a direct influence on the colour stability of a muscle through its effect on the penetration depth of the oxygen; therefore the findings here are in agreement with O'Keeffe and Hood (1982) who found that the PM is the most colour unstable, has a high OCR and a colour shelf-life significantly shorter than that of any other muscle. On the other hand they found that the LD was the most colour stable. Madhavi and Carpenter (1993), when comparing the biochemical characteristics of LD and PM discovered that PM had a significantly higher OCR and lower NAD ($P<0.05$) than LD steaks up to day 7 post mortem. However, on days 14 and 21 they did not detect a significant difference between the LD and PM, which is not in agreement with the present work.

Conclusions

The OCR of the PM is higher than that of the LD, with the PM continuing to have an active OCR up to 21 days post mortem. The OCR of both the LD and PM stabilised from 7 days post mortem onwards. From this it can be concluded that the optimum storage time prior to anoxic packaging of beef steaks is on or after 7 days post mortem.

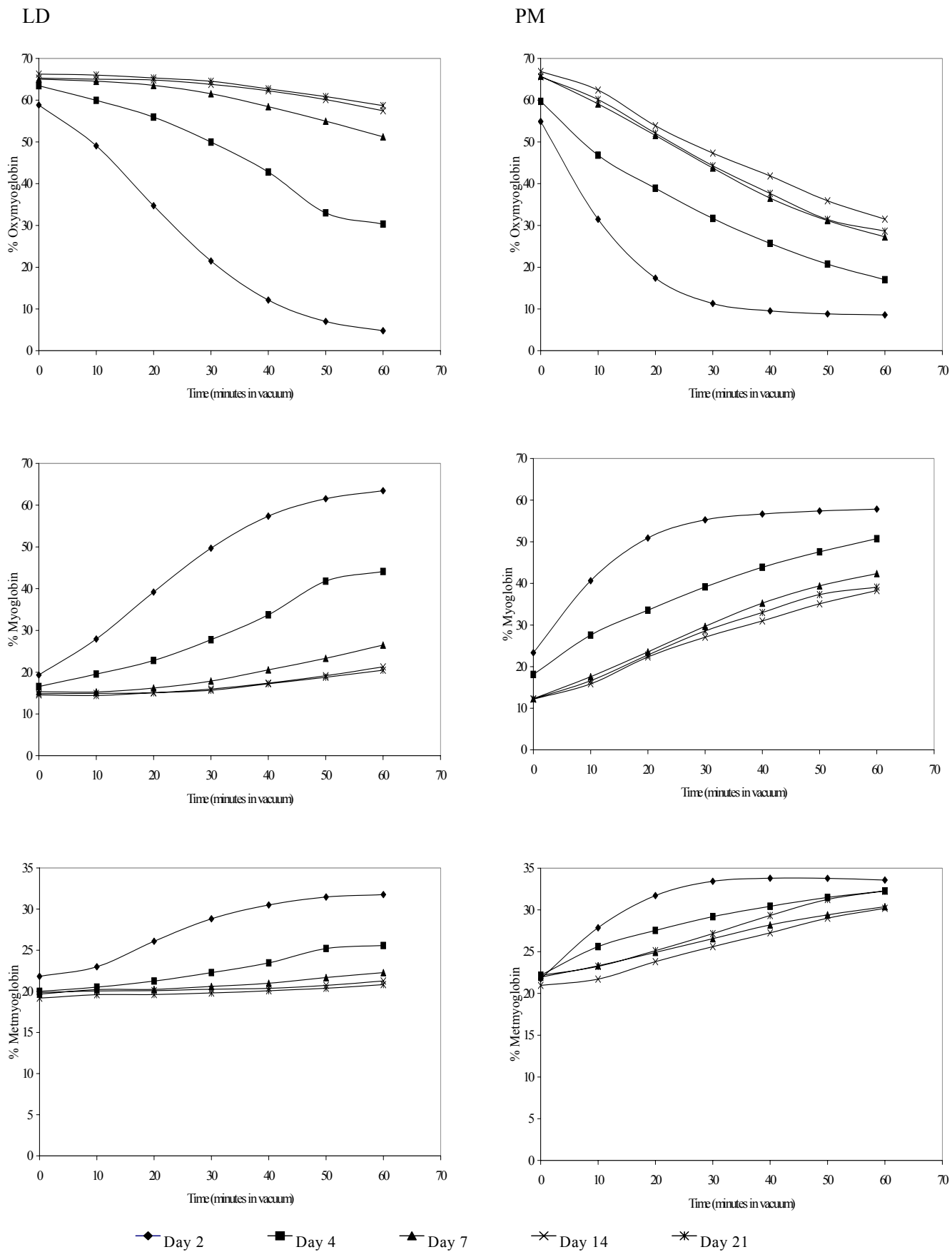

Figure 1: The concentration of the pigments oxymyoglobin, myoglobin and metmyoglobin over 60 minutes in vacuum in the LD and PM muscles.

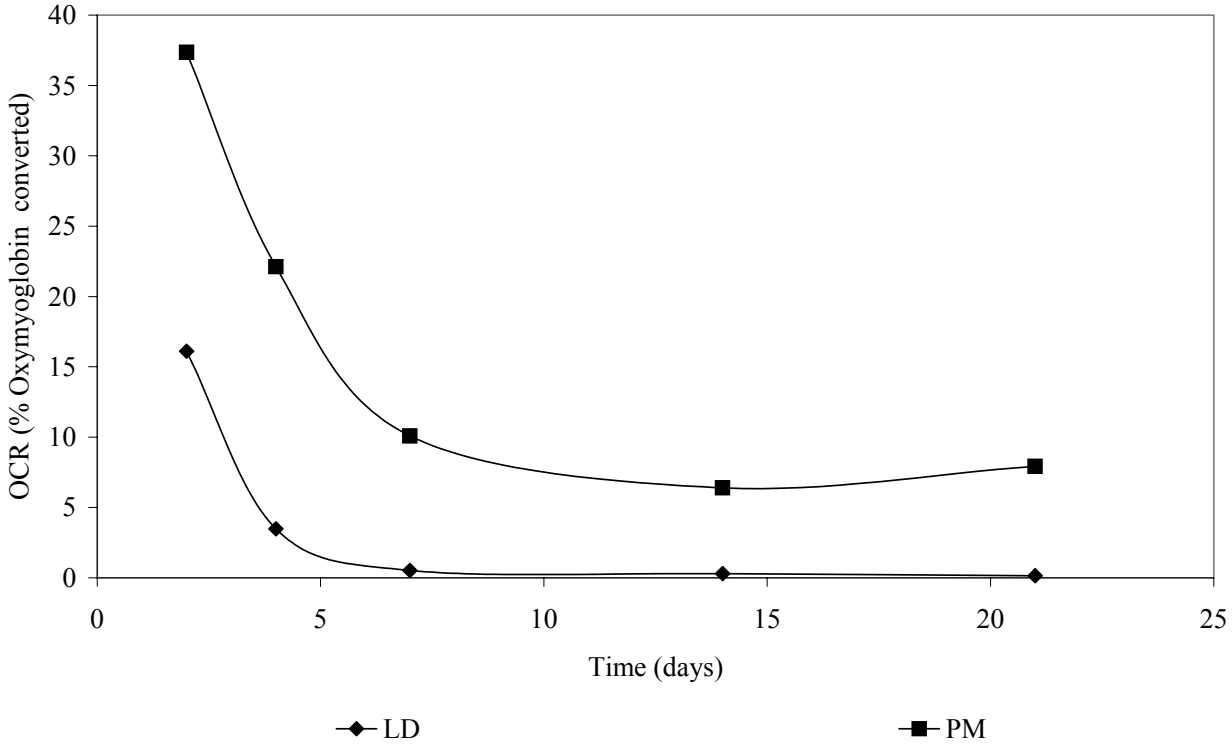

Figure 2: Effect of post mortem time on the oxygen consumption rate (% oxymyoglobin converted in the first 10 minutes in vacuum) of the LD and PM muscles.

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THE CORRELATION OF SLIGHT, MEDIUM AND EXTREME RIGOR MORTIS CARCASS TO HALOTHANE GENOTYPE, SARCOPLASMIC CALCIUM AND PROTEIN FUNCTIONAL CHARACTERISTICS IN ABATTOIR SLAUGHTERED PIGS

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Background

Halothane gene (HAL gene) is a genetic defect that can be triggered by stress during hog's handling treatments . The clinical symptoms of Halothane gene are gross muscular rigidity, high temperature, and arrhythmia. After the exsanguination of the animals, the blood circulation stops, and consequently, the hogs muscle couldn't obtain energy by respiration. Then, the energy is depleted which results in the development of carcass's rigor mortis. Glycogen transfers to ATP as an energy resource during rigor mortis and consequently affects the rigor development, because of the accelerated rigor mortis caused by the lack of calcium ion recovery activity during rigor mortis. After the hogs have been hanged and have been dressed, shaking hand method (Swatland, 1995) might give information about softening and tendering of the meat in which softening and tendering are in relation to muscle protein functional characteristics.
There have been shown three kinds of rigor mortis degrees (extreme, medium and slight) in abattoir slaughtered pigs in Taiwan. The different kinds of developed rigor mortis carcasses are measured by shaking hand method in early abattoir slaughtered pigs. The sarcoplasmic calcium concentration and glycolysis may be involved in extreme, medium and slight rigor mortis carcasses. The sarcoplasmic calcium concentration and glycolysis at postmortem carcasses play important roles in the formation of normal or pale, soft and exudative meat. The rate of glycolysis is controlled by sarcoplasmic calcuim levels; the carcass's rigor mortis might be affected by sarcoplasmic calcium concentration and rate of glycolysis. Rapid glycolysis within the first hour post mortem results in a low muscle pH value and high body temperature, leading to denaturation of muscle protein and development of inferior meat quality.

Objectives

The objective of this study was to determine the correlation of slight, medium and extreme rigor mortis carcass to halothane genotype, sarcoplasmic calcium and protein functional characteristics in abattoir slaughtered pigs.

Materials and methods

Materials

The live weight of the hogs were approximately 100-110 kg. They were slaughtered at local abattoir and stunned electrically. Within 45 min after exsanguination, the three groups of rigor mortis degree carcasses (slight, medium and extreme) were determined by tension load tester (Fig. 1). Longissimus dorsi (LD, from 4th to 5th rib) samples were subsequently excised within 45 min and 24 h after post mortem from 10 carcasses of three groups of rigor mortis degree, respectively, and immediately stored at -70℃ until analysis.

Methods

Halothane genotype identified

Halothane genotype of three kinds of rigor mortis degree carcasses were identified by mutagenically separated polymerase chain reaction (MS-PCR) (Liaw *et al.*, 2000).

Measurements of sarcoplasmic calcium concentration and the products of glycolytic metabolic response

Sarcoplasmic Ca^{2+} concentration in LD muscle was determined by using 10 g sample in 10ml of 150mM KCl (Cheach *et al.*, 1984). The Ca^{2+} was analyzed by an atomic absorption spectrophotometer at 422.7 nm. The concentration of glycogen was analyzed by a method of Hartschun *et al.* (2002), and glucose, glucose-6-phosphate, adenosine triphosphate（ATP), and creatine phosphate（CP）concentration were analyzed by a enzyme analyze method (Yang，1993). The R value was analyzed by a method of Thompson *et al.* (1987).

Measurements of protein functional characteristics

The pH, lightness value, protein solubility, myofibrillar ATPase activity, water-holding capacity(WHC), and myofibril fragmentation index (MFI) were determined at 45 min and 24 h post mortem, respectively. The protein solubility and WHC were measured by Joo *et al.* (1999). The assayed method of myofibrillar ATPase activity was modified from Lin *et al*. (1999). MFI was determined by Hopkins and Thompson (2002).

Statistical analysis

The data from the three kinds of rigor mortis degree carcasses were compared by analysis of variance (ANOVA) using the General Linear Model (GLM) of SAS（1988).

Results and discussion

The results showed that there was one heterozygote in the samples of extreme rigor and medium rigor mortis carcasses; however, the Halothane gene did not exist in the samples of the slight rigor mortis carcasses. Within 45 min of post mortem, both sarcoplasmic Ca^{2+} and R value in the extreme rigor mortis carcasses were significantly higher than those in the others ($p<0.05$); on the other hand, the adenosine triphosphate (ATP) and the creatine phosphate (CP) content in the extreme rigor mortis carcasses were lower than those in the slight rigor mortis carcasses ($p<0.05$), and the sarcoplasmic Ca^{2+} concentration did not have difference among the three groups at 24 h of post mortem. The results were similar to Cheah *et al.*(1984).

The increased level of sarcoplasmic Ca^{2+} in extreme rigor mortis carcasses was due to a disturbed regulation of calcium release channel in sarcoplasmic reticulum, and Ca^{2+} was fast releasing in sarcoplasmic reticulum. Cheah *et al*.(1986) indicated that Ca^{2+} release of sarcoplasmic reticulum might be caused by the mechamism of Ca^{2+}-induced Ca^{2+} released. Monin *et al*.(1980) indicated that the rigor mortis score was higher in Halothane positive pigs at 1 hour post mortem, for explaining that the extreme rigor mortis might to have a mutational Halothane gene. The faster rate of glycolysis in extreme rigor mortis carcasses is caused by the higher sarcoplasmic Ca^{2+} concentration they have. The rate of glycolysis was controlled by Ca^{2+} concentration which stimulated the degradation of glycogen. This study showed that the extreme rigor mortis carcasses were higher in free sarcoplasmic Ca^{2+} concentration and the ATP as well as the CP content to be lower. The results also indicated that the rate of glycolysis was faster within 45 min post mortem due to the extreme rigor mortis carcasses which had a higher Ca^{2+} concentration in sarcoplasmic.

Additionally, the solubility of sarcoplasmic protein, total protein, myofibrillar ATPase activity and WHC in the extreme rigor mortis carcasses were lower than those in the slight rigor mortis carcasses ($p<0.05$), but the extreme rigor mortis carcasses had significantly higher lightness value, drip loss and MFI than the other rigor mortis carcasses ($p<0.05$). Protein denaturation was quantified by myofibrillar ATPase activity and protein solubility. Within 45 min post mortem the extreme rigor mortis carcasses had a faster rate of glycolysis and it would result in lactate accumulation, pH value decrease and protein denaturation in muscle. Our study showed that extreme rigor carcasses had lower protein solubility and ATPase activity, in which it supported that extreme rigor carcasses had lower protein function characteristics than the others.

Conclusions

The results showed that although the relationship between hog carcasses and halothane genotypes did not reach to a significant level, extreme rigor mortis carcass had significantly higher sarcoplasmic Ca^{2+} level and caused rapid glycolysis and decreased pH value in pork muscle. Thus, the meat qualities were seriously affected.

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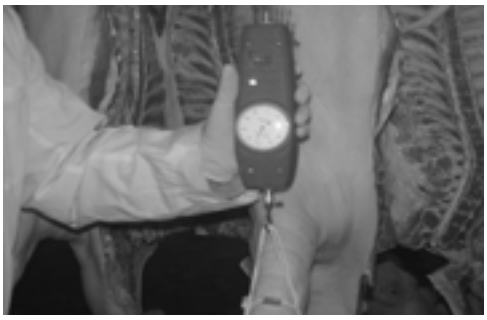

Fig. 1. The degree of rigor mortis carcass was measured by tension load tester.

Table 1. Sarcoplasmic Ca^{2+}, glycolytic metablic response concentration and R value in LD muscle at 45 min post mortem of different degree rigor mortis carcass

	extreme rigor	medium rigor	slight rigor
sarcoplasmic Ca^{2+} (μg/g)	12.41±1.45^{a}	12.35±1.88^{a}	8.75±2.34^{b}
Glycogen(mg/g)	109.58±11.28	110.27±11.90	111.83±11.44
Glucose (mg/g)	7.293±2.41	6.690±2.24	8.111±3.50
G-6-p (mg/g)	0.1213±0.044^{a}	0.0549±0.013^{b}	0.0363±0.020^{b}
ATP (μmol/g)	0.1368±0.039^{b}	0.1673±0.031^{a}	0.1717±0.025^{a}
CP (μmol /g)	4.126±1.41^{b}	6.591±2.7^{a}	6.206±2.28^{a}
R value	1.2943±0.035^{a}	1.2862±0.027^{ab}	1.2687±0.017^{b}

Different superscripts(a, b)indicate significant differences between means.($p<0.05$)

Table 2. Protein solubility, pH_1, WHC, MFI, myofibrilar ATPase activity, lightness in LD muscle at 45 min post mortem of different degree rigor mortis carcass

	extreme rigor	medium rigor	slight rigor
pH_1	6.19±0.33^{b}	6.37±0.10^{a}	6.43±0.06^{a}
protein solubility(mg/g)			
sarcoplasmic protein(mg/g)	71.47±7.03	74.39±9.07	74.06±6.14
total protein (mg/g)	212.94±23.12	220.29±15.57	216.36±11.75
WHC(%)	69.66±1.95^{b}	71.57±0.78^{a}	71.71±1.22^{a}
MFI	0.138±0.012^{a}	0.116±0.008^{b}	0.115±0.008^{b}
myofibrillar ATPase activity (μmol/min/mg protein)	0.2958±0.077^{c}	0.3680±0.064^{b}	0.4286±0.099^{a}
lightness value	34.36±0.97^{a}	33.17±1.73^{b}	32.96±0.98^{b}

Different superscripts(a, b, c)indicate significant differences between means.($p<0.05$)

Table 3. Sarcoplasmic Ca^{2+}, pHu, protein solubility, WHC, drip loss, myofibril fragmentation index, myofibrilar ATPase activity and lightness in LD muscle at 24 h post mortem of different degree rigor mortis carcass

	extreme rigor	medium rigor	slight rigor
pHu	5.84±0.20^{a}	6.10 ±0.21^{b}	6.11±0.36^{b}
sarcoplasmic Ca^{2+} (μ g/g)	8.25±1.74	8.83±2.90	7.81±1.38
protein solubility			
Sarcoplasmic protein (mg/g)	64.64±1.74^{b}	67.27±2.90^{ab}	70.73±1.38^{a}
total protein (mg/g)	169.70±15.87^{b}	174.39±10.70^{ab}	181.27±13.04^{a}
WHC(%)	66.17 ±0.82	68.37 ±0.90	68.93 ±1.06
drip loss	3.16±0.83^{a}	1.78±0.35^{b}	1.21±0.19^{b}
MFI	0.187 ±0.010^{a}	0.166 ±0.008^{b}	0.145 ±0.004^{c}
myofibrillar ATPase activity(μ mol/min/mg protein)	0.281±0.11^{b}	0.362±0.08^{ab}	0.425±0.139^{a}
lightness value	42.75 ±1.54^{a}	40.73 ±0.87^{b}	37.79 ±2.04^{c}

Different superscripts(a, b, c)indicate significant differences between means.($p<0.05$)

EFFECT OF HCG STIMULATION ON ANDROSTENONE, SKATOLE AND INDOLE LEVELS IN ENTIRE MALE PIGS

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Background

Skatole, indole and androstenone are the compounds responsible for boar taint, an offensive odour detectable when cooking pork. Due to their lipophilic properties, they accumulate in adipose tissue of some entire male pigs. Skatole and indole are formed from tryptophan in the colon of pigs (Claus et al., 1994). Skatole passes through the liver where most of it is metabolised by cytochrome P450. The activity of this enzyme system, therefore, affects skatole levels (Babol et al., 1998). Environmental factors and nutrition are also important in the regulation of skatole levels (Walstra et al., 1999).

Androstenone is a testicular steroid and its production is linked to the synthesis of anabolic testicular hormones. Puberty development results in increased androstenone levels (Claus et al., 1994). Environmental factors such as season, photoperiod (Andersson et al., 1998) and nutrition levels can influence the time of puberty and thus androstenone levels in adipose tissue at slaughter.

HCG injection causes an abrupt increase in androstenone both in plasma and fat (Carlström et al., 1975; Andresen, 1975; Bonneau et al., 1982), but to our knowledge an eventual effect on skatole has not been studied. High level of androstenone may inhibit the expression of CYP 2E1 in liver (Doran et al., 2002), one of the enzymes involved in skatole metabolism. Subsequently, much skatole remains unmetabolised and can then accumulate into fat. Some researchers showed that androstenone levels correlated positively with skatole levels in adipose tissue at slaughter age as reviewed by Walstra et al. (1999). A positive correlation between androstenone and skatole levels in plasma at 20 weeks of age was also found (Zamaratskaia et al., 2004a). Generally, however, results vary on the relationship between androstenone and skatole levels.

Objectives

The aim of the present study was to investigate the effect of hCG stimulation and the relationships of androstenone, skatole and indole levels in plasma and fat after hCG stimulation in entire male pigs.

Materials and methods

Animals

A total of 34 entire male pigs of a crossbred (Swedish Yorkshire dams x Landrace sires) were used in this study. Animals were raised in single-sex pens with 7 pigs in each. All pigs were fed the same commercial diet according to the standard feeding regimen for finishing pigs in Sweden (restricted, 12 MJ ME per kg, digestible CP 13%).

HCG injection was performed (Pregnyl, 30 IU/kg body weight) 4 days prior to slaughter. Control pigs were injected with sterile saline the same day. Blood samples were taken from all pigs twice: before injection and the day before slaughter. Plasma samples were kept in -80°C until analysis. Back fat was taken at slaughter and kept in -20°C until analysis.

Analysis of skatole, indole and androstenone

Skatole and indole levels in plasma were measured with HPLC as described by Zamaratskaia et al. (2004a). Skatole levels in fat were measured with a colorimetric procedure (Mortensen and Sørensen, 1984). Androstenone levels in plasma and fat were measured with an ELISA method described by Squires and Lundström (1997). Androstenone was extracted from plasma with ethyl acetate and from fat with methanol.

Statistical analysis

All data were analysed with the Statistical Analysis System, version 8.2 (SAS Institute, Cary, NC, USA). Procedure Mixed was used for evaluating the results. The model included treatment and time of sampling as fixed factors, and individual pig within sire, dam and treatment as random factors. A logarithmic transformation was applied to the levels of skatole, indole and androstenone to normalise the distributions of observed values. Results are presented after back transformation.

Results and discussion

Androstenone analysis in plasma with ELISA method has been used by many researchers. The direct plasma analysis (without extraction), however, led to overestimation and extreme values could be observed (above 200 ng/ml). To avoid this overestimating effect, extraction procedure was used prior to analysis in this study.

Three pigs were used in a pilot study to evaluate the experimental design. The blood samples were taken at five occasions: before hCG injection, each day during three days after injection and the day prior to slaughter. The results showed a rapid increase in androstenone level, a plateau on the second day and a subsequent decline. This is in line with the former Swedish results (Carlström et al., 1975), where maximum levels of androstenone in plasma were found the second day after hCG-injection. Overall, no effect on skatole or indole levels was observed; however, a pronounced increase in both compounds was observed within some individuals causing a high variation (Figure 1).

HCG stimulation significantly increased androstenone levels in plasma ($P < 0.001$; Table 1), whereas skatole levels in plasma did not differ between treatments. Surprisingly, plasma indole levels increased significantly ($P = 0.03$; Table 1). This increase is difficult to explain. Probably, high androstenone levels inhibit the enzymes involved in indole metabolism more efficiently than skatole levels. Both skatole and indole are metabolised in the liver with the same enzymatic system (Gillam et al., 2000). However, the precise metabolism of indole has not been the subject of intensive research, and factors affecting indole metabolism are not yet known. The effect of hCG administration on both skatole and indole levels needs to be further studied.

HCG injection also caused a significant increase in androstenone levels in fat ($P < 0.001$; Table 1). The mean androstenone level for the control group was 1.03 µg/g and for hCG treatment group 4.22 µg/g, approximately four fold higher than the control. Skatole levels in fat were significantly higher in the hCG-treated group compared to the control. It should be noted that skatole levels in fat in this study were measured by the colorimetric method. This method is not specific for skatole, but measures the total amount of indolic compounds. Therefore, the observed differences between treatments in fat might rather be due to indole than skatole concentrations. Indole may also partially contribute to boar taint (Garca-Regueiro and Diaz, 1989). However, the level above which consumers can perceive indole odour is not specified. It is assumed that indole is not of the same importance for boar taint as skatole and androstenone because of weaker odour.

The mean skatole levels in fat in control and hCG injection groups were 0.09 µg/g and 0.13 µg/g, with 1 and 4 pigs exceeding the rejection level of 0.20 µg/g respectively. The androstenone levels were relatively high even in pigs without hCG-stimulation. The mean value for androstenone in the control group was 1.03 µg/g. In this group, 13 and 8 out of 17 pigs exceeded the threshold levels for androstenone in fat of 0.5 and 1.0 µg/g respectively.

The correlation coefficients between skatole and androstenone levels in plasma at the two sampling occasions were 0.22 ($P = 0.44$) before hCG stimulation, and 0.03 ($P = 0.88$) after hCG stimulation. The relationship between skatole and androstenone levels in plasma after hCG stimulation has not been studied previously. Androstenone might be involved in skatole metabolism by the inhibition of CYP2E1 in vitro (Doran et al., 2002). High androstenone levels would then lead to high skatole levels. However, in an in vivo study, high androstenone levels did not result in immediately increased skatole levels (Zamaratskaia et al., 2004a, b). In these studies, the pubertal increases in testicular steroids preceded the rise in skatole levels. In the present study, skatole and androstenone in plasma were measured three days after hCG stimulation.

Probably, this time was not enough for androstenone to perform its action on liver enzymes. In this case, the increase in skatole levels can be delayed. The other explanation could be that androstenone levels after hCG stimulation still did not reach a sufficient level to inhibit CYP2E1 in vivo. The androstenone levels used in the in vitro study by Doran et al. (2002) were probably much higher than can be achieved in the liver in vivo. If so, no increase in skatole as a result of inhibiting action of testicular steroids could be anticipated.

The positive correlation between skatole and indole levels in plasma (r = 0.49) is probably due to the same intestinal origin and the linked metabolic pathway. Skatole levels in plasma and fat strongly correlated (r = 0.83). This is in agreement with previous studies (Zamaratskaia et al., 2004a). The correlation between androstenone and skatole levels in fat at slaughter was 0.47 in control animals and 0.39 after hCG stimulation. The lower correlation coefficient in the hCG-treated group may be related to the fact that androstenone levels in this group increased rapidly, whereas no such high increase in skatole levels was observed.

Conclusions

HCG stimulation significantly increased androstenone levels in both plasma and fat. Skatole levels in fat were also higher in hCG-treated pigs. This increase might be related to the variations in indole levels, since indole but not skatole levels in plasma were higher after hCG stimulation. High androstenone levels did not cause increased skatole levels in plasma with the sampling pattern used in this study. This would have been expected if androstenone inhibited the expression of the main enzymes of skatole metabolism.

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Table 1. Concentrations of androstenone, skatole and indole in plasma and fat from entire male pigs before and after hCG injection or at slaughter (least-square means)

	Control group			hCG injection group		
	Before injection	Day before slaughter / Slaughter day	P-value	Before injection	Day before slaughter / Slaughter day	P-value
Plasma (ng/ml)						
Androstenone	1.01	1.32	0.22	1.19	6.42	0.001
Skatole	2.97	2.75	0.59	3.54	4.10	0.29
Indole	2.25	2.66	0.33	2.74	3.98	0.03
Fat (µg/g)						
Androstenone		1.03			4.22	0.001
Skatole		0.09			0.13	0.03

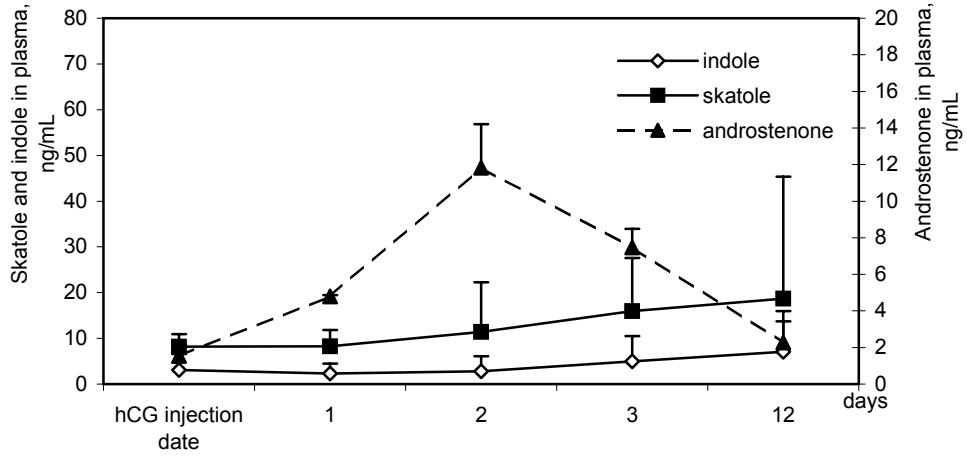

Figure 1. Androstenone, skatole and indole levels in plasma after hCG stimulation in three entire male pigs (mean value + standard deviation).

EFFECT OF LACTOFERRIN LEVELS ON TBARS AND NONHEME IRON OF GROUND PORK

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Background

Lactoferrin is an iron-binding glycoprotein that belongs to the transferring family, and mainly exists in mammalian milk. Iron ions are an important catalyst of lipid oxidation which could be bound by lactoferrin (Naidu, 2002). Probably due to this reason, the natural bovine lactoferrin could inhibit lipid oxidation in corn oil emulsions and lecithin liposome systems (Huang *et al*., 1999). Lactoferrin could be used as a dual-purpose additive in infant formulas and similar food products for its antioxidant and antimicrobial properties (Satué-Gracia *et al*., 2000). In the USA, lactoferrin is permitted at levels of 65.2 mg/kg of beef. In Taiwan, lactoferrin may be used in special nutritional foods under the condition "only for supplementing foods with an insufficient nutritional content and may be used in appropriate amounts according to actual requirements". Currently, the European Union does not have a specific regulation for lactoferrin. Higher levels of bovine lactoferrin had been administered orally to mice and rats, as high as 20 g/L of milk for fourteen days and 20 g/kg for thirty weeks, respectively, with no known side effects (Naidu, 2002).

Objectives

Lactoferrin was reported to inhibit various microorganisms, but only limited information has been reported on the effect of lactoferrin on lipid oxidation in food systems and to what extent the antioxidative effect of this additive exerts when used in ground pork and what influence it could have. The purpose of this study was to investigate the effect of lactoferrin levels on TBARS values, total and nonheme iron and pH of ground pork during storage at 4 ℃ for 9 days.

Materials and methods

Bovine lactoferrin was obtained from DMV International (Veghel, the Netherlands).
Ground meat samples were divided into three batches (1 kg/batch). To each batch was added 0, 40 or 80 mg lactoferrin/kg meat, respectively. Samples were stored at 4 ℃ for 0, 3, 6 and 9 days. TBARS (2-thiobarbituric acid reactive substances) values of meat samples were determined by using the distillation method (Ockerman, 1985). TBARS values were expressed as mg malonaldehyde/kg meat. Total iron concentration of the samples was determined on dry ashed samples by an atomic absorption spectrophotometry (Hitachi Z-8000, Tokyo, Japan) according to the AOAC method (1995). A modification of the method of Rhee and Ziprin (1987) as described by Schricker *et al*. (1982) was used to determine nonheme iron. The pH values of ground pork were determined according to Ockerman (1985).

Results and discussion

Mean total and nonheme iron concentration of samples is shown in Fig. 1. Due to that lactoferrin is an iron-containing protein; therefore, the treatments with the addition of lactoferrin (40 and 80 mg/kg) had higher (P<0.05) total iron concentration than the controls. The higher the levels of lactoferrin added, the higher the total iron. However, the differences in total iron concentration between the treatments with the addition of 40 and 80 mg/kg lactoferrin were not significant (P>0.05). On the contrary, nonheme (free) iron concentration was higher for the controls (14.2 μg/g), compared to the treatments with the addition of 40 mg/kg (12.0 μg/g) or 80 mg/kg lactoferrin (10.5 μg/g). The higher the levels of lactoferrin added, the lower were the nonheme iron. The differences in nonheme iron between the controls and the treatment with the addition of 80 mg/kg lactoferrin were significant (P<0.05). Lactoferrin used in this study is partially saturated with iron, which has the ability to bind free iron; therefore, nonheme iron concentration was lower, but total iron concentration was higher, in the treatments with the addition of lactoferrin when compared to the controls. In general, nonheme iron in all treatments increased slightly during storage at 4 ℃ for 9 days; however, the results were not consistent (data not shown). Kanner *et al*. (1988) reported that free iron in raw turkey and chicken muscles increased during storage at 4 ℃, and suggested that this free iron increase was important in lipid oxidation in stored meat.

TBARS values were lower (P<0.05) in lactoferrin-treated samples (40 and 80 mg/kg) than in controls at 3, 6 and 9 days of storage, except at day 0 (Fig. 2). This suggested that lactoferrin addition could inhibit lipid oxidation and decrease TBARS values in ground pork. This was due to the iron-binding properties of lactoferrin (Naidu, 2002). From our results, it seemed that nonheme (free) iron was probably more important than total iron in lipid oxidation in ground pork. Satué-Gracia *et al*. (2000) reported that lactoferrin could be used as an additive in infant formulas and similar food products for its antioxidant properties. The differences in TBARS values between the treatments with the addition of 40 and 80 mg/kg lactoferrin were not significant at each interval of storage time. Increasing the concentration of lactoferrin at 80 mg/kg could only slightly enhance its antioxidant activity in ground pork. This suggested that the addition of 40 mg/kg lactoferrin in ground pork (20% fat) was probably sufficient to retard the lipid oxidative rancidity. TBARS values in controls increased as the storage time increased up to 9 days. TBARS values in the treatments with the addition of 40 and 80 mg/kg lactoferrin were not significantly changed at 3 days of storage; however, the TBARS values significantly increased at 6 and 9 days of storage. It seemed that lactoferrin could be more effective in inhibiting lipid oxidation in ground pork at the early storage time. All samples with or without the addition of lactoferrin had TBARS values below 0.5 during 9 days of storage, which were relatively low and well below the threshold value (1.0 mg malonaldehyde/kg meat) for detection of warm-over flavor (Boles and Parrish, 1990).

The differences in pH values among the treatments were not significant (P>0.05) at each storage interval (Table 1). The results indicated that lactoferrin addition has little effect on pH values of ground pork. The pH values of all meat samples slightly increased (from 6.19 to 6.31) during 9 days of storage; however, the differences were very small.

Conclusions

Ground pork containing 20% fat was treated with 0, 40 and 80 mg/kg bovine lactoferrin and stored at 4 ℃ for 0, 3, 6 and 9 days. Total iron concentration was lower, but nonheme iron was higher in controls than in lactoferrin-treated samples. The higher the levels of lactoferrin added, the higher were the total iron, and the lower were the nonheme iron. The addition of lactoferrin decreases TBARS (thiobarbituric acid reactive substances) in ground pork. The antioxidant activity increased with lactoferrin concentration.

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Table 1

Effect of lactoferrin on pH value of ground pork during storage at 4 ℃ (n=12)

	Lactoferrin (mg / kg meat)		
Days	0	40	80
0	6.19±0.16	6.19±0.16	6.19±0.16
3	6.26±0.17	6.26±0.17	6.26±0.18
6	6.28±0.18	6.26±0.18	6.26±0.18
9	6.31±0.16	6.31±0.16	6.31±0.16

Each value is the mean ± 1 standard deviation.

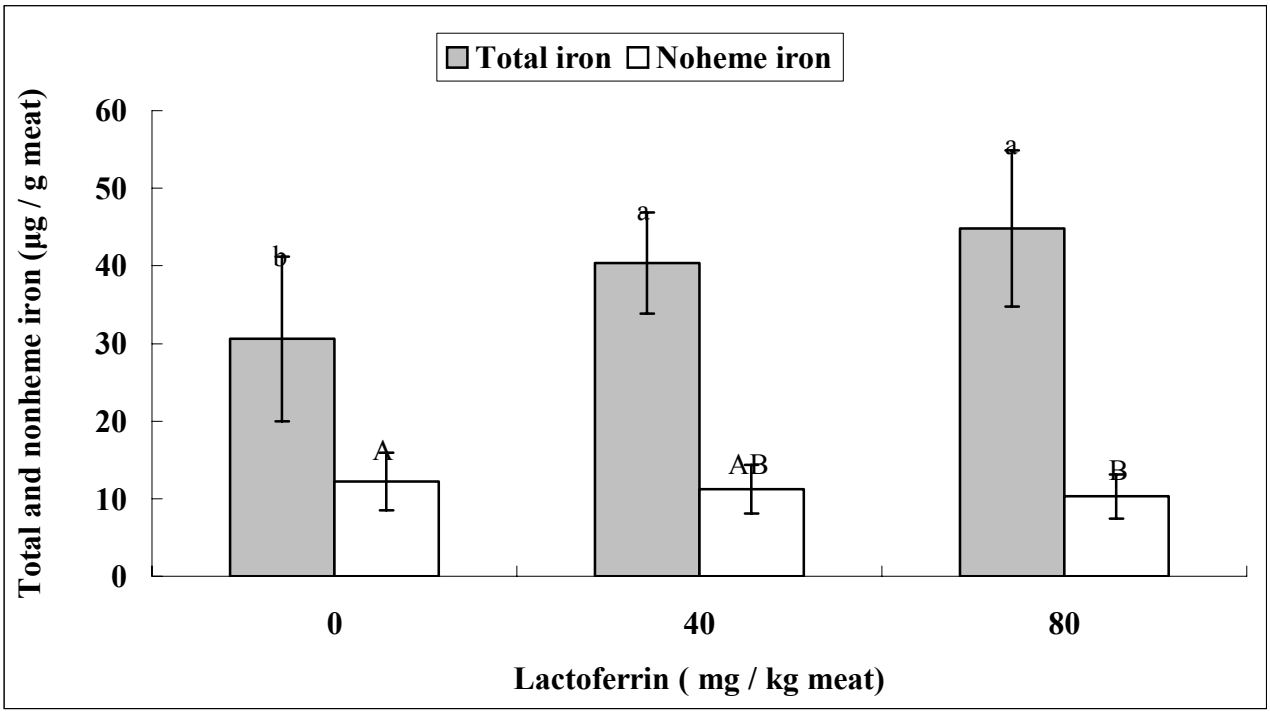

Figure 1. Effect of lactoferrin levels on total and nonheme iron of ground pork during storage at 4 ℃ (n=27). Error bars represent ± 1 standard deviation. Each bar represents the average of three replicates. Bars with different letters are significantly different ($P<0.05$).

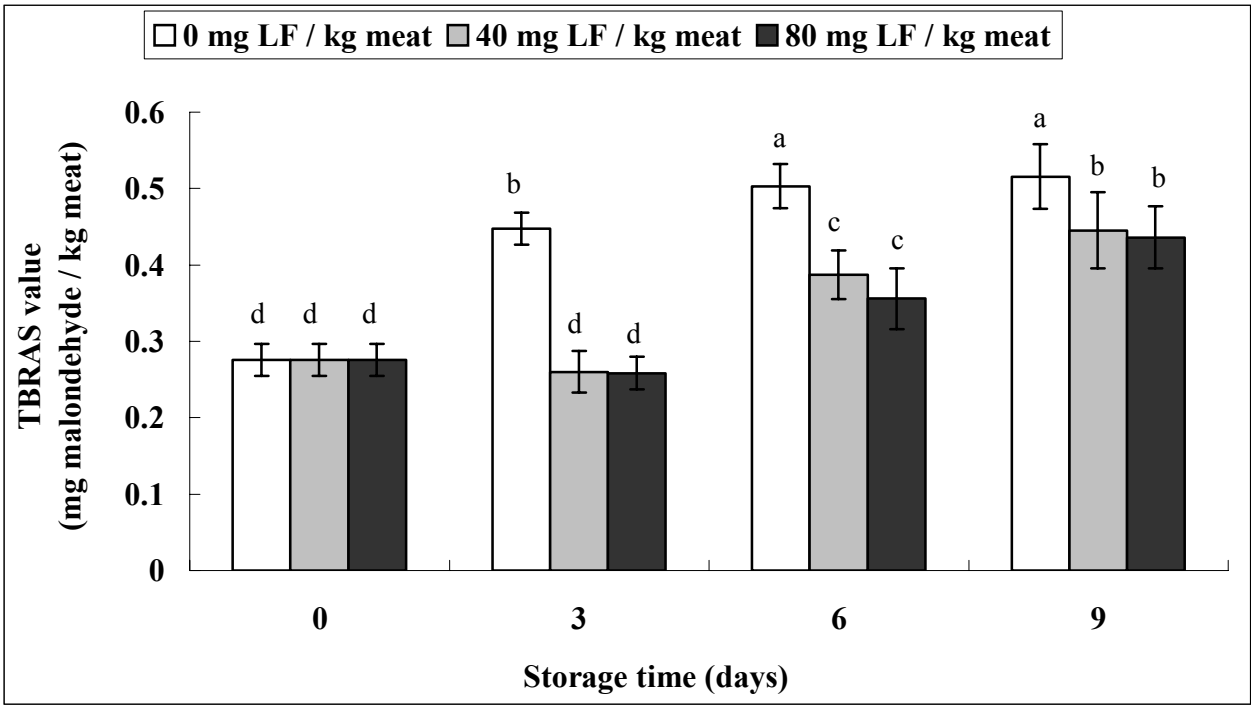

Figure 2. Effect of lactoferrin (LF) levels on TBARS values of ground pork during storage at 4 ℃ (n=8). Error bars represent ± 1 standard deviation. Each bar represents the average of three replicates. Bars with different letters are significantly different ($P<0.05$).

ASSOCIATION BETWEEN DNA MARKERS OF CANDIDATE GENES AND CARCASS COMPOSITION AND MEAT QUALITY IN KOREAN CATTLE

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Background

The challenge to the beef industry is the production of cattle that exhibit favorable or superior carcass composition and meat quality due to the increasing demand of the consumer for high quality meat. Advances in molecular techniques have made it possible to select animals based upon desirable genotypes to meat production, regardless of age or sex of individual. It has been proposed that candidate gene analysis can be used to identify individual genes affecting the carcass traits and meat quality in beef cattle (Beever et al., 1990; Sellier, 1994; Haegeman et al., 2003). Candidate genes are selected on the basis of known relationship between physiological or biochemical processes and quantitative traits of interest. Leptin is a hormone in the fat metabolism pathway that has been shown to affect the amount of marbling in beef (Willis et al., 1998). The myogenic factor 5 (MYF5) gene plays a role in myogenic lineage determination and/or myocyte differentiation (Li et al., 2004). Heart fatty acid-binding proteins (H-FABP) are involved in fatty acid transport from the cell membrane to the intracellular sites of fatty acid utilization (Gerbens et al., 1999). Therefore, the leptin, MYF5 and H-FABP genes are important candidate genes for the identification of genetic markers for carcass composition and meat quality in Korean cattle.

Objectives

The objectives of this study were to analyze DNA markers of three candidate genes and to investigate their possible association with carcass composition and meat quality in Korean cattle.

Materials and methods

A total of 215 Korean cattle registered in the official performance-testing program from the National Livestock Research Institute, R.D.A. were used in this study. Carcass traits studied were carcass weight (CW), dressing percentage (DP), eye muscle area(EMA), backfat thickness(BMT) and marbling score(MS). The meat quality was classified according to Korean beef carcass grading from 1(high quality grade) to 3(low quality grade). Genomic DNA was extracted from blood samples and dissolved in TE buffer and kept at -20℃. Genotyping of the leptin, MYF5, and H-FABP genes were carried out using the PCR-RFLP technique. Primer sequences and restriction enzymes used in PCR-RFLP analysis are presented in Table 1.
Allele frequencies in the two groups selected for high and low quality grades were tested using chi-square test. The GLM procedure of SAS was used to test the association between the genotypes of the candidate genes and the carcass traits.

Table 1. Primer sequences and restriction enzymes of candidate genes used in PCR-RFLP analysis

Candidate gene	Primer sequence (5′ to 3′)	Enzyme	Fragment size (bp)
Leptin	GTCACCAGGATCAATGACAT AGCCCAGAATGAAGTCCAA	BgI Ⅱ	1,820
MYF5	ACAGCGTCTACTGTCCTGATG CGTGGCATATACTAAGGACAC	Taq Ⅰ	890
H-FABP	TACCTGGAAGTTAGTGGACAGC CTTGGCTCTGCTTTATTGACCT	Msp Ⅰ	612

Results and discussion

The three candidate genes of leptin, MYF5 and H-FABP were genotyped using PCR-RFLP method to determine their association with carcass composition and meat quality in Korean cattle. Representative results of PCR-RFLP analysis detected on polyacrylamide gel electrophoresis are shown in Figure 1, 2 and 3.

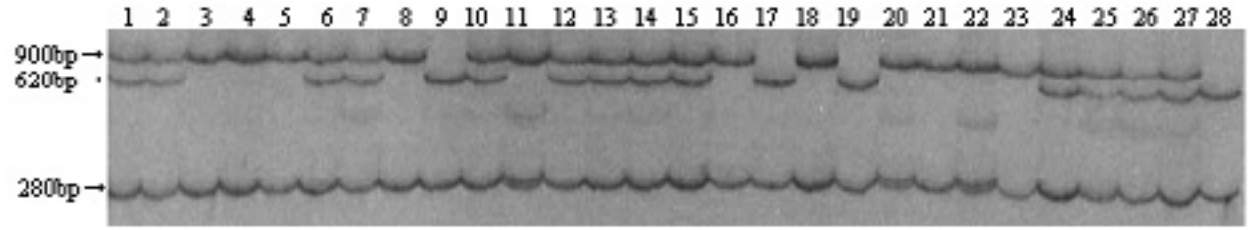

Fig. 1. RFLP genotype markers of leptin gene in 12% polyacrylamide gel following digestion with *BgI II* restriction enzyme of PCR products. In the gel, lanes 3~5, 8, 11, 16, 18, 20~22 and 23 represent AA genotype, lanes 1, 2, 6, 7, 10, 12~15, 24~26 and 27 represent AB genotype and lanes 9, 17, 19 and 28 represent BB genotype.

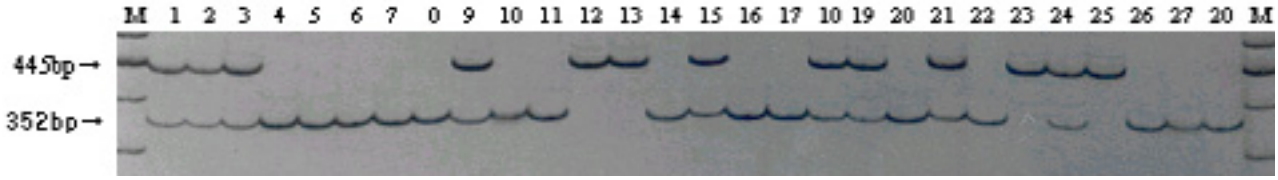

Fig. 2. RFLP genotype markers of MYF5 in 13% polyacrylamide gel following digestion with *Taq I* restriction enzyme of PCR products. In the gel, lanes 12, 13, 23 and 25 represent AA genotype, lanes 1~3, 9, 15, 18, 19, 21 and 24 represent AB genotype and lanes 4~8, 10, 11, 14, 16, 17, 20, 22, 26, 27 and 28 represent BB genotype. M : molecular size marker(100bp DNA ladder)

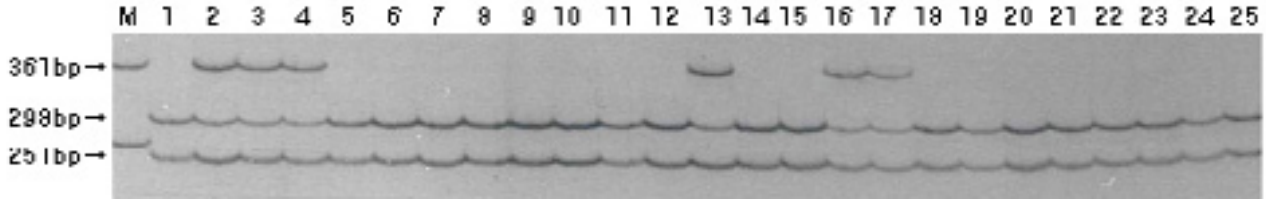

Fig. 3. RFLP genotype markers of H-FABP in 12% polyacrylamide gel following digestion with *Rsa I* restriction enzyme of PCR products. In the gel, lanes 1, 5~12, 14, 15, 18~24 and 25 represent AA genotype, lanes 2, 3, 4, 13, 16 and 17 represent AB genotype. M : molecular size marker(100bp DNA ladder)

The gene frequencies for A and B alleles in all animals were 0.57 and 0.43 for leptin, 0.61 and 0.39 for MYF5 and 0.90 and 0.10 for H-FABP, respectively. The difference in allele frequencies between the two groups selected for high and low quality grades was significant (P<.05) at leptin and MYF5 genes (Table 2).
Least squares means and standard errors of carcass traits for different genotypes of three candidate genes are given in Table 3. The gene frequencies for A and B alleles in all animals were 0.57 and 0.43 for leptin, 0.61 and 0.39 for MYF5, and 0.90 and 0.10 for H-FABP, respectively. The difference in allele frequencies between the two groups selected for high and low quality grades was significant (P<.05) at leptin and MYF5 genes(Table 2).
The effect of leptin gene was significant for backfat thickness (P<.01). The backfat thickness of animals with the BB genotype (1.43±0.64cm) was significantly higher than that of animals with the AA genotype (0.66±0.52cm). The MYF5 gene was found to be significantly associated with eye muscle area (P<.05). Animals with the AA genotype (98.82±7.92cm^2) produced a higher eye muscle area than animals with the BB genotype (81.94±8.37cm^2).

Table 2. Comparisons of allele frequency between the two groups selected for high (n=52) and low(n=43) quality grades using chi-square test

Candidate gene	χ^2	df	P
Leptin	5.6461	1	P<.010
MYF5	4.4731	1	P<.025
H-FABP	1.7412	1	P<.100

Table 3. Least square means and standard errors of carcass composition for leptin, MYF5 and H-FABP genotypes in Korean cattle

Candidate gene	Genotype	Carcass traits				
		CW(kg)	DP(%)	BFT(cm)	EMA(cm^2)	MS
		LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE
Leptin	AA	481.43±78.47	60.80±2.35	0.66±0.52^{b}	91.63±7.92	3.92±1.01
	AB	479.43±78.02	60.54±1.56	0.94±0.38^{ab}	89.23±8.53	3.83±0.94
	BB	486.82±77.25	60.51±2.84	1.43±0.64^{a}	88.26±8.23	3.82±0.48
MYF5	AA	482.35±81.71	61.29±2.13	0.94±0.38	98.82±7.92^{a}	3.94±0.98
	AB	487.58±94.47	60.27±2.22	1.04±0.45	86.52±8.22^{ab}	3.77±0.96
	BB	479.75±90.46	61.26±2.52	1.03±0.54	81.94±8.37^{b}	3.59±0.72
H-FABP	AA	485.39±94.25	59.27±2.46	0.98±0.42	93.00±7.60	3.71±0.83
	AB	488.76±98.94	61.66±2.04	1.09±0.53	96.13±7.60	3.64±0.88

Superscripts with different letters in the same column significantly differ (P<.05).
CW=Carcass weight; DP=Dressing percentage; BFT=Backfat thickness; EMA=Eye muscle area; MS=Marbling score (ranges 1-5)

However, there were no significant effects of H-FABP gene on carcass traits. The significant association between the specific gene and quantitative traits suggests that the gene may be one of the causative genes or that the gene is very close to the causative gene(s)(Li et al., 2004). These results suggest that the leptin and MYF5 may be candidate genes that influences some carcass traits and meat quality in Korean cattle. The gene-specific RFLP markers of leptin and MYF5 genes could be used as genetic markers to select for increased backfat thickness and eye muscle area in breeding programs. Further investigations are needed in other populations of Korean cattle to verify the associated effects of the candidate gene-specific DNA marker.

Conclusions

In other to find DNA markers to improve the carcass and meat quality of Korean cattle we studied the association between genotypes in the three candidate genes (leptin, MYF5 and H-FABP) and carcass composition and meat quality. Genotypes of three candidate genes were determined for 215 animals using PCR-RFLP method. Allele and genotype frequencies were calculated for each genes. The allele frequencies were different between the two groups selected for high and low quality grades at leptin and MYF5 genes (P<.05). The leptin genotype was found to have significant association (P<.05) with backfat thickness. A significant association (P<.05) was also found between the MYF5 genotype and eye muscle area. No significant association, however, was defected for the H-FABP genotype. The results indicate that the leptin and MYF5 gene-specific RFLP markers could be used as DNA markers to select animals with desirable meat quality.

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ASSOCIATION OF NEW CALPASTATIN ALLELES WITH MEAT QUALITY TRAITS OF COMMERCIAL PIGS[1]

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Background

The calpain system, a Ca^{2+}-activated protease family, and indirectly calpastatin (*CAST*), a calpain inhibitor, plays an important role in postmortem tenderization of skeletal muscle due to its involvement in the degradation of important myofibrillar and structural proteins (Koohmaraie, 1992; Sensky et al., 2001; Ciobanu et al., 2002). Two new *CAST* polymorphisms, *CAST Arg249Lys* and *Ser638Arg*, are located in or close to subdomain C of their respective domains. This subdomain potentiates the inhibitory activity of CAST. In order to investigate whether the alleles/haplotypes of the *CAST* gene are associated with meat quality traits such as ultimate pH, Minolta L*, cooking loss, tenderness and juiciness, a large number of pigs was harvested at commercial processing plants and screened for these two markers.

Objectives

Estimate the association of the *CAST* alleles with meat quality traits using a large number of commercial pigs harvested at commercial processing plants.

Materials and methods

Tissue Sampling and DNA Isolation. Tail and muscle samples and phenotypic data were collected from four different groups of commercial pigs. Genomic DNA was isolated using the DNeasy tissue kit (Qiagen, Valencia, CA).

Phenotypic measurements. Four separate experiments were conducted on four different commercial pig crosses: Group A: N=205; Group B: N=161; Group C: N=691; Group D: N=1829. All test pigs were harvested at two different commercial processing plants. *Longissimus dorsi* and *Gluteus medius* (ham) muscle pH was measured 24 hours post-mortem directly on the carcass using a Start Probe pH meter. Additional meat quality measurements such as Minolta L*, a*, a, b* (both muscles), instrumental tenderness (shear force, star-probe), cooking loss, sensory measurements (juiciness and tenderness), and pH (Longissimus muscle) were evaluated after 14 days of ageing. In experiment A, shear force was measured with an Instron 1222 Universal Testing Machine (Instron, Canton, MS) fitted with a Warner-Bratzler shear attachment. In experiment B, two chops were evaluated for instrumental texture using a circular, five-pointed star-probe attached to a texture analyzer (Texture Technologies, Scarsdale, NY). The star probe attachment was used to determine the amount of force needed to puncture and compress the chop 80% of the sample height. Subjective juiciness and tenderness were scored from 1 to 10 with higher values indicating more tender/juicier meat. For cooking loss measurements, a chop was cut from the muscle after ageing, weighed, cooked to 70C^{o} in a Faberware open hearth electric grill, refrigerated until cool, and re-weighed. During the time of cooking, temperature in each chop was monitored in its center with thermocouples. Cooking loss was calculated from weights taken before and after cooking and was expressed as a percentage.

Genotyping and PCR-RFLP Analysis. The region flanking each missense polymoprhism (*Arg249Lys* and *Ser638Arg*) was amplified by PCR and then digested with *Hpy*188I (*Arg249Lys*) and *Pvu*II (*Ser638Arg*).

Statistical Analysis. Haplotypes defined by the *CAST Arg249Lys* and *CAST Ser638Arg* polymorphisms were assigned based on inferences derived from the genotypes of homozygous individuals: haplotype 1, *249Lys – 638Arg*; haplotype 2, *249Arg – 638Arg* and haplotype 3, *249Arg – 638Ser.* All the predicted haplotypes were

present in all four groups of commercial pigs analyzed. The substitution effects of the haplotypes were estimated using a mixed model (SAS procedure MIXED, SAS Institute Inc., Cary, NC) and were considered as deviations from the effect of haplotype 3, which was arbitrarily set to zero. Analysis of haplotype associations was based on a model with genetic background of the four commercial pig groups included as a fixed effect.

Results and discussion

CAST Arg249Lys and *Ser638Arg*, are located in or close to subdomain C of their respective domains. This subdomain potentiates the inhibitory activity of CAST (Takano and Maki, 1999). Both substitutions (*Arg249Lys* and *Ser638Arg*) are outside the most conserved area of subdomain C: KPxxEDDxIDALSxDF (reviewed by Takano and Maki, 1999), but *Ser638Arg* is separated by just one amino acid from this sequence. An additional substitution, the *Ser66Asn,* is situated in Domain L. The function of this domain is not clear even though its sequence is well conserved among mammalian species. Recently, a role of the L domain was identified in regulation of L-type Ca^{2+} channels in guinea pig cardiac myocytes, suggesting the involvement of CAST in restoring Ca^{2+} channel activity, which facilitates Ca^{2+} influx and subsequently activation of calpain (Hao et al., 2000).

Detailed results representing Group A test pigs (N=205) are provided in Table 1. For all traits measured in this group of pigs, haplotype 1 was the favorable one and it was associated with higher pH, better tenderness and juiciness, lower shear force and cooking loss (Table 1). For instance, the effect of haplotype 1 was significantly different from the effect of haplotype 2 for shear force ($P < 0.04$), and for percentage of cooking loss ($P < 0.001$). The effect of haplotype 1 was also significantly different from that of haplotype 3 for muscle pH ($P < 0.03$), cooking loss ($P < 0.002$), juiciness ($P < 0.008$), and tenderness ($P < 0.09$) in this test group.

The results were not so clear for Group B test pigs (N=161), although some associations were detected. For example, for meat firmness, haplotype 2 had a higher substitution effect than that of haplotype 1 ($P<0.05$), and haplotype 3 ($P<0.01$). A suggestive association was also discovered for star probe with haplotype 1 being associated with a better meat quality than haplotype 2 (as for Group A). No significant differences were found for meat pH at 24hr and cooking loss.

Data from Group C pigs (N=691) revealed that haplotype 1 and haplotype 3 were again associated with higher quality pork. These haplotypes were significantly different from haplotype 2 for loin pH at 24 hr ($P<0.01$), and ham Minolta L* ($P<0.05$).

Commercial pigs from Group D (N=1829) were characterized by a significant association of Loin Minolta a* with haplotype 1 ($P < 0.0001$) and haplotype 3 ($P < 0.05$). Haplotype 1 and 3 were associated with lower value for this component of color and were significantly different from haplotype 2.

Conclusions

The results indicate that the porcine calpastatin variants have significant effects on meat ultimate pH, tenderness, cooking loss, and other economically important pork quality traits. It remains to be further demonstrated if the observed effects were caused by these polymorphisms alone or by their linkage disequilibrium with the causative mutations. Genetic background may be responsible for the differences in effects observed in the different commercial populations analyzed. The polymorphisms, or DNA markers, can potentially be incorporated into breeding programs to improve overall meat quality and hence the economic value for the pork supply chain and quality products for consumers.

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[1]The authors express their appreciation to Jessica Magrin and Kyong Hee Blakeman for technical support. Partial financial support was provided by Sygen International, PIC USA, USDA/CSREES IFAFS grant # 00-52100-9610, and the Iowa Agriculture and Home Economics Experiment Station project number 3600 & 3700 and supported by Hatch Act and State of Iowa funds.

Table 1. Haplotype substitution effects for *Longissimus dorsi* meat quality traits

Trait	Mean (s.e.)	s.d.	Estimate[A]			Contrast P values		
			Haplotype			Haplotypes		
			1	2	3	1 vs 2	1 vs 3	2 vs 3
pH	5.73 (0.02)	0.18	0.055	0.026	0	0.34	0.03	0.37
Shear Force	1.92 (0.04)	0.55	-0.074	0.097	0	0.04	0.28	0.22
Cooking loss %	24.23 (0.36)	5.01	-1.916	0.727	0	0.001	0.002	0.30
Tenderness score[B]	7.33 (0.10)	1.32	0.276	0.199	0	0.69	0.09	0.29
Juiciness score[B]	8.02 (0.08)	1.14	0.381	0.253	0	0.45	0.008	0.12

[A] haplotype 1: *249Lys – 638Arg* (frequency = 0.48); haplotype 2: *249Arg – 638Arg* (0.21); haplotype 3: *249Arg – 638Ser* (0.31);

[B] Tenderness and Juiciness scores are assigned using a subjective method;

Number of observations = 158 – 205 depending on the trait measured

STUDY OF THE EFFECT OF THE SEXUAL CONDITION ON THE pH AND TEMPERATURE OF MEAT FROM CROSSBRED BRAHMAN TO THE 0 AND 24 HOURS POSTSLAUGHTER

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Background

The carcass and meat quality of ruminants is influenced by various factors such as transport (vehicle type, animals/m^2, and journey times), loading and unloading, stay of the animals in the slaughterhouses, type of stockyard, climate, and breed, among others. In a wide review, Ciria and Asenjo (2000) indicates that the genetic type, feeding, sex, age, season, handling during breeding or prior to slaughter can affect that quality. The low checking of these factors is a constant threat to the organoleptic, technological and sanitary quality of meat in Venezuela, and particularly in Lara state, opposed to the European Union and USA where some minimum conditions prior to the sacrifice are imposed by means of the "Animal Well-being" and the "Law of Humanitarian slaughter (Humane Slaughter act)", respectively. On the other hand, transport is an event highly stressor for the bovine (Van de Water et al., 2003), and might have a tremendous significance in Venezuela because of the existence of lines of high crossbred Brahman that they are easily excitable and susceptible to suffer of stress. Preslaughter conditions, therefore, can affect muscle pH, color, texture and water-holding capacity of meat.

Objectives

With the purpose of evaluating in the Venezuelan tropic the influence of the preslaughter parameters on the carcass and meat quality of crossbred Brahman, a series of experiments are carrying out. The pH and temperature at 0 and 24 hours postslaughter of young bulls, cows, heifers, and steers that arrive to the slaughterhouse were observed in this particular study.

Materials and methods

This study was developed at the Western Center Slaughterhouse localized in the semi-arid climate of Lara state, Venezuela with an annual half temperature between 22º and 28º Celsius, annual precipitations greater than 1000 mm and a relative humidity between 77 and 85%. A total of 78 crossbred Brahman animals were slaughtered as discriminated as followed. Thirty-eight young bulls (whole males) of 2.5 to 3.5 years old and average half carcass weight of 135.6 Kg and 15 steers (castrated males) of 2.5 to 3.5 years and average half carcass weight of 134.4 Kg. Twelve 2.5 year-old heifers with average half carcass weight of 83.4 Kg, and thirteen 3 to 6 years old cows with average half carcass weight of 95.8 Kg. The animals were loaded among 14:30 to 17:00, and traveled for 4 to 6 hours. Arrival in slaughterhouse occurred between at 20:00 and 20:30. The animals were placed by discharge lots in stockyard separated by metallic rails with free readiness of water. Stay time in slaughterhouse went from 11 to 12 hours. After giving a shower with cold water, the animals were stunned with gun of captive bullet in the cranial region. Chronological age was estimated through the dental characteristics, and approximately 25 minutes after the stunned state of the animal, both initial pH and temperature (pH O and Temp O) were measured in the Gracilis muscle of the half left carcass by using a pHmeter (3030N Neukum) with penetration electrode and sensor of temperature. A final pH and temperature (pH 24 and Temp 24) were also measured after 24 hours refrigerated. Values obtained were analyzed by the general lineal model of variant analysis of computer software SPSS, version 10.0 for Windows.

Results and discussion

Temp 0 of carcass were lower ($p < 0.01$) for young bulls than for cows, heifers and steers probably due to differences in the way genders handle the preslaughter stress. Sañudo (1992) explains that the females of calmer character increases the corporal temperature when suffering stress in the moment prior to the

sacrifice, accelerating glycogen degradation and glycolisis. The fat cover of the carcass might also affect the initial and final temperature by avoiding heat dispertion, since according to Aalhus et al. (2001), thicker back fat corresponded with slower temperature decline. Young bulls may deposit less fat cover because of their hormonal condition. On the other hand, Temp 24 was higher ($p < 0.01$) in steers and cows as compared to heifers and young bulls.

Table 1.Values of pH 0, pH 24, Temp 0, and Temp 24 in crossbred Brahman of different gender

	Young bulls	Cows	Heifers	Steers	Signification
pH 0	6,66a	6,52a,b	6,48b	6,51b	**
	±0,21	±0,26	±0,15	±0,21	
pH 24	5,90a	5,85a	5,64b	5,63b	**
	±0,20	±0,14	±0,08	±0,19	
Temp 0	35,01a	38,38 b,c	37,59b	39,50c	**
	±2,22	±1,91	±0,60	±0,99	
Temp 24	3,86a	6,58b	3,09a	9,58c	**
	±2,36	±1,79	±0,58	±1,93	

* * Significative difference (p = 0.01)
Differents letters in the same row means significant differences between young bulls, cows, young bulls and heifers. (p = 0.01).

The pH 0 and pH 24 were higher ($p < 0,01$) for the young bulls than all the other groups evaluated. These results suggest a greater glycogen consumption preslaughter (Mojto et al, 1998), because of greater physical activity, biggest excitability, maintained muscular contraction and catecolamines hipersecretion before the sacrifice (Tarrant, 1981). In fatigued animals prior to sacrifice, the pH descends little and very slowly, because glycogen depletion which causes elevated values of final pH (Sañudo 1992).

The steers and heifers had lower values of pH 24 ($p < 0,01$) as compared to the cows and young bulls, however, the total average of pH 24 (5,79) is higher than the observed in other studies (Page et to the, 2001), which could indicate that the preslaughter conditions are significantly affecting muscle glucogen in this group of animals.

Conclusions

With the material and used methods, and starting from the results obtained in this study and lower these experimental conditions, we can conclude that the sexual condition has influence in the initial and ultimate pH and temperature of the meat of crossbred Brahman.

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Acknowledgements

To the directive meeting of the Slaughterhouse Western Center C.A. for the gracefulness of allowing that this work was carried out in its facilities, very especially to Mr. Bernardo Ortega Laughed and the M.V. Margarita Arizpe who was possible to reach the yearned goal thanks to their valuable support.

EFFECTS OF GROWTH RATE, SEX AND SLAUGHTER WEIGHT ON CARCASS COMPOSITION AND MEAT QUALITY IN COMMERCIAL PIGS

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Background

Little is known about the combined or interactive effect of growth rate and final weight of pigs on pork carcass and meat quality characteristics. Increasing slaughter weight has the advantage of reducing overhead costs per unit weight of output for producer, slaughterer and processor, increasing carcass yields, improving meat to bone ratio and reducing chilling and processing losses (Ellis and Bertol, 2001). However, research showed that starting from 100 kg live weight each increment of 10 kg in body weight leads to a slightly lower average daily gain, a significant deterioration in feed efficiency, reduced lean deposition and poorer meat quality (Albar et al., 1990; Cisneros et al., 1996; Latorre et al., 2004). To get to heavier weights without affecting carcass leanness, the simplest approach is to restrict the amount of feed supplied to the animal, especially in the later stage of the finishing period when fat deposition rates increase dramatically (Ellis et al., 1996; Candek-Potokar et al., 1998). However, this practice may eventually lead to negative age-related effects, such as slower growth rate and reduced intramuscular fat content and, consequently, poorer quality of pork meat (Candek-Potokar et al., 1998). The use of modern, high-lean growth potential genotypes may represent a valid solution as in theory they can be taken to heavier weights without compromising growth performances and carcass traits.

Objectives

The aim of this project was to evaluate the effects of growth rate, sex and slaughter weight on carcass and meat quality traits in commercial pigs.

Materials and methods

A total of three hundred and forty (340) Duroc x (Landrace x Yorkshire) crossbred piglets were allotted into 28 pens and raised until slaughter at the experimental farm of the Centre de développement du porc du Québec (CDPQ) in Deschambault (QC, Canada). The animals were equally distributed according to an experimental design including the following independent variables: 1) growth rate, which was set according to two different EBVs (estimated breeding values) for age at 100 kg (fast growth: around -10 days and slow growth: around + 2 days) of the sire-line, 2) the sex (barrows and gilts) and 3) the liveweight at slaughter (107, 115 and 125 kg). The selected sire-lines had similar EBVs for backfat thickness at 100 kg slaughter weight. At the end of the finishing period, a sub-population of 119 pigs (10 carcasses per treatment) was selected for the carcass and meat quality evaluation trials.
After slaughter, warm carcass weight (kg) and loin eye area (cm^2) between the 3rd and 4th last ribs level were recorded. After conventional overnight chilling, one side of each carcass was dissected in order to estimate the effects of the treatments on the proportions of the lean, fat and bone tissue in the carcass. Full dissection did not include the front shank and the belly. Meat quality was assessed on the *longissimus* (L) muscle by measuring pH1 on the day of slaughter (45 min. post mortem) and ultimate pH (pHu), drip loss (%) and light reflectance (Minolta Chromameter CR 300) at 24 h post mortem. Furthermore, L muscle chops were ground and frozen pending the analysis of dry matter,

collagen and collagen heat-solubility, protein and intramuscular fat (IMF) content (Soxtec extraction with ethanol and dichloromethane). All analyses were conducted as described by AOAC (1990). Data were analysed according to a 2 x 2 x 3 factorial design by using the GLM Procedure of the SAS System (SAS, 1999).

Results and discussion

The selection by the EBV for growth rate of the sire-line successfully led to a difference in the age at slaughter, with pigs from the high growth rate line being a week younger than the slower growth rate ones. As liveweight at slaughter increased there were significant increases in hot carcass weight ($p<0.001$) and dressing percentage ($p<0.05$) (Table 1). These results are in agreement with those reported by Garcia-Macias et al. (1996) and Candek-Potokar et al. (1998). However, contrary to Garcia-Macias et al. (1996) and Beattie et al. (1999), in our study the increase of carcass weight did not result in a variation of lean and fat proportions (%). Globally, loin eye area (LEA) increased ($p<0.001$) with weight and gilts showed greater loin eye area than barrows. Loin eye area was dependant on a significant triple interaction of treatments. LEA increased with weight and this effect was more pronounced and larger in gilts. Main variation, however, arose from fast growing barrows which have readily completed their cross sectional muscle growth as they reached 115 kg, while, in opposite, a late but rapid development occurred from 115 to 125 in the slow growing ones which attained a LEA comparable to that of gilts of the same group. Final LEA of fast growing barrows was much smaller at 125 kg than that of the three other sex and growth rate combination of treatments at the same weight. Bone proportion was globally affected ($p<0.05$) by sex and growth rate, with gilts and slower growing pigs having higher proportions than barrows and fast growing pigs, respectively. However, a significant triple interaction also characterizes bone content (%). Percentage in gilts increased with weight, but the slope was more pronounced in the fast growing group, although slower gilts displayed a larger overall content. Slower growing barrows had lower bone content than their female counterpart and the changes from 107 to 125 kg followed a "V" shape pattern, while the opposite characterized faster growing barrows. These results are in contrast with Beattie et al. (1999) who reported a clear effect of weight increase on bone proportion in the carcass.

As already observed by Cisneros et al. (1996) and Eggert et al. (1996), gilt carcasses had higher ($p>0.001$) lean proportion and, thus, lower ($p>0.001$) fat proportion than barrow. Furthermore, slower growing pigs had a higher lean and lower fat proportion compared to high growth rate pigs, similar to McGloughlin et al. (1988) who have reported a negative relationship between high growth rate and carcass quality in Duroc parental line of pigs.

Muscle pH, drip loss and reflectance values were not affected by growth rate, sex and slaughter weight (Table 2). Except for Latorre et al. (2004), who reported a higher initial pH in the semimembranosus muscle and a lower L* value in the longissimus muscle with a weight increase from 116 to 133 kg, no consistent pattern of changes in these pork quality traits with weight have been reported in the literature (Eggert et al., 1996; Ellis and Bertol, 2001).

Only protein, IMF and collagen contents were affected by the factors under study. Protein was affected by slaughter weight and sex. Indeed, a lower ($p<0.05$) muscle protein content was found at 107 kg compared to 115 and 125 kg in the L muscle of both growth rate pigs. The effect of weight on protein content is in disagreement with a number of studies which showed inconsistent changes in protein content with slaughter weight (Ellis and Bertol, 2001; Latorre et al., 2004). In accordance with Latorre et al. (2004), muscle protein content was higher ($p<0.05$) in gilts than in barrows. As reported by several authors (Beattie et al., 1999; Ellis and Bertol, 2001; Latorre et al., 2004), no change in IMF was observed with increasing slaughter weight. On the other hand, IMF content was higher in barrows ($p<0.01$) than in gilts, as found by Barton-Gade (1987) and Van Oeckel and Warnants (2003). Contrary to Candek-Potokar et al. (1998) and Beattie et al. (1999), slaughter weight did not influence dry matter and total collagen content. However, the content of heat-soluble collagen content decreased ($p<0.001$) with weight. This may mean that tenderness of cooked pork

could be somewhat reduced in heavier animals, as already reported by Ellis et al. (1996) and Candek-Potokar et al. (1998).

Conclusions

Generally, the results of this study suggest that pigs can be slaughtered at heavier weights without compromising carcass quality and meat quality. However, given the lower lean proportion found in the fast growing genotypes used in this study, the increase in slaughter weight should be combined with appropriate genetics in order to avoid economical losses.

Acknowledgements

We are grateful to Agriculture and Agri-Food Canada, the CDPQ, the Fédération des producteurs de porcs du Québec, the Fond québecois d'adaptation des enterprises agroalimentaires, the Conseil pour le développement de l'agriculture du Québec and to the Canadian Meat Council for providing the financial support. Many thanks to the swine company F. Ménard for allowing J.A. Correa to conduct his M.Sc. project.

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Table 1. Least Squares Means for growth rate, sex and slaughter weight for carcass composition.

Slaughter weight (W)[c]	107 kg				115 kg				125 kg				Significance			Interactions
Growth rate (GR)[a]	A		B		A		B		A		B					
Sex (S)[b]	B	G	B	G	B	G	B	G	B	G	B	G	GR	S	W	GR*S*W
Carcass weight (kg)	85,6	85,6	86,3	84,7	92,0	92,2	92,2	91,7	100,2	99,1	101,4	99,2	NS	NS	***	NS
Dressing (%)	79,2	79,8	80,1	79,7	80,2	80,0	80,5	80,4	80,7	79,8	80,8	80,5	NS	NS	*	NS
Loin eye area (cm^2)	42,4	45,3	43,2	45,7	46,4	48,1	43,8	49,0	45,4	54,4	48,9	50,1	NS	***	***	**
Total lean (%)	42,9	45,5	46,1	47,1	43,7	46,0	44,3	48,1	41,8	46,8	45,0	48,4	***	***	NS	NS
Total fat (%)	23,6	20,5	20,5	19,8	22,1	20,8	21,9	19,1	24,4	19,9	22,0	19,0	***	***	NS	NS
Total bone (%)	8,1	8,0	8,4	8,5	8,5	8,2	8,0	8,6	7,7	8,5	8,2	8,6	*	*	NS	*

[a] GR : Growth rate = A :fast, B : slow, [b] S : Sex = B : barrows, G : Gilts [c] W :Slaughter weight

NS : Not significant, * : p < 0,05, ** : p < 0,01, ***: p < 0,001.

Table 2. Least Squares Means for growth rate, sex and slaughter weight for meat quality characteristics

Slaughter weight (W)[c]	107 kg				115 kg				125 kg				Significance		
Growth rate (GR)[a]	A		B		A		B		A		B				
Sex (S)[b]	B	G	B	G	B	G	B	G	B	G	B	G	GR	S	W
45min. pH	6,2	6,3	6,1	6,2	6,2	6,3	6,3	6,3	6,2	6,3	6,3	6,3	NS	NS	NS
Ultimate pH	5,6	5,7	5,7	5,6	5,6	5,6	5,7	5,6	5,7	5,6	5,6	5,6	NS	NS	NS
L*	51,5	50,8	49,9	51,1	50,9	50,3	50,2	50,0	50,3	51,0	50,5	50,0	NS	NS	NS
Drip loss (%)	5,1	4,5	4,9	5,4	5,6	5,8	4,9	5,8	4,9	5,1	4,8	5,5	NS	NS	NS
Dry matter (%)	26,1	26,2	26,1	26,0	26,0	25,7	26,2	26,2	26,4	26,1	26,4	25,9	NS	NS	NS
Protein (%)	23,3	23,7	23,2	23,5	23,8	23,6	23,6	23,9	23,4	23,8	23,9	23,9	NS	*	*
IMF (%)	2,2	2,0	2,2	1,8	1,7	1,5	2,0	1,8	2,3	1,7	2,2	1,5	NS	**	NS
Soluble collagen (%)	13,5	13,3	13,2	13,4	12,9	11,9	12,4	11,8	11,4	11,5	10,8	11,2	NS	NS	***
Total collagen (g $100g^{-1}$)	0,4	0,4	0,4	0,4	0,4	0,4	0,4	0,5	0,4	0,5	0,5	0,5	NS	NS	NS

[a] GR : Growth rate = A :fast, B : slow, [b] S : Sex = B : barrows, G : Gilts [c] W :Slaughter weight

NS : Not significant, * : p < 0,05, ** : p < 0,01, ***: p < 0,001.

ANALYSIS OF THE RELATIONSHIP BETWEEN DNA POLYMORPHISMS IN CANDIDATE GENES AND BEEF TENDERNESS

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Background

The factors underlying meat quality, while poorly understood, have a molecular basis, which are responsive to nutrition, environment and animal breeding. Major progress is being made worldwide in identifying variations in DNA (polymorphisms) which are related to genes influencing meat quality, growth and carcass yield. Some of these polymorphisms may have the potential to serve as markers of meat quality. These include polymorphisms in the calpain I and II and growth hormone genes. The calpain family are postulated to affect meat quality by initiating muscle protein degradation. Exons 9 and 14 of the calpain I gene were found to contain single nucleotide polymorphisms (SNPs), predicted to alter the protein sequence of calpain I. Analysis of these polymorphisms with Warner Bratzler shear force (WBSF) values showed that a relationship existed between the alleles containing the SNP and decreased meat tenderness (Page et al., 2002). A restriction fragment length polymorphism (RFLP) in the calpain II gene has also been identified (Zhang et al., 1996), which may have an effect on meat quality. Growth hormone has proven to be the major regulator of postnatal growth and metabolism in mammals and thus affects growth rate, body composition, health, milk production, and aging by modulating the expression of many genes. PCR-RFLP analysis was used to identify a polymorphism in intron 3 of the bovine growth hormone gene (Zhang *et al.,* 1993). A population of Piedmontese cattle was genotyped for this growth polymorphism and an association was found between WBSF at day 11 post-mortem and the polymorphic allele of bovine growth hormone (Di Stasio *et al.,* 2003).

Objective

The objective of this study was to evaluate the association of polymorphisms in bovine Calpain I exon 9 and 14, Calpain II and Growth Hormone genes with tenderness in Irish bovine *M.longissimus dorsi*.

Materials and methods

Genomic DNA was isolated from 25mg of *M. longissismus dorsi* muscles (n=284) on which quality attributes were also characterised, using the QIAamp® DNA minikit. Tenderness was measured by WBSF (Shackelford, 1991). Compositional analysis was conducted to determine any variation in intramuscular fat, protein and moisture levels. Sarcomere lengths were determined according to the laser diffraction method (Cross *et al.,* 1980). Polymerase Chain Reaction (PCR) was performed using primers specific for calpain I exon 9 and 14 (Costello et al., in preparation), calpain II (Zhang et al., 1996) and growth hormone genes (Zhang et al., 1993). Restriction digests were carried out using the restriction enzymes *Btg*I (calpain I exon 9), *DpnII* (caplain I exon 14) *HhaI* (calpain II) and *Msp*I (growth hormone). Digests were analysed on agarose gels using electrophoresis (Figure 1).

Associations between the four polymorphisms and WBSF day 14 values were tested using the GLM procedure of SAS. Association analysis was performed between the observed genotypes and WBSF at day 14 post-mortem. In a controlled data set, confounding factors were excluded, in particular animals with extreme values of sarcomere length and intramuscular fat (IMF). For sarcomere length, cut off points of 1.4µm and 1.85µm were employed. Samples with percentage of IMF greater than 5% were also removed from the sample set.

Results and discussion

A significant association was observed between the calpain I exon 9 genotypes and WBSF in both the controlled ($P = 0.0333$) and uncontrolled ($P = 0.0033$) data-sets (Tables 1 and 2). Animals with the

GA genotype exhibited decreased WBSF when compared to animals with the GG genotype. No association was observed between WBSF and calpain I exon 14 genotypes in either sample set. The V to I transition occurred in domain III of the protein, representing a conservative substitution of non-polar amino acids with no apparent function in terms of calpain enzyme activity. In contrast the G to A transition, which occurs in domain II of the protein, which has been identified as the proteolysis domain, thus this polymorphism could alter calpain I enzyme activity.

No significant association was observed between the growth hormone or calpain II polymorphisms, and WBSF. Di Stasio *et al.,* (2003) observed a significant association between the C allele of growth hormone and WBSF at day 11, but cautioned that the statistical model used to calculate the effect could lead to biased estimates of single gene effects, and that the highly unbalanced genotype distribution observed was undesirable for association analysis. However, our observed genotypic and allelic frequencies indicate that there is a higher frequency of C alleles over D alleles in general. Low frequencies for the D allele have previously been observed across Northern European breeds (Lagziel *et al.,* 2000), suggesting difficulties in finding a "balanced" genotype distribution in the Irish national herd.

Conclusions

It was found that the calpain 1 exon 9 genotypes had a statistically significant association with WBSF such that animals with the GA genotype exhibited decreased WBSF and increased tenderness, when compared to animals with the GG genotype. This observation concurs with that of earlier studies (Page *et al.,* 2002), strongly suggesting that this polymorphism is a functional marker for increased beef tenderness. None of the other genotypes examined were observed to have an effect on tenderness.

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Acknowledgements

This project is part funded by the US-Ireland Co-operation Progamme, managed by The Irish Department of Food and Agriculture.

Figure 1: Agarose gels showing RFLP patterns and corresponding genotypes for each of the polymorphisms. M = marker, UC = uncut fragment, bp = base pairs.

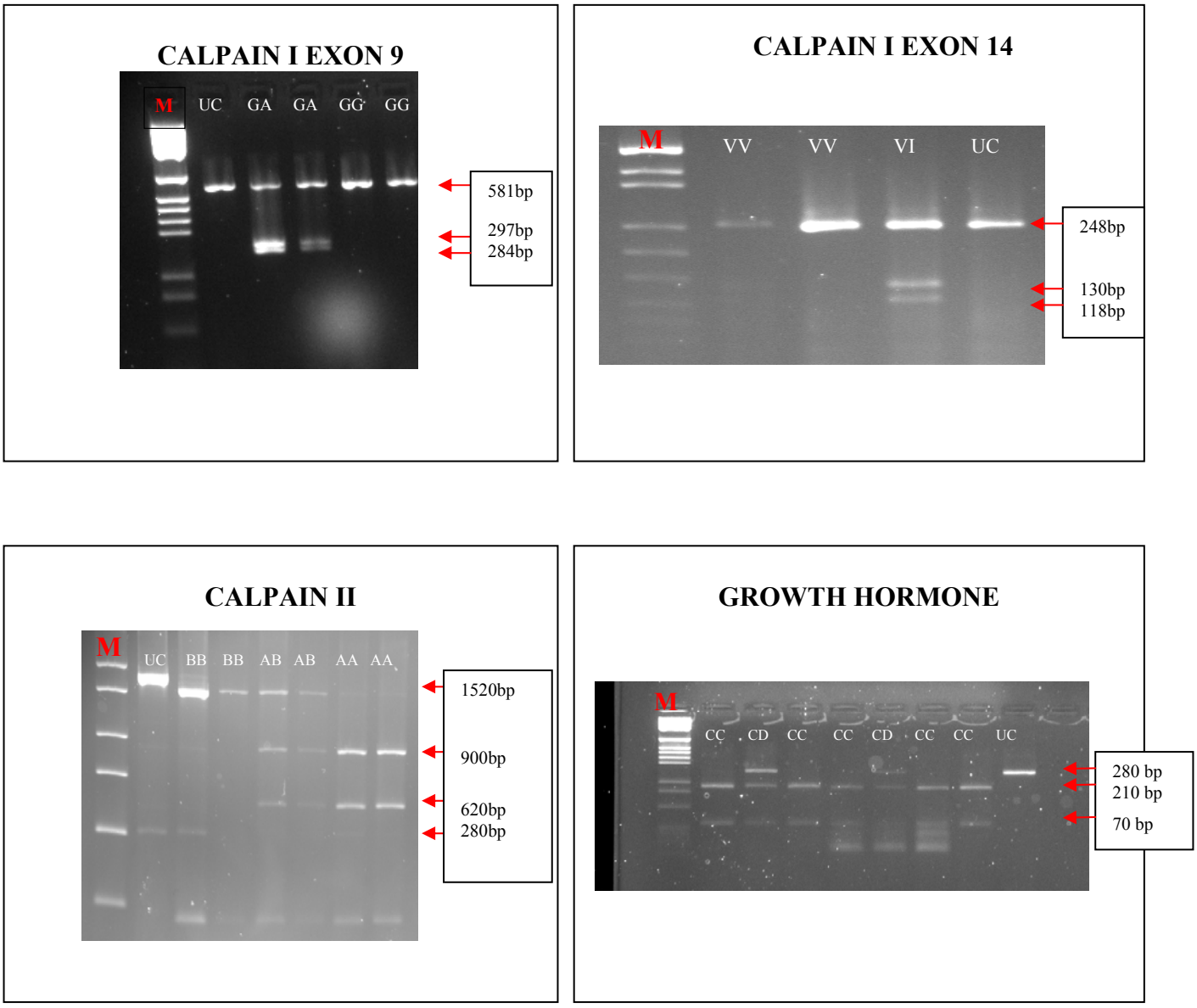

Table 1: Association analysis between observed genotypes and Warner Bratzler shear force (WBSF) values in bovine *M.longissimus dorsi* - Uncontrolled data-set

Gene	Genotype	Warner-Bratzler Shear Force (N) (mean±SDEV)	n	*P*
Calpain I, exon 9	**GG**	49.42 ± 23.61	208	0.0033
	GA	39.05 ± 11.50	63	
	AA	34.15	1	
Calpain I, exon 14	**VV**	46.20 ± 19.3	177	NS
	VI	51.82 ± 30.58	57	
Calpain II	**AA**	46.02 ± 16.36	41	NS
	AB	43.80 ± 16.63	143	
	BB	49.06 ± 27.58	86	
Growth Hormone	**DD**	33.53 ± 11.44	5	NS
	CD	46.61 ± 27.12	43	
	CC	47.59 ± 20.89	220	

NS = non significant; N = newtons

Table 2: Association analysis between observed genotypes and Warner Bratzler shear force (WBSF) values in bovine *M.longissimus dorsi* - Controlled data-set

Gene	Genotype	Warner-Bratzler Shear Force (N) (mean±SDEV)	n	*P*
Calpain I, exon 9	**GG**	51.34 ± 17.60	82	0.0333
	GA	42.24 ± 12.81	27	
	AA	34.15	1	
Calpain I, exon 14	**VV**	47.60 ± 15.04	77	NS
	VI	48.62 ± 15.74	28	
Calpain II	**AA**	46.68 ± 17.36	18	NS
	AB	46.90 ± 16.77	58	
	BB	50.72 ± 16.19	34	
Growth Hormone	**CC**	48.62 ± 16.24	99	NS
	CD	50.38 ± 21.72	13	

NS = non-significant. N = newtons

Note: Genotypes were assigned according to published literature. Genotypes for calpain I exon 9 and exon 14 refer to the coded amino acid (ie Exon 9: G refers to the amino acid glycine, A refers to alanine; exon 14: V refers to valine, I refers to isoleucine). Genotypes for calpain II and growth hormone were arbitrarily assigned.

FEEDING PROTEIN DEFICIENT DIETS DURING THE GROWER PHASE INCREASES INTRAMUSCULAR FAT DEPOSITION AND IMPROVES EATING QUALITY OF PORK

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Background

The Australian pork industry has implemented significant changes to its production systems to produce leaner and 'healthier' pork. This has seen the introduction of leaner genetic lines, improved feed formulations, and the use of metabolic modifiers to improve carcass leanness. The gains in 'carcass leanness' have resulted in reduced intramuscular fat (IM fat) levels or marbling, and the perception is that pork is now tougher, less moist and has reduced flavour. Recent studies have shown that IM fat levels in male and female pigs are as low as 1% in some genotypes while in others, levels can vary between <1% to 4% (Channon *et al*., 2001).

Cisneros *et al*. (1996) investigated the use of nutrition to improve the quality of pork and reported that reducing the protein to energy ratio of the finisher phase increased IM fat deposition, but was also associated with a corresponding increase in carcass fat and decreased feed conversion. However, results from a recent pilot experiment (D'Souza *et al*., 2003) indicated that feeding individually housed pigs grower diets with 15% reduced protein to energy ratio, and grower and finisher diets restricted in Vit A improved the IM fat levels in the *Longissimus* muscle from 1.3% to 2%. Also, the improvements in IM fat did not negatively affect the subcutaneous fat levels of the pigs. The highest IM fat levels (2.7%) were obtained when pigs were fed a 30% reduced protein to energy ratio diet. However, this increase was accompanied by a negative impact on growth performance and increased subcutaneous fat.

Objectives

The objectives of this experiment was to (i) determine if feeding pigs either a grower diet with 15% reduced protein to energy ratio, or grower and finisher diets restricted in Vit A increased IM fat in pigs under commercial conditions without affecting growth performance and carcass quality, and (ii) to determine if nutritionally increased IM fat in pigs resulted in improved eating quality of pork.

Methods

A total of 63 Large White x Landrace x Duroc crossbred female pigs of similar age were used in this experiment. At approximately 14-16 days of age the pigs were transported to the Department of Agriculture, WA, Research Unit, Medina, and group housed in an environmentally controlled weaner room for three weeks (15-20kg LW), after which they were moved to a naturally ventilated experimental grower/finisher shed. The pigs were group-housed (7 pigs per pen). The pigs were stratified on a weight basis and randomly allocated to one of the 3 diet treatments. The dietary treatments were (i) Control (commercial grower and finisher diets), (ii) 15% reduced protein to energy ratio during the grower growth phase (commercial diet during the finisher growth phase), and (iii) Vit A restricted diet during the grower and finisher growth phase (commercial finisher diet without any added Vit A). All pigs had *ad libitum* access to feed, and water via nipple drinkers. At 23 weeks of age, the pigs were transported to a commercial abattoir and slaughtered according to standard commercial procedures.

Carcass weight and depth of backfat (Hennessy probe) were measured on the hot carcass as per commercial practice. Intramuscular fat in the *Longissimus thoracis* was chemically determined using the method of direct soxhlet extraction of fat by a solvent (hexane) and expressed as the weight percentage of wet muscle tissue (AOAC, 1990). The pH of the *Longissimus thoracis* muscle between the 12th and 13th rib was determined at 24h (pH_u) post-slaughter using a portable pH/temperature meter (Jenco Electronic Ltd, Model 6009) fitted with a polypropylene spear-type gel electrode (Ionode IJ42S, Brisbane, QLD) and a

temperature probe. Drip loss from the *Longissimus* muscle was measured using the filter paper method (Honikel, 1987). Surface lightness (L^*) of the *Longissimus* muscle was measured using a Minolta Chromameter CR-400, using D_{65} lighting, a 2^o standard observer and a measuring aperture of 8mm, standardised to a white tile. Loin samples were also used to measure cook loss and shear force. Shear force of the cooked sample was determined using a Warner Bratzler shear blade fitted to an Instron Universal Testing Machine.

One hundred and fifty boneless *Longissimus thoracis* steaks were used for the sensory analysis in this study. The steaks were individually vacuum packaged and frozen approximately 24 hours post-slaughter. Ten pigs per treatment were used. Five steaks per loin (12cm portion between the 10th and the 14th rib, 2 cm thick) were used. Thirty-two boar taint free *Longissimus* steaks (20mm thickness) were also collected for use as "warm-up steaks" during the cooking of pork samples. The steaks were thawed and cooked according to a standard protocol. The pork steaks were cooked using a Silex flat-plate grill for approximately five minutes to a standardised degree of doneness (medium/well-done, 190°C until an internal temperature of 75°C was reached). The cooking method ensured that steaks were not contaminated with flavour components from other steaks being tested concurrently. The cooked steaks were rested for 2 min, cut into half (width-ways) and immediately presented to consumers for evaluation. Consumers assessed the steaks for odour, tenderness, juiciness, flavour and overall acceptance using a line scale where 1 = dislike extremely to 100 = like extremely. The consumer taste panel were also asked to grade the pork into one of 5 quality grades where, 1 = unsatisfactory, 2 = below average, 3 = average, 4 = above average, 5 = premium

Results and Discussion

The results indicate that there was no significant difference ($P>0.005$) in carcass weight and carcass dressing % between the dietary treatments. There were no significant differences ($P>0.05$) in P2 backfat depth between the dietary treatments. The results indicate that pigs fed the 15% reduced protein:energy diet had significantly higher ($P<0.05$) IM fat levels (1.8%) compared to pigs fed the control (1.4%) and the Vit A restricted diets (1.5%). These results are in contrast to that reported by D'Souza *et al*. (2003) where pigs fed either the 15% reduced protein to energy ratio diet, or the vitamin A restricted diet had significantly higher IM fat levels compared to pigs fed the control diets.

It has been hypothesised that the effect of Vit A on IM fat deposition is mediated by retinoic acid, a derivative of Vit A, which regulates the adipogenic differentiation of fibroblasts, inhibiting the terminal differentiation of intramuscular adipose tissue in cattle (Kuri-Harcuch, 1982). It has also been proposed that retinoic acid regulates growth hormone gene expression (Bedo *et al*., 1989), which in turn decreases fat deposition and marbling in steers (Dalke *et al*., 1992). The results from the previous study by D'Souza *et al*. (2003) indicated that feeding pigs a Vit A restricted diet during the grower and finisher phase significantly lowered liver Vit A levels compared to the pigs fed a control diet. Hence it is possible that the Vit A levels in the Vit A restricted pigs in this experiment were not depleted sufficiently to result in increased IM fat levels. A similar variable response to Vit A restricted diet has been observed in cattle. Vitamin A depletion was found to be dependant Vit A levels at birth of the new born calf (in turn dependant on its the mother's Vit A levels) and hence time taken to deplete the Vit A levels sufficiently to increase IM fat levels was significantly different (D. Pethick, Personal communication).

Pork from the 15% reduced protein:energy treatment had significantly lower ($P=0.010$) ultimate muscle pH, was paler ($P=0.004$) (higher L*) and had a higher ($P=0.006$) b* value (yellowness-blueness) compared to pork from the control treatment group. There was no significant difference ($P>0.05$) in surface exudate, cook loss % and shear force of pork between the dietary treatments. The sensory pork quality results indicate that there was no significant difference ($P>0.05$) in aroma and flavour of pork from the different dietary treatments, although pork from pigs fed the 15% reduced protein:energy diet tended to have better flavour ($P=0.098$) scores compared to pigs fed the Vit A restricted diet. Pork from pigs fed the 15% reduced protein:energy diet was considered to have the best ($P=0.001$) juiciness, tenderness, and overall acceptability, followed by pork from the control treatment, while pork from the Vit A restricted diet was least preferred. Although significantly higher ($P=0.004$), consumers rated the pork from pigs fed the 15% reduced protein:energy diet with the same quality grade (ie Grade 3) compared to pork from control pigs.

Threshold levels of IM fat required for optimal eating quality have been reported to be about 2.5 % (Bejerholm and Barton Gade, 1986). While the IM fat levels in the 15% reduced protein to energy diet group in this experiment were below the threshold levels required for optimal eating quality, the pork was considered to have better juiciness, tenderness and overall acceptability compared to pigs fed the control and the Vit A restricted diet. However, the quality grade assigned by the consumer panel in this experiment suggests that consumers still class the eating quality of pork from the 15% reduced protein to energy diet treatment as having average eating quality and being similar to pork from the control diet treatment. This suggests that the IM fat levels needs to be near or above the threshold of 2.5% to significantly improve the eating quality of pork to a higher quality grade (ie quality grade 4 or 5).

Conclusions

The results from this experiment indicate that feeding pigs a 15% reduced protein to energy ratio diet significantly increased IM fat levels compared to pigs fed a control diet and pigs fed a Vit A restricted diet during the grower and finisher growth phase. Pork from pigs fed the 15% reduced protein to energy ratio diet were found to have better tenderness, juiciness and overall acceptability scores compared to pork from the control and Vit A restricted diet treatments.

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ACKNOWLEDGEMENTS

The authors are appreciative of the funding provided by Australian Pork Limited, Canberra, Australia (Project DAW 1636). The technical assistance provided by Stacey McCullough, Megan Trezona, Roland Nicholls, Hugh Payne and Bob Davis is gratefully acknowledged.

Table 1. The effect of grower and finisher phase dietary treatments on carcass quality and objective and sensory (consumer taste panel) pork quality of the *Longissimus thoracis* muscle of group housed female pigs.

	Dietary Treatments				
	Control	**- 15% P:E**	**-Vit A**	**L.s.d.**	**P-values**
No. of Pigs	21	21	21	-	-
Liveweight (kg)	107.0	103.4	106.1	6.59	0.520
Carcass weight (kg)	72.3	70.7	71.4	4.78	0.798
P2 (mm)	12.7	12.3	13.4	1.46	0.304
IM FAT %	1.4	1.8	1.5	0.255	0.007
Muscle pH (24h)	5.63	5.44	5.54	0.121	0.010
Muscle colour					
L*	48.9	52.9	51.0	2.31	0.004
a*	6.09	6.79	5.98	0.853	0.127
b*	4.17	5.27	4.48	0.678	0.006
Surface exudate (mg)	55.6	49.8	40.2	16.4	0.172
Cook loss (%)	32.5	32.8	30.8	2.27	0.163
Shear force (kg)	5.3	4.7	4.7	0.825	0.185
Consumer taste panel:					
Aroma[A]	68	66	65	6.74	0.635
Flavour[A]	68	72	65	6.83	0.098
Juiciness[A]	57	67	49	9.09	0.001
Tenderness[A]	55	69	52	8.96	0.001
Overall acceptability[A]	67	74	58	7.85	0.001
Quality grade[B]	3.4	3.7	3.1	0.358	0.004

[A]Line score; 1 = dislike extremely; 100 = like extremely
[B]Quality grade score; 1 = unsatisfactory, 2 = below average, 3 = average, 4 = above average, 5 = premium

INFLUENCE OF SEASONS ON INCIDENCE OF DIFFERENT *M. SEMIMEMBRANOSUS* QUALITY OF PIG HALVES OF THREE-RACE HYBRIDS

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Background

Climate conditions may affect significantly the quality of meat post mortem. Pigs are particularly sensitive to high tempertures (Lengerken and Hennebach, 1980; Church and Wood, 1992). Ludvigsen, according to Briskey (1964) has found pale, soft and exudative (PSE) muscle incidence to be higher in warmer periods. Patricia Barton-Gade (1971), in Denmark, found more PSE meat in summer and autumn, than in winter. Krzecio et al. (2001), were investigating different HAL genotypes in different seasons and pointed to higher incidence of PSE meat in spring-summer compared to autumn-winter. However, Schepper (1971), in Germany, found PSE and DFD meat incidences to be higher in autumn and winter than in spring and summer. Danica Manojlović and Rahelić (1978) found almost two times higher incidence of PSE meat in the period when the climare factors are changing, e.g. spring and autumn than in summer and winter. Okanović et al. (1992) showed that the highest incidence of PSE meat, regarding total mass of ham, is in spring.

Objectives

The objective of this study was to investigate the influence of seasons on quality of halves and meat of three-race pigs determining the incidence of different quality *M. semimembranosus* (RFN, RSE, PSE, DFD) on the basis of technological parameters: pH_i, pH_u, WHC and L* and our criteria for quality estimation (Tomović, 2002; Natalija Džinić et al., 2003, 2004)).

Materials and methods

The investigations included 43 pigs of three-race hybrids, from the crosbreeding program performed at a farm in Serbia and Montenegro. The pigs were slaughtered and investigated in autumn (n=14), winter (n=13), and spring (n=16). The pigs were fed standard feed during fattening, and heads of approximately uniform age and mass were transported to the slaughterhouse. After a night rest, the pigs were stunned, debleeded and processed by the standard technological procedure.

Meat yield (%) was determined by partial dissection method (Walstra and Merkus, 1996) of cooled left halves.

The pH_i was determined on right halves 45 min post mortem, and pH_u 24 hrs p.m. in *M. semimembranosus* (MSM), caudo-medial part, using the pH-meter ULTRA X, type UX 390, Gronert (Germany) INGOLD penetrating electrode.

Samples (200 – 300 g) taken from the caudo-cranial part (MSM) were used 24 hrs p.m. for the determination of colour, water holding capacity (WHC), marbleness and chemical composition. The color was determined sensorily (1 – very pale; 7 – very dark) and with MOM Color 100 and the color characteristics were expressed in CIE L*a*b* system (Robertson, 1977). WHC was determined by compression method and expressed as % of bound water (Grau and Hamm, 1953). Marbleness was determined sensorily, applying the analytical descriptive test (1 – without marbleness; 10 – very expressed marbleness).

The content of moisture, proteins, free fat and total ash was determined by standard methods (AOAC, 1999).

The incidence of different MSM quality in different seasons was determined on the basis of parameters and criteria for MSM quality: PSE: pH_i<5,8, pH_u<5,4, WHC<50%, L*>55; RSE: pH_i=5,8-6,0, pH_u=5,85-6,2; WHC=50-60, L*=50-55; RFN: pH_i>6,0, pH_u=5,4-5,85, WHC=60-70, L*=45-50; DFD: pH_u>6,2, WHC>70, L*<45).

Results and discussion

Significantly higher meat yield (P≤0,01) was found in halves of hybrids in spring (55,83%), compared with meat yield in halves of hybrids in winter (55,06%). However, it was not significantly higher in halves in autumn (55,32%) (P>0,05).

Table 1. Meat yield in halves determined in different seasons by partial dissection

Characteristic	Season			t – test		
	Autumn	Winter	Spring	A – W	A – S	W – S
Mass of halves (kg)	38,28±1,77	40,77±1,88	36,67±2,49	*	NS	NS
Meat yield in halves (kg)	21,18±1,6	22,77±1,41	20,43±1,32	**	NS	**
Meat yield in halves (%)	55,32±2,33	55,06±3,40	55,83±2,61	**	NS	**

NSP>0,05 *P≤0,05 ** P≤0,01

The investigation of technological characteristics (Table 2) showed that the average pH_i (6,27) and pH_u (5,63) measured in MSM of three-race hybrids were significantly higher (P≤0,01) in autumn compared with the same parameters in winter (6,08 and 5,53), but not in spring. The average pH_i values of MSM respond to the values of muscles which are potentially of normal quality (pH_i>6,0) (Tomović, 2002). WHC (%) of MSM in autumn was significantly higher (P≤0,01) compared with values determined in winter, but not compared with results in spring. The lowest WHC value (39,18%) was found in winter. On the basis of criterion for WHC, all investigated MSM are of PSE quality, on the average (WHC<50%).

Table 2. Effect of seasons on technological quality of *M. semimembranosus*

Characteristic	Season			t – test		
	Autumn	Winter	Spring	A – W	A – S	W – S
pH_i	6,27±0,22	6,08±0,24	6,06±0,31	**	NS	**
pH_u	5,63±0,11	5,53±0,07	5,53±0,15	**	NS	**
WHC (%)	44,55±7,9	39,18±6,73	41,64±7,27	**	NS	**
Marbleness	4,44±1,44	3,00±1,07	3,33±0,98	**	NS	*
Colour (sensory)	3,57±0,77	2,99±0,52	3,54±0,75	**	NS	**
L^*	53,15±2,49	51,39±2,49	51,02±3,41	**	**	NS
a*	10,99±2,93	9,86±1,14	4,28±4,29	*	**	*
b*	13,81±1,26	8,97±1,02	7,33±1,87	**	**	NS
Moisture (g/100g)	76,09±0,36	75,92±0,41	75,56±0,71	**	*	**
Protein (g/100g)	21,65±0,35	22,14±0,39	22,35±0,44	**	**	**
Free fat (g/100g)	1,12±0,26	0,72±0,22	0,85±0,31	**	*	**
Total ash (g/100g)	1,18±0,05	1,24±0,22	1,24±0,2	**	NS	NS

The color – lightness L* – of MSM was also determined (Table 2). It was found that the investigated MSM were significantly lighter in autumn (L*=53,15) compared to winter (51,39%) and spring (L*=51,02). According to our criteria for L*, the investigated MSM were of PSE characteristics, on the average, in all investigated seasons. The moisture content of MSM in autumn was significantly (P≤0,05) and highly significantly (P≤0,01) higher (76,09) compared to winter, e.g. spring. The sensory assesment showed that the

average color of MSM was significantly lighter (P≤0,01) in autumn (3,57), compared to winter, however not significantly lighter compared to spring. The average grade for marbleness of MSM in winter (3,0) was significantly lower (P≤0,01) and (P≤0,05) compared to autumn, e.g. spring. The differences in protein content of MSM in different seasons are significant (P≤0,01), however, it was also found that the protein content was higher than 21% in all MSM samples. This is an important finding, since this aim has to be fulfilled in contemporary breeding of pigs (Vidović, 1999).

The analysis of average technological quality of MSM of three race hybrids, in different seasons, confirmed that the seasons affect the quality of meat.

On the basis of criterion for pH_i, it was found (Table 3), that the highest potential incidence of PSE characteristics of MSM was in spring, 31,25%, and significantly lower in autumn (7,7%). Further, on the basis of criterion for pH_u, all investigated MSM were of RFN quality in winter, 7,2% of MSM were of RSE quality in autumn, and 6,7% of MSM were of DFD characteristics in spring. According to criterion for L*, it was found that the highest incidence of PSE characteristics in MSM was in spring (50%), and the highest incidence of RSE characteristics of MSM, 64,3%, was found in autumn.The obtained results further show that according to criterion for WHC, all MSM in winter were of PSE quality, e.g. 81,2% and 71,6% in spring and autumn, respectively.

Table 3. Effect of seasons on incidence of different meat quality of *M. semimembranosus* in halves of three race hybrids

Parameters	Quality	Season		
		Autumn	Winter	Spring
pH_i	PSE (%)	0	7,7	31,25
	RSE (%)	21,4	23,1	12,5
	RFN (%)	78,6	69,2	56,25
pH_u	PSE (%)	0	0	0
	RSE (%)	7,2	0	0
	RFN (%)	92,8	100	93,3
	DFD (%)	0	0	6,7
L*	PSE (%)	28,6	15,4	50,0
	RSE (%)	64,3	46,2	37,5
	RFN (%)	7,1	30,7	12,50
	DFD (%)	0	7,7	0,0
WHC	PSE (%)	71,6	100	81,2
	RSE (%)	21,3	0	12,5
	RFN (%)	7,1	0	6,3
	DFD (%)	0	0	0

Conclusions

The highest average meat yield in halves of three-race hybrids was found in spring (55,83%), and the lowest in winter (55,06%) (P≤0,01). The investigated MSM, in all seasons, were on the average of PSE and RSE quality regarding criteria for WHC e.g. color L*, respectively. The highest incidence of normal quality 100% (according to criterion for pH_u) and 30,7% (criterion for L*) was in winter. The incidence of MSM of normal (RFN) quality was 7,1%, according to criteria for color L* and WHC in autumn. In spring, 12,5% and 6,3% of MSM were of normal quality, according to criteria for color L* and WHC, respectively. Accrding to criterion for color L*, the highest incidence of PSE quality (50%) was found in spring. According to criterion for WHC, 100%, 81,2% and 71,6% of MSM was of PSE quality, from carcasses of pigs slaughtered in winter, spring and winter, respectively.

Acknowledgment

This work was supported by the Serbian Ministry of Science, Technologies and Development through the Project BNT.5.2.1.7101.B "Production of cooked ham"

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MECHANICAL STIMULATION OF C2C12 CELLS INCREASES M-CALPAIN EXPRESSION, FOCAL ADHESION PLAQUE PROTEIN DEGRADATION AND CELL DIFFERENTIATION

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Background

The process of muscle growth is a central issue in the business of producing animals for meat. At the most fundamental level, the process of muscle development and growth is a complex sequence of events whereby muscle cells respond to a number of stimuli in order to form organised muscle tissue. Increase in muscle mass is greatly influenced by the rate of skeletal muscle protein synthesis, a process that can be altered by mechanical forces. Stretch- or load-induced signaling is now beginning to be understood as a factor which affects the mass and phenotype of muscles as well as the expression of a number of proteins within muscle cells (Carsen *et al.,* 1996; Winchester *et al.,* 1991). Use of magnetic field to produce mechanical forces to stimulate cell populations has been well documented (Glogauer *et al.,* 1998). Magnetic field stimulation has been shown to affect transcription of specific gene sequences, protein synthesis, the immune system and increase in Ca^{2+} influx (Tatsumi *et al*, 2002).

The past 10 years has seen a dramatic increase in the understanding of how proteolytic enzymes such as calpains can affect the growth of muscle (Goll et al, 1992). In vivo studies have shown that m-calpain is necessary for myoblast fusion leading to the formation of muscle fibres (Barnoy et al., 1998; Joffroy et al., 2000), and that inhibition of this enzyme restricts myotube formation (Temm-Grove et al., 1999). Whether there is a link between stretch- or load induced signaling and m-calpain expression and activation is not known.

Objectives

The purpose of this study was to investigate the role of mechanical signals in m-calpain induced muscle cell fusion. The specific goal of this work was to determine whether a mechanically stimulated cell population showed differences in expression and localization of m-calpain, an enzyme required for myotube formation in vitro.

Materials and methods

C2C12 cells from ATCC (American Type Culture Collection, Manassus, VA), were maintained under a 5% CO_2 stream at 37°C, plated at 7,500 cells/cm^2 on tissue culture surfaces and grown to 75% confluence in DMEM containing 10% foetal calf serum (FCS).

For stimulation experiments, cells were incubated with laminin or fibronectin coated 1 μm Encapsulated Super-Paramagnetic Microspheres (EMI- 100/40) for 30 minutes to allow for bead attachment and then rinsed to remove unattached beads. Stimulated cultures were placed on a heating plate maintained at 37 °C under the electromagnet for a period of 6 hrs. Control cultures were kept in incubator at 37 °C during that period. Where indicated, cells were incubated for 1 h at 37°C in PBS containing a 1:100 dilution of anti-integrin blocking antibody prior to the attachment of the microspheres.

A magnetic field of 0.5 mT was generated by an electromagnet (Power Generator 0-30 Volts, 0.1-100 Hz; Elcanic A/S, Denmark). The magnet produced alternating MF at frequency of 1 Hz. Magnet was placed 10 mm over the monolayer of cells during the stimulation period. Cells lacking beads but placed under the magnetic field were used as an additional control.

For immunohistochemical analysis, cells were fixed with 4% paraformaldehyde, permeabilised with saponin and stained the indicated primary antibodies, followed by Alexa 488 secondary antibodies (Molecular Probes, Eugene Oregon). Images were obtained using a Leica DmIRB inverted microscope (Leica, DK) couples to a Coolsnap digital camera (Roper Scientific, DE). Images were obtained and analysed using the Image Pro Plus system (Image House, DK).

The activity of the enzyme creatine phosphokinase (CPK) increases as myoblasts fuse into myotubes. The level of CPK was therefore assayed as a measure of cell differentiation and myotube formation in the different culture conditions, using the CPK assay kit (Sigma, St. Louis, MO), on cell suspensions whose total protein concentration was determined using a BCA kit (Pierce, Rockford, IL), on homogenised cell suspensions.

Results and discussion

Cells mechanically stimulated with laminin coated microspheres show increased CPK activity. The activity of CPK increases as myoblasts differentiate into myotubes. When cells were stimulated with laminin coated microspheres, the CPK activity seen in the culture rose approximately 40% (Figure 1). Similar increases were not seen in any of the control cultures, whether they were exposed to the magnetic field in the absence of spheres or had the microsperes present without being exposed to the magnetic field. No differences in CPK activity were seen when cells were stimulated with fibronectin coated beads. or in cell populations that had been stimulated with fibronectin coated microspheres. These results indicate that mechanical stimulation of myoblasts through laminin but not fibronectin receptors can significantly stimulate myoblast differentiation. Additionally, the response was not due to the simple binding of spheres to ECM receptors or to the exposure of cells to a magnetic field.

Expression of m-calpain but not μ-calpain is up regulated in cells exposed to mechanical stimulation. M-calpain is thought to be an essential enzyme in the differentiation and fusion of myoblasts. Cells mechanically stimulated with laminin coated microspheres express a higher amount of m-calpain, while the expression of the related enzyme μ-calpain is unaffected by this stimulation (Figure 2). Similarly, cells mechanically stimulated with fibronectin coated spheres do not show an increase in m-calpain expression (data not shown).

Mechanical stimulation through laminin receptors causes a re-localization of m-calpain to focal adhesion, followed by a disappearance of the enzyme from these regions. In control cells, m-calpain is found throughout the cytoplasm and in small quantities at the cell surface (Figure 3a). After 30 min. of mechanical stimulation, the amount of enzyme is increased in these focal adhesions, as it is in the whole cell (Figure 3b). 3 hours later, the amount of m-calpain in the cells remains high, but less is found in the focal adhesion sites (Figure 3c). The decrease in staining may be due to autolysis of the enzyme after activation.

Mechanical stimulation via laminin receptors leads to a breakdown of paxillin in focal adhesion complexes. m-calpain is known to associate with focal adhesion complexes at the cell membrane of muscle cells and focal adhesion proteins are known substrates for this enzyme. After mechanical stimulation of C2C12 cells via the laminin receptor, staining for paxillin is not longer found in punctuate regions on the cell surface (Figure 4). Similar results have been seen when cells are stained for talin, and β1-integrin, also known substrates for m-calpain (data not shown). Previous work in our laboratory has shown that blocking m-calpain activity with specific enzyme inhibitors blocks the breakdown of focal adhesion proteins on the cell surface.

Conclusions

Muscle cells are exposed to both a chemically and mechanically active environment during differentiation. During the developmental process, myoblasts come in contact with different extracellular matrix proteins, through which mechanical signals can be generated. We have shown that mechanical signals transmitted through the C2C12 cells interaction with laminin cause an increase in cellular differentiation. At the same

time, this signaling results in not only an increase in the expression of the proteolytic enzyme m-calpain, but a resultant breakdown of focal adhesion proteins on the cell surface. This breakdown may well be due to the activation and subsequent autolysis of the enzyme in this region.

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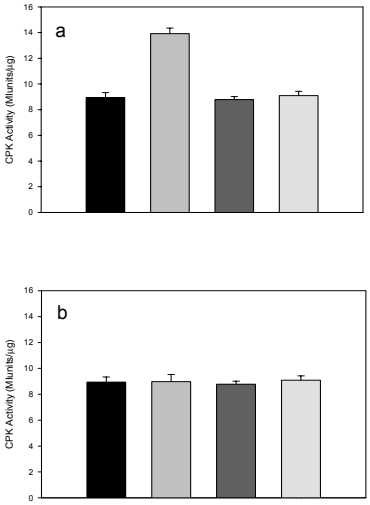


Figure 1. The effect of mechanical stimulation on CPK activity in C2C12 cells. C2C12 cell cultures stimulated with laminin coated microspheres showed a higher CPK activity than controls (a). CPK activity was unaffected by either the presence of, or stimulation by fibronectin coated microspheres (b).

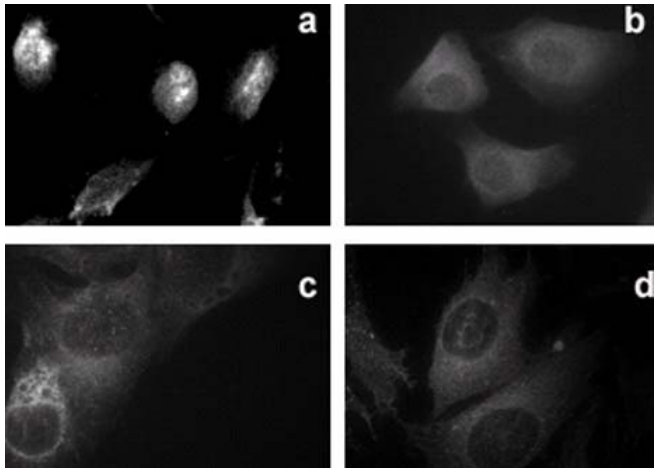


Figure 2. The effect of mechanical stimulation via laminin receptors on m-calpain expression in C2C12 cells. Mechanically stimulated (a,c) and un-stimulated (b,d) myoblasts stained for either m-calpain (a,b) and m-calpain (c,d). Mechanical stimulation increases m-calpain but not m-calpain expression.

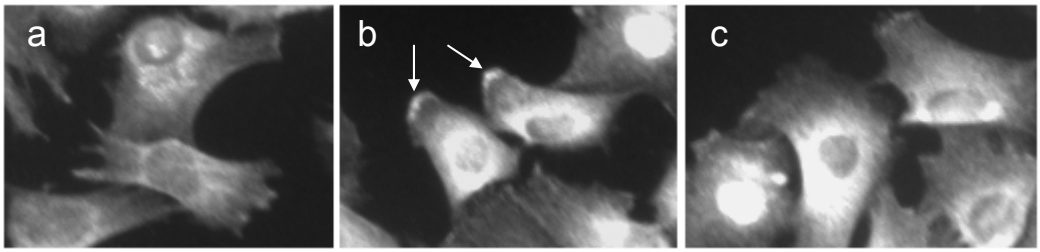


Figure 3. Localization of m-calpain after mechanical stimulation with laminin coated spheres. A. Prior to stimulation. B. 30 min of stimulation. C. 4 h of stimulation. Arrows - focal adhesion complexes. Expression of m-calpain increases shortly after mechanical stimulation begins, and the enzyme relocates to the cell surface. After a longer stimulation time, the staining at the cell surface disappears.

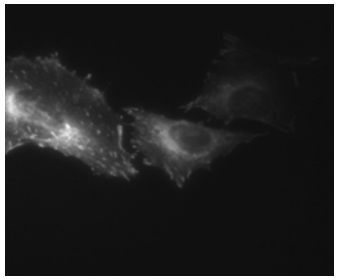
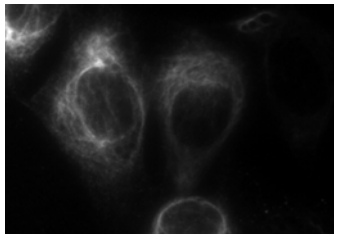


Figure 4. Breakdown of focal adhesions after mechanical stimulation of myoblasts. Stimulated (Bottom) and un-stimulated (Top) myoblasts were stained for the focal adhesion plaque protein paxillin. Paxillin staining can be seen in punctate focal adhesions in un-stimulated cells but not in stimulated cells.



BEHAVIOR OF CHICKEN BREAST MUSCLE MYOSIN SOLUBILIZED IN NEUTRAL AND LOW IONIC STRENGTH SOLUTIONS

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Background

Meat, an essential food for humans, is a very rich source of proteins and contains all essential amino acids. However, its usage is limited because it is maintained in a solid state even after cooking. If the proteins in meat could be solubilized in water or a solution of low ionic strength, utilization of meat could be extended in various ways, as a liquid diet for elderly people. We have already established a method to solubilize more than 80% of chicken breast muscle myofibrillar proteins in water (Ito *et al*., 2003). To accomplish solubilization, it is essential to maintain myofibrillar suspensions at neutral pH with L-histidine (L-His) and low ionic strength and to disrupt the high-ordered structures of myofibrils by ultrasonication. Furthermore, we have shown that myosin prepared from chicken skeletal muscle, one of the major myofibrillar proteins, is also solubilized in a solution of low ionic strength by ultrasonication (Ito *et al*., 2002). However, it has not been determined whether ultrasonication and presence of L-His are essential for solubilization of myosin in a solution of low ionic strength.

Objectives

The objective of this study was to examine the behavior of myosin solubilized in neutral and low ionic strength solutions without ultrasonication.

Materials and methods

Myosin was prepared from chicken breast muscle (Perry, 1955) and solubilized in 0.6 M KCl, pH 6.5. Myosin was dialyzed against neutral and low ionic strength solutions containing 1 mM KCl and various concentrations of L-His. Each dialyzed myosin suspension was centrifuged for 120 min at 100,000×g, and the obtained supernatant was defined as water-soluble myosin. The solubility of myosin in low ionic strength solutions was determined.

SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was performed according to the method of Laemmli *et al*. Water-soluble myosin molecules stained by the method of negative staining and subjected to rotary shadowing were observed under a transmission electron microscope. Gel-formation ability of water-soluble myosin was tested by heating for 10 minutes in a water bath at 70 °C.

Results and discussion

In the presence of L-His, myosin was solubilized in neutral and low ionic strength solution without ultrasonication (Figure 1). As shown in the SDS-PAGE patterns (Figure 2), water-soluble myosin contained intact heavy chains and light chains. The solubility increased with increase in the concentration of L-His to 7 mM. However, the solubility decreased when the concentration of L-His exceeded 10 mM. This indicates that the solubility is dependent on L-His concentration. The solubility was also dependent on protein concentration of myosin. When a water-soluble myosin solution was dialyzed against a solution of physiological ionic strength, myosin molecules aggregated and were precipitated by centrifugation. Therefore, it is concluded that water-soluble myosin maintains the ability of self-assembly under physiological conditions.

Using the method of negative staining, we observed that water-soluble myosin formed a very thin filament-like structure (Figure 3A, B). This structure clearly differed from the thick filament observed under the physiological condition. Also, using the method of rotary shadowing, we confirmed the existence of myosin monomers that have two heads and a long rod as do native myosin molecules (Figure 3C). The rod of water-soluble myosin was longer than that of native myosin soluble in a solution of high ionic strength (Figure 4). These observations indicate that water-soluble myosin in neutral and low ionic strength solutions

exists in two forms, separated monomers and very thin filament-like structures. Heating could not induce any gelation of water-soluble myosin.

Conclusions

Myosin is soluble in a solution of low ionic strength in the presence of L-His without ultrasonication. In such a condition, myosin molecules exist in two forms, separated monomers and very thin filament-like structures. The rod of the separated monomer is longer than that of the native myosin molecule. These results suggest that low ionic strength and L-His cause some conformational changes in myosin molecules resulting in lengthening of its rod portion. Water-soluble myosin could not be changed into gel by heating.

Acknowledgements

We thank Mr. Toshiaki Ito of the Electron Microscope Laboratory, Graduate School of Agriculture, Hokkaido University for his technical assistance in taking electron micrographs.

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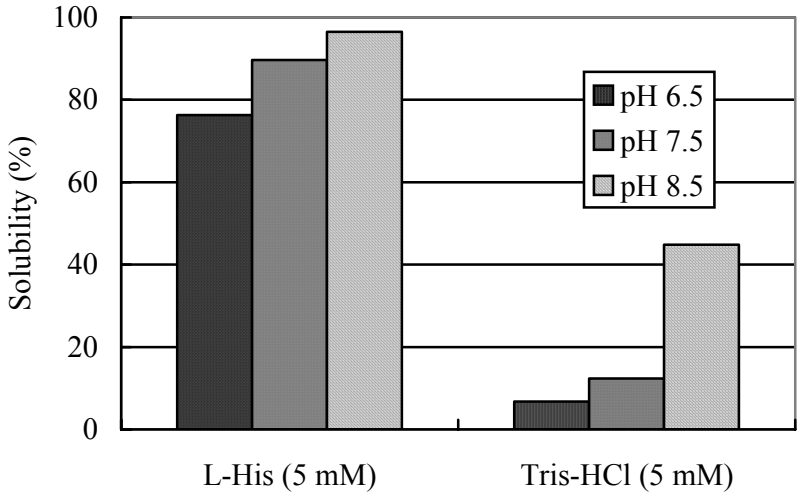

Figure 1. Effect of L-His on solubilization of myosin in neutral and low ionic strength solutions. Myosin (5 mg/ml) was dialyzed against 1 mM KCl, 5 mM L-His or Tris-HCl.

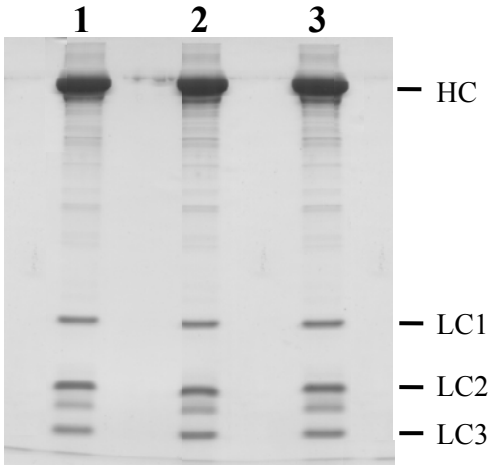

Figure 2. SDS-PAGE patterns of dialyzed myosin suspension (lane 1), water-soluble myosin (lane 2) and precipitation (lane 3). HC: myosin heavy chain, LC: myosin light chain

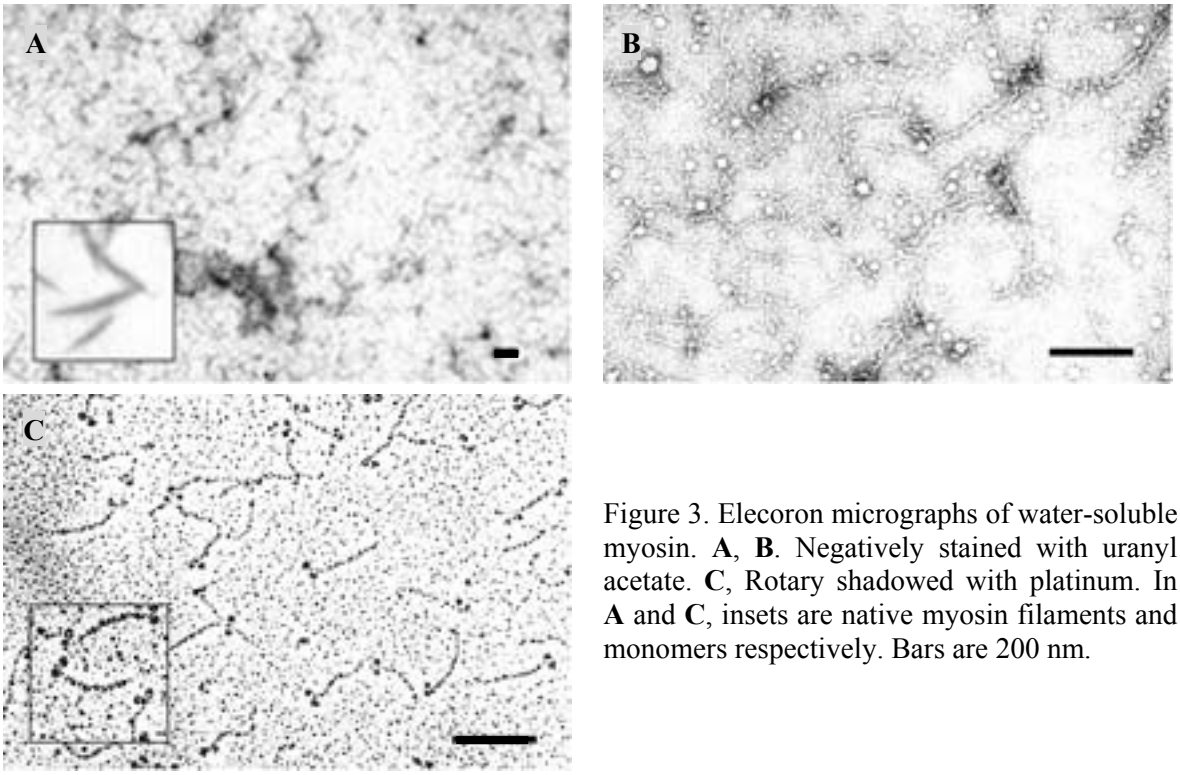

Figure 3. Elecoron micrographs of water-soluble myosin. **A**, **B**. Negatively stained with uranyl acetate. **C**, Rotary shadowed with platinum. In **A** and **C**, insets are native myosin filaments and monomers respectively. Bars are 200 nm.

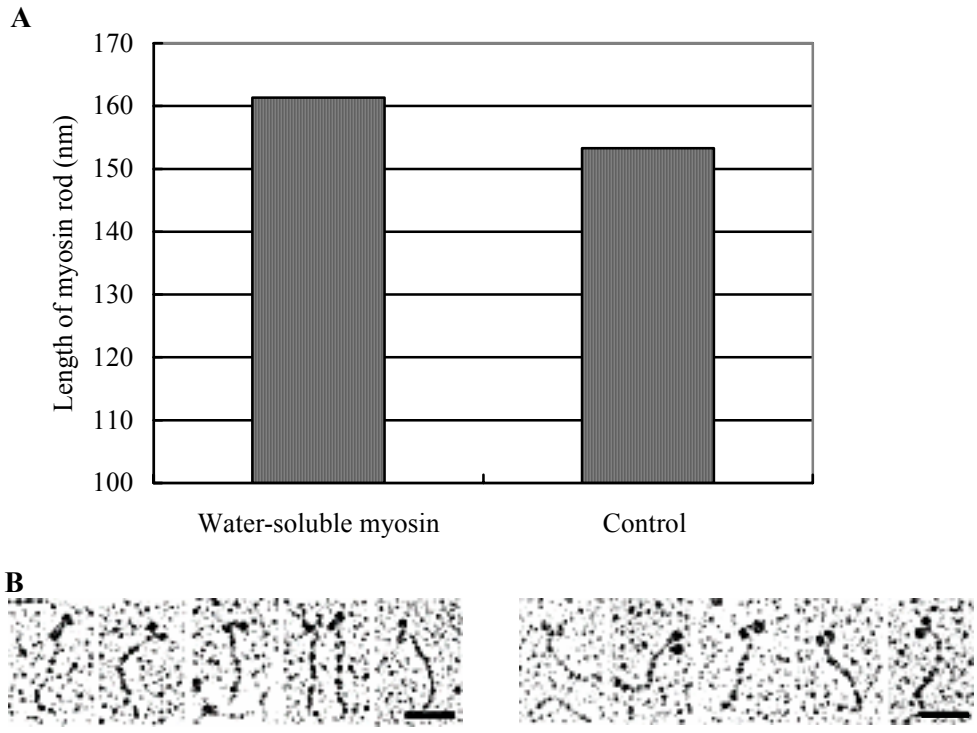

Figure 4. Length of water-soluble myosin rod. **A**, The length of the rod was determined by measurements in electoron micrographs of rotary shadowed water-soluble myosin. **B**, Electron micrographs of water-soluble myosin (left) and native myosin (right). Both are rotary shadowed with platinum. Bars are 100 nm.

THE EFFECT OF SELECTION FOR GROWTH RATE ON SENSORY CHARACTERISTICS OF RABBIT MEAT

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Background

Selection for growth rate is currently practiced in commercial sire lines of genetic schemes for rabbit genetic improvement (BASELGA and BLASCO, 1989; LEBAS et al., 1996). Meat rabbit production is based on three-way crosses. Does are crossbred females from lines selected by litter size, whereas terminal sires come from lines selected for growth rate. Selection for growth rate decreases food conversion rate and improves efficiency, but may decrease carcass and meat quality. In rabbits only two experiments have assessed the consequences of selection for growth rate in carcass and meat quality, PILES et al. (2000) and LARZUL et al. (2003). PILES et al. (2000) comparing a control group and a group selected for growth rate, found a worse water holding capacity of the meat in the selected group and a decrease in the fat content of the carcass, but did not find clear differences in fat content of the hind leg between groups. LARZUL et al. (2003) in a divergent selection experiment on 63 days weight found that, as body weight increases, the percentage of skin decreased, carcass yield was slowly affected and perirenal fat increased. However, at present there are not studies about the influence of selection for growth rate on the sensory properties of rabbit meat.

Sensory analyses are usually performed with small samples. Until now, classical statistics has been the usual way of expressing uncertainty with meat quality analysis, whereas Bayesian analysis have been mainly applied by animal breeders to solve complicated genetic problems (see BLASCO, 2001, for a review). An advantage of the Bayesian approach trough MCMC procedures is the possibility of easy construction of all kind of confidence intervals. This allows asking questions that we could not ask within the classical inference approach, or that had complex procedures to be answered. For example, we can find intervals of the type [k, $+\infty$) having a 95% of the probability area of the marginal posterior distribution. With these intervals we know that the probability of the trait of being lower than k is a 95%. We can also find the probability of finding relevant differences for a trait between two or more treatments. This gives a high flexibility to this type of analyses.

Objectives

Our objective is to study the effect of selection for growth rate on the sensory characteristics of rabbit meat. We use a Bayesian approach.

Materials and methods

Animal material

The animals used in this experiment were originated from a synthetic line selected for growth rate between the 4th and 9th week of life (ESTANY et al., 1992), in the Animal Science Department of Universidad Politécnica de Valencia. Embryos belonging to generation 7th were frozen, thawed and implanted in does in order to produce the control group. The procedure is described by VICENTE et al. (1999). The control group was formed from the offspring of the embryos belonging to the 7th generation, to avoid the effect of cryoconservation. Selected animals belonging to 21st generation were compared with animals of the control group. Control (C) and selected (S) groups were contemporary. Forty animals per group were slaughtered at 9-weeks-old. Animals were slaughtered at the abattoir on the farm, thus they did not suffer stress due to transport. No fasting was practiced. At 24 hours post-mortem the *Longissimus dorsi* muscles were dissected and vacuum packed and frozen at –20ºC until they were required for sensory analysis.

Sensory evaluation
A quantitative descriptive analysis (STONE et al., 1974) was performed by four trained tasters of rabbit meat in 20 sessions. The parameters evaluated were: intensity of rabbit flavour (IRF), aniseed odour (AO), aniseed flavour (AF), liver flavour (LF), tenderness (T), juiciness (J), fibrousness (F). The sensory analysis was carried out on samples of the *Longissimus dorsi* muscle following a complete block design (STEEL and TORRIE, 1980). Samples were vacuum packed and cooked in a water bath at 80ºC for 1 hour. Samples were cut into four pieces and distributed in such way to the panellist to eliminate any location effect within the loin.

Statistical analysis
As panellists had different ranges when scoring sensory traits, variables were transformed dividing by the standard deviation of each panellist, as recommended by (BROCKHOFF et al., 1996). The model used included, group (with two levels, S and C corresponding to selection and control groups respectively), panellist (four panellists), session (20 levels), muscle location (four zones) and sex effects. A Bayesian analysis was performed. Bounded flat priors were used for all unknowns. Data were assumed to be normally distributed. Marginal posterior distributions of all unknowns were estimated by using Gibbs Sampling. After some exploratory analyses we used one chain of 10,000 samples, with a burning period of 2,000, thus marginal posterior distributions were estimated with 8,000 samples each one. Convergence was tested for each chain using the Z criterion of Geweke. Details of the procedure can be found in SORENSEN and GIANOLA (2002).

In sensory analyses, it is difficult to determine what a relevant difference is, thus instead of assessing the differences between the selected and control populations, the ratio of the selection and control effects is analyzed. This is easily made from the results of the Gibbs sampling chains and allows expressing the superiority of the selected over the control population (or conversely the superiority of the control over the selected population) in percentage.

Results and discussion
Features of the estimated marginal posterior distributions of the sensory properties are presented in tables 1 and 2. Monte Carlo standard errors were very small. The Geweke test did not detect lack of convergence in any case. Posterior distributions of sensory properties were symmetrical. This is reflected in the similar values for means and medians, and in the symmetrical high posterior density interval around the mean.

Table 1 shows the features of the marginal posterior distributions of the ratio of the group effects, selection/control (S/C). When S/C >1, we consider that selected and control groups are different if the probability of S/C > 1 is more than 0.95 (*P>1* in table 1 more than 0.95). When S/C <1 we consider that selected and control groups are different if the probability of S/C < 1 is more than 0.95; i.e., when *P>1* in table 1 is less than 0.05. According to the values of *P>1* in table 1, there is a difference between selected and control groups for intensity of rabbit flavour (IRF), aniseed odour (AO), aniseed flavour (AF) and liver flavour (LF). Conversely, no differences were found in tenderness (T), juiciness (J) and fibrousness (F) between groups.

Selected group had a 3% higher value of IRF than control group, with a HPD(95%) from 1.00% to 1.07%. We consider that a relevant difference appears when one group is at least a 10% higher than the other one with a probability higher than 0.95 (Pr: probability of relevance). Although a selection effect appeared for IRF, this effect was not relevant, since the probability that the selected group being at least a 10% higher than the control group was only 0.01.

Conversely, a relevant effect of selection on aniseed odour (AO) and aniseed flavour (AF) appeared (Pr=1), with lower values for selected animals. By calculating the interval $(-\infty, k]$ of the marginal posterior distribution containing a 95% of the probability, we can assess the maximum value that the ratio S/C can have with a probability of 0.95. This value was a 0.69 and a 0.63 for AO and AF, respectively, which means that the probability of the selected group being higher than a 69% and a 63% of the control group respectively is only 0.05. These sensory attributes have been previously described in rabbit meat by OLIVER et al. (1997) and HERNANDEZ et al. (2000). The greater intensity of these attributes could provide positive

aromatic notes. In these sense, we could consider that selection for growth rate has a negative effect for aroma characteristics, although it is not clear that these differences could be detected by consumers.

An effect of selection for growth rate on liver flavour is also shown in table 1, being a 23% higher the selected group than the control, with a HPD (95%) from 1.03 to 1.44. This attribute is a common descriptor in meat flavour and it has been described previously in beef (FONT et al., 1995). OLIVER et al. (1997) and HERNANDEZ et al. (2000) reported the same descriptor in rabbit meat. These authors considered that an increase of liver flavour could have a negative effect on consumer acceptability. However, the probability that the selected group being at least a 10% higher than the control group was only 0.88, lower than 0.95 that it is minimum value that we have considered relevant.

Table 2 shows the features of the marginal posterior distributions of the ratio of the group effects, males/females (M/F). According to the values of *P>1* in table 2, there is a difference between males and females groups for intensity of rabbit flavour (IRF), with a 4% higher in males than in females. However, this difference was not relevant, since the probability that the selected group being at least a 10% higher than the control group was only 0.01. No differences were found for the rest of the characteristics evaluated.

Table 1. Sensory properties of rabbit meat. Features of the marginal posterior distributions of the ratio of the group effects, selection/control (S/C).

S/C	Mean	Median	HPD(95%)	*P>1*	Pr	k_1	k_2	MCse	Z
IRF	1.03	1.03	1.00, 1.07	0.96	0.01	1.06	1.00	0.0002	-0.11
AO	0.59	0.58	0.47, 0.71	0.00	1.00	0.69	0.49	0.0007	-1.82
AF	0.52	0.51	0.39, 0.65	0.00	1.00	0.63	0.41	0.0007	0.49
LF	1.23	1.22	1.03, 1.44	0.99	0.88	1.41	1.06	0.0011	1.21
T	1.00	0.99	0.96, 1.04	0.49	0.00	1.04	0.97	0.0002	-0.18
J	1.01	1.01	0.95,1.07	0.57	0.01	1.06	0.96	0.0004	-0.32
F	1.02	1.02	0.95, 1.09	0.70	0.01	1.08	0.96	0.0003	1.18

IRF: intensity of rabbit flavour. AO: aniseed odour. AF: aniseed flavour. LF: liver flavour. T: tenderness. J: juiciness. F: fibrousness.
HPD (95%): high posterior density interval at a 95% of probability. *P>1*: Probability of S/C > 1. Pr: Probability of relevance, the probability that one group being at least a 10% higher than the other group. k_1: limit of the interval $(-\infty,k_1]$ containing a probability of 95%. k_2: limit of the interval $[k_2,+\infty)$ containing a probability of 95%. MCse: Monte Carlo Standard error. Z: Z-score of the Geweke test.

Table 2. Sensory properties of rabbit meat. Features of the marginal posterior distributions of the ratio of the group effects, males/females (M/F).

M/F	Mean	Median	HPD(95%)	*P>1*	k_1	k_2	MCse	Z
IRF	1.04	1.04	1.00, 1.07	0.98	1.07	1.01	0.0002	-0.81
AO	1.03	1.02	0.83, 1.22	0.42	1.20	0.87	0.0010	0.14
AF	1.11	1.10	0.87, 1.37	0.80	1.34	0.91	0.0016	-0.56
LF	0.98	0.98	0.81, 1.14	0.40	1.13	0.84	0.0009	1.12
T	0.97	0.97	0.93, 1.01	0.09	1.01	0.94	0.0003	-1.54
J	0.97	0.91	0.91, 1.03	0.16	1.02	0.92	0.0003	-0.21
F	1.04	1.04	0.97, 1.10	0.84	1.09	0.98	0.0003	-0.47

IRF: intensity of rabbit flavour. AO: aniseed odour. AF: aniseed flavour. LF: liver flavour. T: tenderness. J: juiciness. F: fibrousness.
HPD (95%): high posterior density interval at a 95% of probability. *P>1*: Probability of S/C > 1. k_1: limit of the interval $(-\infty,k_1]$ containing a probability of 95%. k_2: limit of the interval $[k_2,+\infty)$ containing a probability of 95%. MCse: Monte Carlo Standard error. Z: Z-score of the Geweke test.

Conclusions

Selection for growth rate has not affected the main characteristics of meat rabbit, like tenderness and juiciness, but it has a negative effect for some flavor characteristics.

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EFFECT OF ORGANIC RAISING AND BREED CROSS ON CARCASS AND TECHNOLOGICAL MEAT QUALITY OF GROWING/FINISHING PIGS

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Background

The promotion of organic pig production is an EU-wide political goal. However, expansion of organic pig production is slow, possibly due to insufficient certitude about final product quality, such as carcass and meat quality. Carcass and technological meat quality traits of outdoor raised pigs are widely discussed in literature. Further, the breed cross may affect carcass and meat quality traits and therefore interact differently in indoor and outdoor housing systems.

Objectives

The aim of this study was to compare organic and conventional production systems of growing/finishing pigs with regard to their carcass and technological meat quality. Further, the influence of two different types of breed crosses on these quality traits was investigated.

Materials and methods

During two years, 280 growing/finishing pigs were raised to approximately 107 kg live weight in four different production systems; they were equally distributed to housing system (outdoor/indoor), breed cross (D*LW/L*LW) and gender (castrates/females). Outdoor pigs were fed *ad libitum*, with either an organic diet diluted with 20% alfalfa roughage throughout (org.dil.) or with first the diluted diet and thereafter this organic diet undiluted (org. dil./org. undil.). In two indoor treatments, pigs were fed restrictively with either the undiluted organic diet (org. undil.) or a conventional diet (conv.).

Hot carcass weight and back fat thickness over the middle of *M. longissimus dorsi* (LD) were recorded. Lean meat content was estimated as [lean meat percentage = -49.781 + (0.899* ham in carcass) + (0.612* meat and bone in ham) + (0.651*loin in carcass) + (0.252*meat and bone in loin) + 0.249 (for females)] (Hansson, pers. comm.). Ultimate pH (portable pH-meter equipped with a combination gel electrode SE104, Knick, Berlin, Germany, calibrated to chilling room temperature), internal reflectance (FOP, 900 nm; TBL Fibre Optics Group Ltd., Leeds, UK) and surface reflectance (Minolta Chroma Meter CR 300, DP-301, Osaka, Japan) were measured on LD. Drip loss was determined on a 2-cm-thick slice, taken from LD directly in front of the last rib towards the forepart, stored in a plastic bag and hanging on a thread at 4°C for 48 h. Thawing loss was determined as the difference between the weight of fresh and thawed 300-g piece of LD after frozen storage at -20°C. On the same piece of meat, cooking loss was determined as the weight difference before and after cooking in a water bath at 70°C during 90 min. Maximal Warner-Bratzler (WB) shear force and total WB-work were measured on 8 strings (10x10x50 mm), sheared across the fibre direction of cooked LD (speed: 55 mm/min, TA-HDI texture Analyser; Stable Micro Systems, Surrey, UK).

Statistical analyses were performed with the MIXED procedure in SAS (SAS Institute Inc., Cary, N.C., USA, version 8.02) with treatment, breed cross and gender as fixed factors. Sire within breed cross and dam within breed cross and sire were treated as random.

Results and discussion

Carcass quality

From both years, average carcass weight of indoor raised pigs was higher compared with outdoor raised pigs (Table 1). The lower dressing percentage of outdoor pigs might depend on the higher gut filling, because the diluted diet contained more indigestible fibre than the undiluted diet; moreover, the outdoor pigs had access to pasture. Lean meat content is often reported to be higher in outdoor raised pigs, compared with indoor pigs, when receiving identical diets (Stern et al., 2003). In our study, outdoor pigs fed the diluted organic diet

had significantly higher lean meat content (year 2), probably due to the lower energy content of the diet, and consequently lower daily weight gain. The higher energy requirement of outdoor pigs due to their higher agility could not be covered by the diluted diet, even when given *ad libitum*. The uniformity between indoor raised pigs in dressing percentage, lean meat content and back fat thickness indicates that an organic diet can give carcass quality results comparable with those from a conventional diet.

Between breed crosses, no differences in carcass quality could be observed (Table 2). This is not in accordance with other studies, where higher back fat in Duroc breed crosses compared with Large White breed crosses have been reported (Enfält et al., 1997).

Technological meat quality

Technological meat quality traits, such as pH, internal and surface reflectance, WB shear forces and water-holding capacity, in terms of drip, thawing and cooking losses, differed only in some cases between the production systems (Table 1). However, pH values of both muscles did not differ between the treatments, whereas FOP_{BF} values were higher in outdoor pigs, compared with indoor pigs. The pH and FOP values seemed not to be related to each other, which might indicate that these values depend less on production system than on e.g. slaughter conditions. Meat in LD was paler (higher L* values) in indoor raised pigs (year 1), which could be a consequence of the higher FOP values in that muscle. However, Lindahl et al. (2001) stated, that L* values depended mostly on pigment content and myoglobin forms than on internal reflectance. In our study, water-holding capacity was not affected by production system, which might partly be explained by the consistent pH_{LD}. Shear forces in meat from indoor and outdoor raised pigs are widely investigated with various results. Olsson et al. (2003) found significantly higher, whereas Stern et al. (2003) found lower WB shear forces for outdoor pigs. In our study, WB shear forces were mostly unaffected; solely year 2, outdoor raised pigs had lower maximal shear force, compared with indoor raised pigs, given the organic diet.

Generally, pH, FOP and colour values did not differ between Duroc and Landrace breed crosses, whereas water-holding capacity was higher and WB shear force values were lower (year 1) for the Duroc breed crosses (Table 2). This is in accordance with Blanchard et al. (1999), who reported a decrease in shear force with increasing proportion of Duroc in the final breed cross.

Conclusions

It can be concluded that the production system influenced mainly carcass composition and, to a lesser extent, technological meat quality. Indoor raised pigs fed either organic or conventional diet did not differ in carcass and meat quality traits. Breed cross did not influence carcass traits and colour, pH and FOP values. Indications of higher water-holding capacity and lower WB shear force for D*LW pigs could be found.

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Acknowledgements

The study is part of the EU-project “Sustainability in the production of pork with improved nutritional and eating quality using strategic feeding in out-door production”. QLK5-2000-00162.

Table 1. Carcass and technological meat quality traits (LS-means and pooled standard error) of growing/finishing pigs raised outdoors and indoors with different diets

	Year 1					Year 2					
	Outdoor		Indoor			Outdoor		Indoor			
	org. dil.	org. dil./ org. undil.	org. undil.	SE	P-value	org. dil.	org. dil./ org. undil.	org. undil.	conv.	SE	P-value
No. of animals	40	37	40			40	39	39	40		
Hot carcass weight, kg	81.7^{b}	82.5^{ab}	83.5^{a}	0.44	**0.0187**	79.3^{b}	80.1^{b}	84.6^{a}	83.3^{c}	0.46	**0.0001**
Dressing percentage	75.8^{b}	76.6^{c}	77.5^{a}	0.35	**0.0001**	74.2^{b}	75.4^{c}	78.5^{a}	77.6^{a}	0.47	**0.0001**
Estm. lean meat, %	58.3	57.5	57.6	1.19	0.2005	60.3^{b}	58.6^{a}	58.8^{a}	59.5^{ab}	0.40	**0.0044**
Back fat[1], mm	13.2^{b}	14.5^{a}	15.0^{a}	1.47	**0.0055**	12.4^{b}	13.9^{a}	14.2^{a}	14.0^{a}	0.65	**0.0068**
pH value											
LD	5.52	5.53	5.49	0.019	0.1575	5.55	5.52	5.53	5.51	0.022	0.5235
BF	5.65	5.67	5.65	0.022	0.7769	5.65	5.60	5.66	5.65	0.028	0.1557
FOP value											
LD	28.8^{a}	26.2^{b}	29.6^{a}	1.04	**0.0035**	29.0	29.4	27.9	27.9	1.10	0.4985
BF	34.4^{b}	33.2^{ab}	31.7^{a}	0.07	**0.0190**	32.2^{b}	35.6^{c}	27.7^{a}	29.3^{a}	0.79	**0.0001**
Minolta value$_{LD}$											
L* (lightness)	47.8^{b}	46.9^{b}	49.1^{a}	0.60	**0.0001**	47.1	47.5	47.8	47.9	0.44	0.1917
a* (redness)	5.9	5.9	5.7	0.15	0.3361	6.1	6.3	6.1	6.1	0.18	0.8613
b* (yellowness)	2.2	1.8	1.8	0.16	0.0737	2.0^{bc}	2.1^{b}	1.5^{a}	1.7^{ac}	0.12	**0.0009**
Drip loss$_{LD}$, %	4.6	4.0	3.9	0.03	0.1134	4.2	4.9	4.1	4.9	0.35	0.1041
Thawing loss$_{LD}$, %	7.2	7.0	7.3	0.59	0.9521	9.8	10.4	10.7	11.6	0.48	0.0912
Cooking loss$_{LD}$, %	21.4	21.1	22.1	0.57	0.5576	18.8	17.8	18.4	19.2	0.44	0.1359
Warner-Bratzler											
max. shear force$_{LD}$, N	30.5	28.9	30.6	1.39	0.5056	28.1^{b}	27.6^{b}	33.8^{a}	31.7^{ab}	1.83	**0.0064**
total work$_{LD}$, Nmm	175.2	161.9	167.8	5.58	0.2426	150.8	144.6	163.1	155.8	6.01	0.1428

[1]Over the middle of *M. longissimus dorsi* at the cut behind the last rib.
Means with different superscript within row and year differ significantly ($p<0.05$).

Table 2. Carcass and technological meat quality traits (LS-means and pooled standard error) of D*LW and L*LW crossbred pigs

	Year 1				Year 2			
	D*LW	L*LW	SE	P-value	D*LW	L*LW	SE	P-value
No. of animals	57	60			79	79		
Hot carcass weight, kg	82.5	82.6	0.35	0.8683	82.0	81.7	0.32	0.6061
Dressing percentage	77.0	76.3	0.41	0.2564	76.3	76.6	0.23	0.3881
Estm. lean meat, %	57.8	57.4	1.18	0.8573	59.3	58.9	0.32	0.4923
Back fat[1], mm	14.6	13.9	2.01	0.7972	13.6	13.7	0.76	0.8790
pH value								
LD	5.52	5.50	0.018	0.5067	5.56	5.50	0.021	0.0791
BF	5.65	5.66	0.021	0.9639	5.65	5.63	0.032	0.6340
FOP value								
LD	28.7	27.7	0.20	0.5654	27.9	29.0	0.10	0.2442
BF	34.5	31.7	0.53	**0.0069**	32.1	30.3	0.55	0.0505
Minolta value$_{LD}$								
L* (lightness)	47.4	48.5	0.78	0.3978	46.9	48.2	0.50	0.0990
a* (redness)	6.1	5.6	0.16	0.3361	6.1	6.2	0.19	0.5781
b* (yellowness)	2.0	1.8	0.20	0.4666	1.8	1.9	0.11	0.6009
Drip loss$_{LD}$, %	3.6	4.8	0.39	0.0643	3.7	5.3	0.34	**0.0091**
Thawing loss$_{LD}$, %	6.7	7.6	0.47	0.2316	10.0	11.2	0.33	**0.0314**
Cooking loss$_{LD}$, %	20.3	22.7	0.64	**0.0451**	18.1	19.1	0.31	0.0513
WB shear force								
total work$_{LD}$, Nmm	156.5	180.1	4.47	**0.0136**	152.5	154.6	5.21	0.7870
max. shear force$_{LD}$, N	27.8	32.2	1.21	**0.0482**	28.9	31.6	1.93	0.3602

[1]Over the middle of *M. longissimus dorsi* at the cut behind the last rib.
Means with different superscript within row and year differ significantly ($p<0.05$).

EFFECTS OF EXOGENOUS PROTEASE EFFECTORS ON CALPAINS ACTIVITY AND ULTRASTRUCTURAL CHANGES OF BEEF

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Background

Beef tenderness increases gradually during postmortem aging, and as a result, the improved palatability. This is an established phenomenon which has long been discovered, however, to date, which factors contribute to this process and how the tenderization is initiated is an unresolved problem.

Koohmaraie and others(Koohmaraie et al.1996, Dransfield 1999) suggested that postmortem meat tenderization is primarily the result of calpain(especially μ -calpain)-mediated degradation of key myofibrillar and cytoskeletal proteins which can cause weakening of the muscle structure, and therefore, meat becomes tender. On the contrary, Takahashi et al. (1999) supposed that with the postmortem time extended, the concentration of calcium ion in muscle sarcoplasm increased, and the calcium alone could lead to the degradation of myofibrillar proteins and the improved meat tenderness, and that calpain was considered to be inactive due to the low concentration of sarcoplasmic calcium ions, and therefore, has little contribution to the tenderization of meat throughout postmortem aging. Consequently, in order to control and regulate meat tenderization, it is indispensable to elucidate the mechanism of meat aging.

Objectives

The purpose of this study was to, through injection treatments, investigate the role of calcium or calpains in beef aging *in situ*.

Materials and methods

Sampling procedures and treatment of muscle samples

Three 2.5 years old Chinese yellow cattle (Luxi×Limusin with liveweight 500±20kg) were slaughtered according to standard slaughtering practices. After being chilled for 20h, one strip loins were sampled per cattle, and was divided into 21 steaks. After being trimmed of all external fat and connective tissue, the steaks(25±3g) were randomly assigned to seven sample groups, which were injected (10%raw weight) distilled water(control),200mM$CaCl_2$, 200mMEGTA, 200mM$ZnCl_2$, 0.2mg/ml Leupeptin ,0.2mg/ml Leupeptin plus 1%TritonX-100 and 1%TritonX-100 respectively. Then the samples were vacuum packaged individually, and stored at 4℃ for 3, 8, 16 days respectively.

Casein Zymography

Casein Zymography was based on the protocol described by Veiseth et al.(2001) with minor modifications. At each storage time, 300mg of muscle were homogenized in 3vol of extraction buffer (100mMTris,10mMEDTA,0.05%2-mercaptoethanol[MCE],pH8.3) using hand operated homogenizer.The homogenate was centrifuged at 10,000×g_{max} for 20min, and the volume of the supernate was measured and used for casein zymography. Hammersten Casein(0.2%, wt/vol) was incorporaed in 12% separating gels(80:1 ratio of acrylamide to bisacrylamide, 375mM Tris-HCl, pH8.8), and 4% stacking gels(80:1, 125mMTris-HCl, pH6.8) without Hammersten Casein was used. TEMED and ammonium persulfate were used to catalyse the polymerisation. Sample buffer(150mM Tris-HCl, pH6.8, 20% glycerol, 0.8%MCE, 0.02% bromophenol blue) was added to supernate (4:1 ratio of buffer to supernate). The gels(1mm) were prerun at 100V for 15 min, 4℃, with a running buffer containing 25mMTris-HCl, 0.05%MCE,192mM glycine, and 1mMEDTA (pH8.3) before samples were loaded into the wells. The gels were run at 100V for 15h, 4℃, removed, and incubated at room temperature (RT) in 50mMTris-HCl, 0.05%MCE and 4mM $CaCl_2$(pH7.5) with slow shaking for 1h, then followed by 16h incubation in the same buffer at RT, before staining for 1h with Coomassie blue and destaining with 20% methanol and 7%acetic acid.

Muscle ultrastructure

At 3, 8, and 16 days storage periods, samples were cut parallel to muscle fibre and fixed by immersion in cold 2.5% glutaraldehyde in phosphate buffer (pH7.3), postfixed in 1% osmium. dehydrated in ethanol, embedded in Epon812 resin. For all samples, thin sections of the same thickness were cut parallel to the

fibres. Sections were stained with uranyl acetate and lead citrate, and then examined using a JEM-100CX-Ⅱ transmission electron microscope(TEM).

Results and discussion

Effects of injection treatment on calpain activity

Results presented in Figure1 indicate that the μ-calpain activity treated by distilled water, EGTA and TritionX-100 respectively declined quickly with aging time and after 16 days storage, μ-calpain activity could not be detected at all by Casein zymography. In contrast, μ-calpain activity in samples injected with $ZnCl_2$, Leupeptin, and Leupeptin plus TritionX-100 respectively can still be detected up to 16 days postmortem. As compared to μ-calpain activity , m-calpain activity did not changed dramatically at a 16 days storage period in all treatments except for $CaCl_2$ injected group in which both μ-calpain and m-calpain activity almost lost completely even after 3 days storage. Two or more band showing activity also appeared in figure 1. Calpain system occurring in animal skeletal muscle cells has three major components ie. μ-calpain, m-calapin and their specific inhibitor calpastatin. A unique property of the calpains is that, when exposed to sufficient calcium, they undergo autolysis, which initially reduces the 80kDa subunit of calpain to 78 or 76kDa, and the 28kDa subunit to 18kDa (Geensink et al.2000). Further autolysis leads to more extensive degradation of large subnunit and loss of proteolytic activity. Znic chloride, Leupeptin are exogenous inhibitor of calpains, the activity of μ-calpain in muscles incorporated into these two components, therefore, declined more slower compared to other treated groups. Calpains quickly lost activity in $CaCl_2$ injected group, which demonstrated that sufficient calcium can activate calpains and then calpains themselves lose activity through autolysis. Two or more active bands of μ-calpain supported the calpain autolysis theory. Except for $CaCl_2$ treated samples, the activity of m-calpain did not change greatly, which indicated that under the condition of this experiment, m-calpain kept inactivity due to low level of calcium, and hence, is not a potential contributor to beef tenderization.

Effects of injection treatment on beef ultrastructure

These experiments conducted demonstrated that injected treatments had great influence on beef ultrastructural changes (Fig.2). Myofibrils kept intact at d3 postmortem (Fig.2 A). At d16, some breaks in myofibrils appeared in control groups (Fig.2 B). While in Leupeptin treated samples, even after 16 days of storage, there was not any significant changes occurred for myofibrils (Fig.2 C). By contrast, myofibrillar breaks was readily perceived at d3 in $CaCl_2$ treated group (Fig.2 D). It has been well documented that muscle fiber changes were mainly caused by the degradation of key myofibrillar proteins, especially, titin, nebulin desmin etc. (Koohmaeaie 1996). When calpains were inhibited by Leupeptin, muscle ultrastructural changes were also inhibited, while, when calpains were activated by calcium, muscle ultrastructural changes quickly happened, which is consistent with calpain hypothesis. In agreement with Taylor et al.(1995), another change that occured in muscle during postmortem tenderization was the fracture in the I band adjacent to the Z line(Fig.2 E), not in the middle of Z line as suggested by other reporters.

Conclusions

Calpain, especially μ-calpain is the most important factor responsible for meat tenderization, while calcium may has indirect influence on meat aging by activating calpains.

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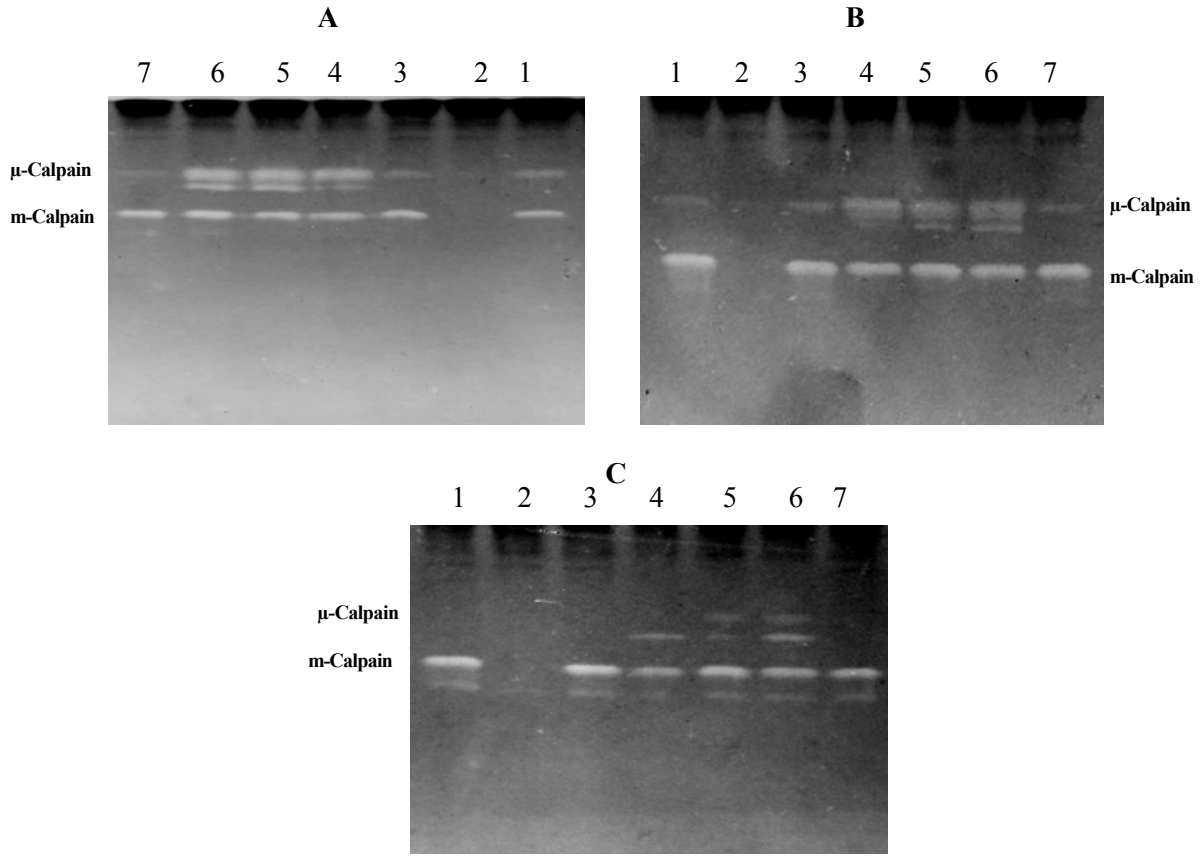

Fig.1 Casein zymography profiles(A, B, C) of beef calpains at 3d, 8d, 16d postmortem respectively. Lane 1 distilled water. Lane 2 calcium chloride. Lane 3 EGTA. Lane 4 Znic chloride. Lane 5 Leupeptin. Lane 6 Leupeptin plus Triton X-100. Lane 7 Triton X-100.

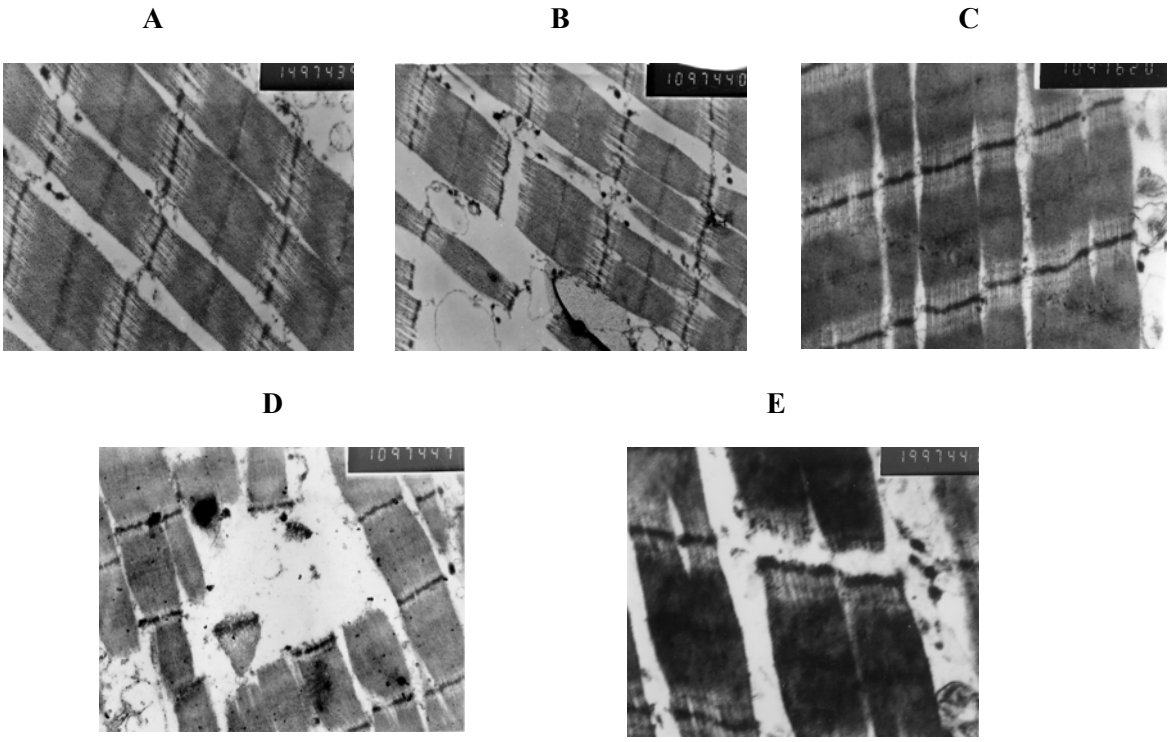

Fig. 2 Micrographs of ultrastructure of different treated beef .
A control sample aged for three days B control sample aged for sixteen days.
C Leupeptin treated sample aged for sixteen days D calcium treated sample aged for three days.
E. location of myofibril breaks.

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EFFECTS OF POSTMORTEM PH/TEMPERATURE DECLINE ON CHANGES IN FREE AMINO ACIDS DURING AGEING IN PIG LONGISSIMUS MUSCLE

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Background

Structural and cytoplasmic proteins of meats are exposed to proteolytic actions of endogenous proteolysis during ageing and result in polypeptides. The degradation of products consequently generate small peptide and free amino acids by subsequent actions of peptidases and aminopeptidases, respectively (Toldra et al., 2000). It has been well documented that interaction between pH and temperature during rigor development directly affects the incidence of PSE meats (Henckel et al, 2000). Moya et al. (2001) showed that changes in free amino acids during ageing varied between PSE, RFN, RSE, and DFD in pork. These changes are related to aminopeptidases activity under a particular class of pork (Toldra and Flores, 2000). The results mirrored that pH/temperature window during the onset of rigor can have an effect on changes in free amino acids during ageing. Lawrie (1991) noted that the accumulation of some free amino acids could result in an undesirable flavor and decrease in the water-holding capacity. However, free amino acids are of great importance in eating quality due to their specific tastes (Nishimura and Kato, 1988). Their subsequent degradation generates volatile compounds (Toldra, 1998; Hernandez-Jover et al., 1996).

Objective

To determine the effects of pH/temperature profile during rigor development on changes in the concentration of free amino acids for 7 days at 1°C.

Materials and methods

Animals, experimental design, and treatment. A total of 20 male pigs weighing an average of 118 kg (10 head of 194-day-old Yorkshire and 10 head of 201-day-old Landrace) were sampled from the National Livestock Research Institute (NLRI) feeding program. The pigs were conventionally transported to the NLRI abattoir, approximately 65 km away, with minimum transit stress, and kept off feed but with access to water, a day before they were slaughtered. To generate a large range of declines in pH and temperature, researchers placed the pigs in a -3°C chiller (five of each breed), or in a 5°C chiller (for the rest of the five of each breed) until the following day. All pigs were conventionally slaughtered over two consecutive days with an electronic stunner (230 volts for 2.5 sec).

pH, temperature, sampling, and objective quality measurements. Muscle temperature was logged at a 5-min interval from approximately 30 min after stunning for 24 hours (Thermo Recorder, TR-50C, Japan) by using thermocouples inserted into the geometrical center of the muscle between the 3rd and 4th lumbar vertebrae. The pH was measured by using a portable needle-tipped combination electrode (NWKbinar pH-K21, Germany) inserted into the center of the muscle between the 3rd and 4th lumbar vertebrae at a 15-min interval from approximately 30 min postmortem, until the muscle was judged to have reached the ultimate pH. Another measurement was made the following day, approximately 24 h postmortem.

The day after slaughter, *m. longissimus* muscles (from the 7th thoracic vertebrae to the last lumbar vertebrae) were removed, cut into three portions, vacuum-packed, and randomly assigned to one of three ageing periods (1, 3, and 7 d). These were used to get the objective measurements of WB-shear force, meat color, drip loss, and cooking loss. The samples were held at 1°C for the relevant ageing period.

For amino acid analysis, approximately 2 g of muscle tissue were taken at the end of the lumbar vertebrae by using a home-made biopsy sampler during breeding (i.e., 0 h), 4, 12, and 24 h postmortem. Similar amounts of muscle tissues were also sampled at 3 and 7 days postmortem from the WB-shear force block. Muscle tissue was frozen in liquid nitrogen immediately after sampling, powdered in liquid nitrogen by using a mortar-based homogenizer (Warning, Dynamics Corp., USA), and stored at -70°C until analysis. WB-shear force, meat color, and cooking loss were determined according to the procedures of Hwang et al. (2004).

Free amino acids. Level of free amino acids was determined at 1 and 7 d postmortem, largely according to the method reported by Moya et al. (2001), but with minor modification. Briefly, one (1) gram of liquid

nitrogen powdered sample was homogenized in 0.01N HCl with a polytron (3x15 sec) at 4C and centrifuged at 10000g for 20 min. About 300 uL of supernatant was filtered and deproteinised for 30 min at room temperature after mixing with 690 uL of acetonitrile and 10 uL of internal standard (L-Citrulline, 250 pmol/uL). Samples were centrifuged at 10000g for 15 min and 16 primary amino acids were determined by online derivatization using o-phthalaldhyde (OPA). Amount of free amino acids were analyzed by using the Agilent 1100 HPLC system (Agilent Tech, Waldbronm, Germany) at diode array UV detector (338 nm, 10 nm band wide) with ZORBAX Eclipse-AAA C18 column (4.6x150 mm, 5 um, Agilent Tech, Waldbronm, Germany). Separation for each sample was completed in 20 min at 40C. A gradient mobile phase between 40 mM Na_2HPO_4, pH 7.8 and acetonitril-methanol-water (45:45:10, v/v) was used and expressed as pmol. The effects of breed and ageing time on objective meat quality and free amino acid contentrations were examined by applying a general linear model (SAS, 1997), where the effects were tested against residual error.

Results and discussion

The temperature treatments (ie., -3°C and 5°C) did not affect pH/temperature profile in longissimus muscle during rigor development, but the experiment design resulted in wide ranges in pH (6.0-6.9 at 3 h pm) and temperature (20-29°C, data not shown). An average sarcomere length of 1.74 µm without any treatment effect indicated that there was no muscle shortening despite the temperature of 0.6 to 36°C with a pH of 6.2 (Table 1). The pH/temperature profile did not significantly differ between breeds, but slow rate in pH decline was noticeable in this study (ie. 6.2 at 4 h pm), with a normal ultimate pH of approximately 5.4 (data not shown). The result was not clearly understood as our previous data showed a pH of ca. 6.1 at 3 h by applying the same procedure (Hwang et al., 2004). But it might be in part related to a delayed transit time with extreme traffic jam. Landrace showed significantly ($P<0.05$) lower WB-shear force and higher hunter L* value than Yorkshire, but there was no correlation between breed and ageing time.

It has been well documented that proteolysis takes place in structural and cytoplasmic protein during ageing, and this significantly is affected by pH/temperature decline during rigor development (Dransfield, 1994; Hwang and Thompson, 2001). The latter study showed a significantly faster reduction in u-calpain activity when muscle had a rapid pH decline with slow pH decline, resulting in early exhaustion of the calpain and slow ageing rate. Despite activities of exopeptidases (ie, dipeptidylpeptidases and aminopeptidases) significantly varying between pig breeds (Armero et al., 1999) and muscles (Cronet and Bousset, 1999), the result of Toldra and Flores (2000) implied that pH/temperature profile at early postmortem might affect the rate and extent of aminopeptidase activities. These consequently affect changes in free amino acids during ageing.

In the current study, two amino acids (Gly and Cyc) out of 16 primary amino acids were significantly ($P<0.05$) affected by breed, where landrace had higher concentrations (Table 1). While concentration of all examined amino acids (except Met) increased over 7 days, the interaction between breed and ageing was not detectable. To examine the effects of pH/temperature profile during rigor development on changes in free amino acids for 7 d, the amino acid concentrations at 0 day (sampled during bleeding) were subtracted from those at the 7th day, and estimated the levels as a function of muscle temperature at pH 6.2 (Tehmph62). Tehmph62 has been used as an important threshold, because it could be an indirect indication of cold and heat shortening (Pearson & Young, 1989), denaturation of myofibril and sarcoplasmic proteins (Offer & Cousins, 1992), and proteolytic and/or autolytic activity of µ-calpain (Dransfield, 1994).

Tempph62 had simple correlation coefficients (r) of -0.9 and –0.93 with pH at 2 and 4 h; respectively, and of 0.4 and 0.5 with temperature at 2 and 4 h , respectively. Tempph62 did not affect changes in extractable concentrations for His, Tyr, Cyc, Gly, Iso, Ala. On the other hand, Tempph62 had significant curvelinear effects on eight amino acids (Asp, Glu, Leu, Lys, Val, Thr, Ser, Arg), varying in magnitude, while it showed only a linear effect on Met and Phe (Figure 1). Although the curvelinear effect was relatively weak for most amino acids, as seen in figure 1, there was a clear tendency of free amino acids to have higher concentration when muscle temperature at pH 6.2 (say during rigor development) was low. Aminopeptidase activities were not determined. However, an early study (Toldra and Flores, 2000) reported that Alanyl had a significantly lower activity in PSE meat than in DFD meat at 2 h pm. Alanyl, arginyl, and leucyl in PSE meat had significantly lower activities than those in DFD meat. Although, in our data set , changes in Ala were not affected by Tempph62, the results implied that pH/temperature interaction during rigor development not only affected the rate of proteolysis (Hwang et al., 2004), but also affected subsequent release of free amino acids.

Conclusion

Prelimarily data of current study show that pH/temperature significantly affect the release of free amino acids during ageing, with higher levels at a lower temperature and pH of 6.2. However, some amino acids are not affected by declines in pH and temperature. This may have a relation to taste development during ageing and cooking.

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Table 1. Least square mean and significance level of carcass traits, objective meat quality and changes in concentration of free amino acids from death to 7 d postmortem as a function of breed and ageing time.

	Breed		Ageing			Model terms	
	Landrace	Yorkshire	1	7	Av. Se	breed	ageing
Carcarss traits and objective meat quality							
pH at 4 h pm	6.2	6.2	na	na	0.07	ns	
Temperature at 4 h pm (°C)	22.1	21.1	na	na	0.09	ns	
Temperature at pH 6.2 (°C)	21.5	20.4	na	na	3.05	ns	
Sarcomere length (um)	1.74	1.74	na	na	0.02	ns	
WB-Shear force (kg)	6.25	7.50	8.04	5.71	0.26	***	***
Hunter L*	45.02	42.34	41.95	45.41	0.91	*	*
Concentration free amino acids (mg/ 100g wet tissue)							
ASP	0.9	0.8	0.0	1.7	0.20	ns	***
GLU	7.1	7.7	5.5	9.3	1.03	ns	*
SER	3.3	2.9	1.2	5.1	0.32	ns	***
HIS	2.4	2.7	0.0	5.1	0.35	ns	***
GLY	6.3	5.4	4.2	7.5	0.29	*	**
THR	2.5	2.3	1.9	2.9	0.28	ns	*
ARG	3.7	3.8	1.9	5.6	0.39	ns	***
ALA	65.8	66.0	14.0	117.8	5.68	ns	***
TYR	7.2	6.4	3.5	10.1	0.64	ns	***
CYC	0.5	0.2	0.0	0.8	0.09	*	***
VAL	3.5	3.1	2.1	4.6	0.30	ns	***
MET	3.3	3.1	ns	ns	0.70	ns	ns
PHE	4.0	3.9	1.8	6.0	0.22	ns	***
ISO	2.9	3.0	1.1	4.8	0.21	ns	***
LEU	3.5	3.4	1.2	5.7	0.34	ns	***
LYS	4.6	4.3	1.3	7.6	0.49	ns	***
df[a]						1/18(1/37)	1/18(1/37_

na-Not applicable, ns- P > 0.05, * P < 0.05, **P<0.01, ***P<0.001.
a -Numerator/denominator degree of freedom (where ageing term was applicable).

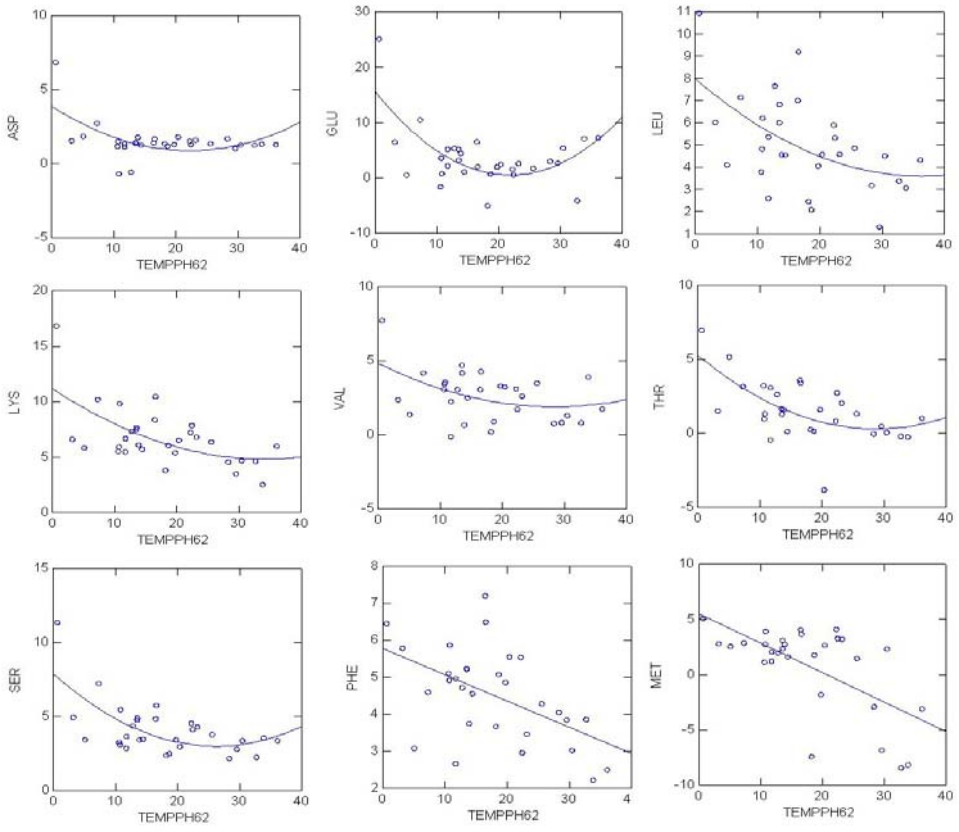

Figure 1. Changes in free amino acids from death to 7 d postmortem as a function of temperature at pH 6.2 (TEMPPH62).

INHIBITON OF POST-MORTEM MUSCLE SOFTENING FOLLOWING *IN SITU* PERFUSION OF PROTEASE INHIBITORS IN FISH

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Background

Fish and shellfish are kept in cold storage after capture and are generally consumed within several days, but the muscle freshness deteriorates even under cold storage. Because Japanese people like fresh slices of raw fish, consumers are interested in the freshness and the muscle texture is an especially important factor for freshness. The muscle structure is known to deteriorate during the 1st day of cold storage, affecting the muscle texture before rigor mortis occurs (Toyohara and Shimizu 1988, Ando *et al.*1991). Such a muscle-softening phenomenon in post-mortem is thought to be caused about by proteolysis of the muscle structure. Several proteases have been studied as being causative factor for fish muscle softening such as cathepsins B, D, H and L (Yamashita 1994), calpain (Tsuchiya *et al.* 1992) and matrix metalloproteinase (Kubota *et al.* 2000). Because of the low level of protease activities in ordinary fish muscle, the material in most of these studies was drawn from particular meat that has an unusually soft texture and extremely high protease activity such as jellied meat from Japanese flounder and matured semelparity fish. In addition, anadromous and amphidromous fish die certainly after spawning and the death is thought to be programmed as apoptosis. If the soft muscle texture at the spawning stage is also programmed as part of apoptosis, there are some possibilities that caspase, a key protease of apoptosis (Yamashita 2003), relates to post-mortem muscle softening.

Objectives

In order to demonstrate involvement of protease in the post-mortem softening in ordinary fish muscle, we have developed an *in situ* perfusion technique, which introduced other chemicals into blood vessels in fish muscle. In the present study, using the *in situ* perfusion technique, protease inhibitors were perfused to identify the type of proteases that led to post-mortem muscle softening in fish, tilapia and yellowtail.

Materials and methods

***In situ* perfusion:** Tilapia *Oreochromis niloticus* and Yellowtail *Seriola quinqueradiata* were obtained from a private fish farm and reared for several months before use. Fish were anesthetized with 200 ppm MS222 (Tricaine methanesulfonate, Acros Organics, NJ, USA) and a cannula connected to a hypodermic syringe that was filled with physiological saline (0.7489% NaCl, 0.1294% KCl, 0.199% $CaCl_2$) was inserted into the bulbus arteriosus. A polyethylene tube (1.5 mm in diameter, 10 cm long) was inserted into the ventricle to bring the venous blood out of the fish body, so that it prevented the circulation of the venous blood and helped the physiological saline perfusion. Soon after the operation, the color of the fish gills changed to white from red and the color of the fluid from the polyethylene tube became clear about 10 min later. Finally after approximately 30 min, the liver color was observed to become whitish (Fig. 2).

Eosin perfusion: Eosin (Wako Pure Chemical Industries, Osaka, Japan) was dissolved in physiological saline to obtain a 10% solution and this solution was perfused into tilapia (359 - 404 g, n = 2) using the *in situ* perfusion technique. After the perfusion of 40 mL of eosin solution (about

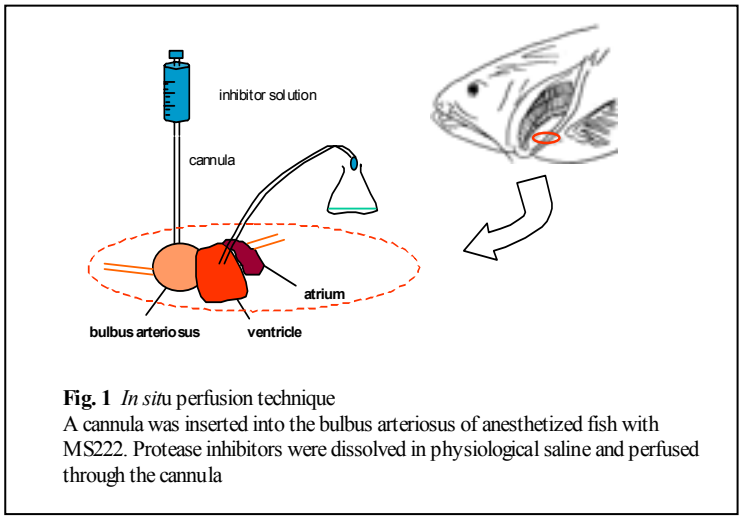

Fig. 1 *In situ* perfusion technique
A cannula was inserted into the bulbus arteriosus of anesthetized fish with MS222. Protease inhibitors were dissolved in physiological saline and perfused through the cannula

40 min), the cannula was removed and the upper dorsal muscle was dissected. The muscle section was observed under a fluorescence stereoscopic microscope (R-400; Edge Scientific Instruments, CA, USA) using ultraviolet light to confirm the occurrence of fluorescence from the eosin.

[^{35}S]-methionine perfusion: [^{35}S]-methionine (Amersham Biosciences, NJ, USA) was dissolved in physiological saline to obtain a 1,000 Bq/mL solution. After the perfusion of 25 mL [^{35}S]-methionine solution into the tilapia (448±71 g, n = 3), fish were individually dissected to obtain four samples of dorsal muscle and one liver sample. Each 1 g-tissue sample was dissolved in 0.1 M NaOH and mixed with scintillation cocktail (PICO-FLOUR 40, Packard Instrument, CT, USA), and the radioactivity was measured using a liquid scintillation analyzer (TRY-CARB 200CA, Packard Instrument).

Protease inhibitor perfusion: Each protease inhibitor, leupeptin (Peptide Institute, Osaka, Japan; 1 mg/mL, a serine and cysteine protease inhibitor), chymostatin (Peptide Institute, 1 mg/mL, a serine protease inhibitor), benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone (Z-VAD-fmk, Peptide Institute, 1 mg/mL, a caspase inhibitor), and E-64 (Peptide Institute; 1 mg/mL, a cysteine protease inhibitor) was dissolved in physiological saline. *o*-phenanthroline (Aldrich Chemical, WI, USA, 1 mM, a metalloprotease inhibitor) was dissolved in a small volume of methanol and diluted with physiological saline. These inhibitor solutions and physiological saline as the control sample were perfused into fish for 60 min. Every fish was perfused with at least 30 mL perfusion solution. Each experimental group consisted of ten tilapias (867±195 g) or six yellowtails (608±84 g). The cannula was removed and the upper dorsal muscle was sliced to give 1-cm-width strips. The sample slices were wrapped in plastic film wrap individually and kept in a 4 °C refrigerator until each measurement time. The breaking strength of the slices as a parameter of muscle toughness was measured using a rheometer (NRM-20002J, Hudoh, Tokyo, Japan) equipped with a cylindrical plunger of 3 mm diameter. The plunger was applied at the interspace between the myocomma membranes at the cut surface of the dorsal muscle. Measurements were taken at five points for each strip and the average value was calculated. Because in our unpublished experiment of tilapia, E-64 was found not to be effective to prevent muscle softening, E-64 was not used for tilapia perfusion.

Results and discussion

***In situ* perfusion**: Approximately 30 min after the perfusion of 10 mL physiological saline, the color of the fluid from the polyethylene tube became clear in the case of a 200-g tilapia (Fig. 2). This technique excludes the effect of blood fluid in fish muscle, in which factors inducing muscle softening may exist (Ando *et al.* 1999), and is able to examine the effectiveness in muscle components than in blood fluid.

Fig. 2 Color change of the fluid from the ventricle. The tilapia was anesthetized with MS222 and physiological saline was perfused by a cannula inserted into the bulbus arteriosus (A). Finally after approximately 30 min (B), the liver color was observed to become whitish.

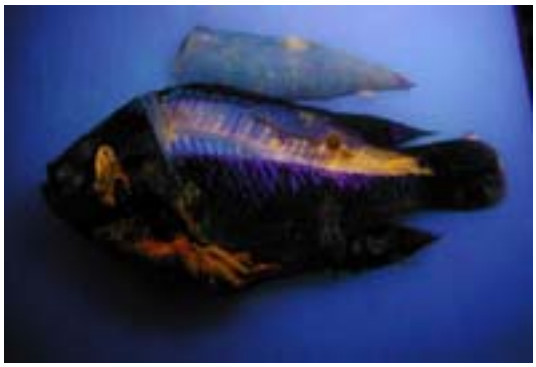

Fig. 3 Fluorescence in the whole body of the eosin-perfused tilapia. Tilapia was anesthetized with MS222 and eosin was perfused by a cannula inserted into the bulbus arteriosus. Orange fluorescence of eosin was observed in the muscle, gill, liver and intestine under ultraviolet light.

Eosin perfusion: Eosin was used for visible confirmation of the *in situ* perfusion technique. Visible fluorescence was observed in the solution from the polyethylene tube, which confirmed the occurrence of eosin, approximately 3 min after the start of the eosin perfusion. Eosin perfusion into the interior organs was confirmed under ultraviolet rays after the upper lateral muscle was removed. Visible fluorescence was observed in the gill, liver, intestine, and dorsal muscle of the eosin treated tilapia (Fig. 3). Eosin perfusion in the blood vessel of the dorsal muscle was confirmed under a fluorescence stereoscopic microscope with ultraviolet rays, and was not observed in the control muscle sample (Ishida *et al*.2003).

[^{35}S]- methionine perfusion: ^{35}S-methionine was used as a marker substance for quantitative analysis. The radioactivity measured in the ^{35}S-methionine perfused tilapia showed that this solution was taken into the dorsal muscle and liver at a rate of 7.8 μL/g and 70.2 μL/g, respectively. As the liver received the radioactive substance much more than the dorsal muscle did, the difference between the two organs possibly depended on the blood circulating volume. From these results of the eosin and [^{35}S]-methionine perfusion experiments, this perfusion technique was concluded to be suitable to introduce chemical substances into fish muscle and was applied to protease inhibitor perfusion in the further experiments.

Protease inhibitor perfusion into tilapia: Figure 4 shows the breaking strength of the tilapia in each inhibitor group. At the beginning of storage (0 h), the breaking strength of all the inhibitor-perfused tilapia was 144 - 197 gw with a large variance. At 23 h storage, significant difference was found in the leupeptin (140±36 gw, P = 0.0140) and Z-VAD-fmk (139±36 gw, P = 0.0162) - perfused tilapia, whereas no remarkable effect was seen in the chymostatin-perfused fish (104±25 gw). At 50.5 h storage, the breaking strength was 86 - 98 gw and statistic difference was not seen between the inhibitor-perfused tilapia. On the other hand, those of *o*-phenanthroline-perfused fish at even 0 h was significantly different from other inhibitors by analysis of variance. Then all the *o*-phenanthroline-perfused experiments were not analyzed statistically (23 h, 106±20 gw, 50.5 h, 86±11 gw) and were not able to be compared with the Kubota *et al*. (2001) that showed involvement of metalloprotease in muscle softening.

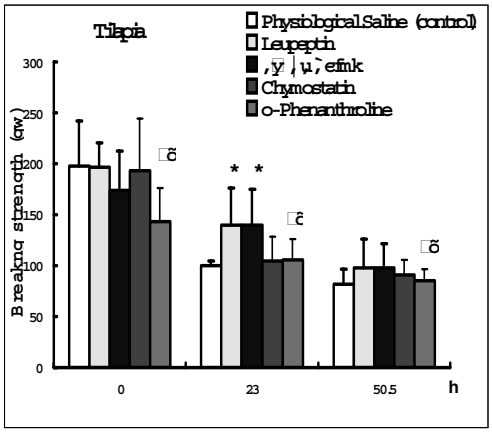

Fig.4 The breaking strength of the tilapia muscle stored under 4°C after the perfusion of the four types of protease inhibitors (leupeptin, Z-VAD-fmk, chymostatin and o-phenanthroline) into the replicate samples (n = 10). Vertical bars represent standard deviation.

* The breaking strength at 23 h storage of leupeptin and Z-VAD-fmk were significantly different by analysis of variance.
† The effect of o-phenanthroline at 23 and 50.5 h were not analyzed statistically, because the breaking strength of o-phenanthroline at 0 h was significantly different from other inhibitor groups by analysis of variance.

Protease inhibitor perfusion into yellowtail: Figure 5 shows the breaking strength of the yellowtail in each inhibitor group. At the beginning of storage (0 h), the breaking strength of all the inhibitor-perfused yellowtail was 191 - 235 gw with a large variance. The E-64 perfused yellowtail was slightly different at 9 h (199±31 gw, P = 0.1102) and significantly different at 46 h (143±13 gw, P = 0.0412) from other perfused fish.

Conclusions

In tilapia, leupeptin (serine and cysteine protease inhibitor) was effective to prevent the muscle softening and it is suggested that trypsin-like protease is the most probable candidate for causative protease of muscle softening. In addition, caspase-3 (Yabu *et al*. 2001) activity was induced in tilapia muscle after death and apoptosis might occur in muscle cells. In yellowtail, E-64 (cysteine protease inhibitor) was effective and cysteine protease was thought to be causative protease in muscle softening. These findings also showed that proteolysis was the one of causative factors for post-mortem muscle softening.

Acknowledgment

This work was supported in part by a grant of the Fisheries Agency, the Ministry of Agriculture, Forestry and Fisheries of Japan.

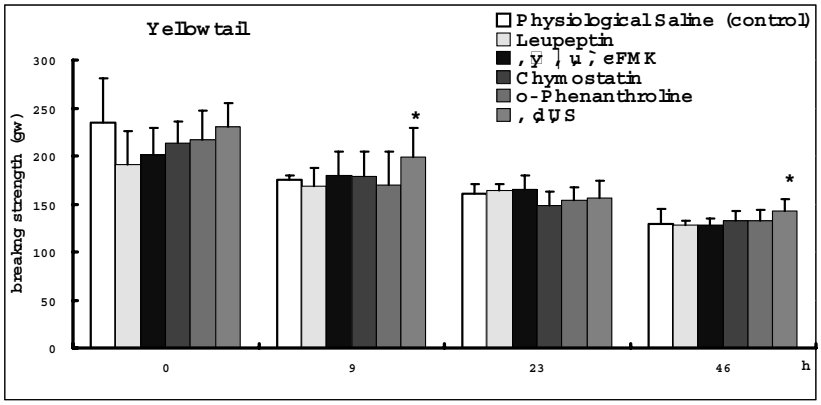

Fig.5 The breaking strength of the yellowtail muscle stored under 4° C after the perfusion of the five types of protease inhibitors (leupeptin, Z-VAD-fmk, chymostatin, o-phenanthroline and E-64) into the replicate samples (n = 6). Vertical bars represent standard deviation.

* The breaking strength of E-64 perfused fish at 9 h and 23 h were different by analysis of variance.

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EFFECT OF FREEZE-THAW PROCESS ON MYOGLOBIN OXIDATION OF PORK LOIN DURING COLD STORAGE

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Background and Objective

Although frozen storage is an important preservation method for muscle foods, quality deterioration cannot be avoid during freezing because of the formation of ice crystals, distorting the tissue structure, mechanical damage and denaturation of protein. Especially, the rapid meat color deterioration has been often observed in thawed meat compared to fresh meat at a commercial meat market. Myoglobin (Mb) oxidation is a major nonmicrobial factor responsible for the quality deterioration of fresh meat, and it is commonly assumed that lipid oxidation is closely related to Mb oxidation. Recently, we observed the formation of MetMb without lipid oxidation in thawed pork chop steak. During retail display, the rate of Mb oxidation is related to many factors, and physical and biochemical factors influence the displayable life of meat. Enzymes and other components are released during freezing and thawing. The release of mitochondrial enzymes such as β-hydroxyacyl CoA-dehydrogenase (HADH) into sarcoplasm have been reported (Gottesmann and Hamm, 1984; Chen et al., 1988; Toldra et al., 1991). Decker and Welch (1990) also reported that the rate of iron release from ferritin was influenced by temperature in muscle foods. In this research, we hypothesized that HADH activity could be partially responsible for the observed formation of MetMb without lipid oxidation in thawed pork loin during cold storage. The objective of this study was to determine whether HADH activity increased by freeze-thaw process could impact myoglobin oxidation without lipid oxidation in pork loin during cold storage.

Materials and methods

Commercial 10 pork loins were selected randomly at 24h postmortem, and the longissimus lumborum was used to make steaks (3 cm thickness). The steaks of each loin were packaged in a polyethylene bag, and samples were subjected to fresh group (Control), one cycle freeze-thaw group (Treatment 1) and two cycles freeze-thaw group (Treatment 2), respectively. All freeze-thaw samples were kept at -65℃ for 12 hours for freezing and stored at 4℃ for 12 hours for thawing. After thawing, samples were stored at 4℃ for 7 days to measure meat color (CIE L*a*b*), percentage of MetMb, thiobarbituric acid reactive substance (TBARS) value and HADH activity at 0, 3 and 7 days of storage.
Meat color (CIE L*a*b*) was measured by using a Minolta Chromameter (Minolta CR 301; Tokyo, Japan). Seven random readings were made from the surface of samples. Myoglobin was extracted from meat samples with phosphate buffer of pH 6.8. Samples were homogenized, centrifuged and filtered to obtain the absorbance of the resulting supernatant solution at 572, 565, 545, and 525 nm, respectively. Lipid oxidation was measured by TBARS value. Five grams of meat was weighed into a 50ml test tube and homogenized with 15 ml of deionized distilled. One ml of homogenate was transferred to a disposable test tube, and butylated hydroxyanisole (50, 10%) and thiobarbituric acid/trichloroacetic acid (TBA/TCA) (2 ml) were added, and then incubated in a boiling water bath for 15 min. The absorbance at 531 nm was used for TBARS value as milligrams of malondialdehyde per kilogram of meat. HADH activity was measured according to the method of Fernandez et al. (1999). Extract was obtained from meat sample by immersion in two volumes (4-6ml) of 0.1M phosphate buffer, pH 6.0, at room temperature. The HADH released in the extracts was assayed by mixing in a methacrylate disposable semi-micro spectrophotometer cell (10mm light path length, nominal working volume 1.5ml), 34 extract, 70 ethylenediaminetetraacetic acid (EDTA; 34.4ml) and 880 phosphate buffer (0.1M, pH 6.0). The mixture was kept at room temperature for 3 min and 20 NADH (1.5mM) and 20 acetoactyl-CoA (5.9mM) were added. The HADH activity was determined by measuring immediately the absorbance of the mixtures at 340nm.

Results and discussion

Sampes of control showed significantly ($p<0.05$) higher CIE a* value compared to those of treatments at 7 days of cold storage. Moreover the a* value of Treatment 1 was higher than that of Treatment 2. On the

contrary, MetMb percentages of Treatments were significantly ($p<0.05$) higher than those of control at 3 and 7 days of cold storage. Treatment 2 showed significantly ($p<0.05$) higher MetMb percentage compared to Treatment 1 at 3 days of storage. There were no significant ($p>0.05$) differences in TBARS values between control and treatments during cold storage. However there were significant ($p<0.05$) differences in HADH activity between control and treatments at 3 days of cold storage. Samples of treatments showed higher HADH activity compared to those of control. There was no significant ($p>0.05$) difference in HADH activity between Treatment 1 and Treatment 2.

These results suggested that the freeze-thaw process could accelerate meat color deterioration, i.e. decreased redness and increased MetMb percentage in pork loin during cold storage. However the freeze-thaw process did not affect on lipid oxidation of pork. This implied that autoxidation of Mb in freeze-thaw pork loin was influenced by somehow except lipid oxidation products. It could be possible that the freeze-thaw process leads to a local concentration effect of metal ions that might accelerate Mb oxidation. The rate of iron release from ferritin could be influenced by temperature in muscle foods (Decker and Welch, 1990). It could be also possible that the thawing process likely makes for a lower solubility of oxygen in the tissue that would lead to decreased OxyMb. The damage to cellular and sub-cellular compartments form physical disruption by ice crystals during freezing and thawing could release enzymes into sarcoplasm, and the enzymes could potentially be involved in MetMb formation in thawed meat finally. In this research, we confirmed the increasing of HADH activity by freeze-thaw process. HADH is involved in generation of NADH that has MetMb reductase activity in mitochondira intermembrane space. Because NAD^+ (Nicotinamide adenine dinucleotide) could not generate NADH (Reduced Nicotinamide adenine dinucleotide) effectively with presence of HADH, MetMb reductase activity might be reduced by increased HADH activity in postmortem muscles. Therefore, results suggested that the rapid Mb oxidation without lipid oxidation could be affected by partially the increased HADH activity during freezing and thawing of pork loin.

Conclusions

Freeze-thaw process of pork loin accelerated color deterioration during cold storage. The oxidation of Mb was not related to lipid oxidation. It was suggested that released HADH into sarcoplasmic from mitochondria could be involved in the autoxidation of Mb in freeze-thaw pork loin.

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Table 1. Changes in CIE a*, MetMb %, TBARS and HADH activity by freeze-thaw cycles of pork loin during 7days of cold storage.

Measurements	Treatments [1)]	Storage period (days)		
		0	3	7
CIE a*	C	7.26±1.15	7.11±0.79	6.24 ± 0.37^{b}
	T1	7.29±0.66	7.20±1.09	5.80 ± 0.64^{ab}
	T2	6.83±1.15	6.58±0.94	5.15 ± 0.44^{b}
MetMb (%)	C	8.03 ± 3.72^{Aab}	8.30 ± 2.31^{Ac}	14.00 ± 4.01^{Bb}
	T1	4.29 ± 0.35^{Bb}	13.35 ± 1.79^{Ab}	15.35 ± 3.83^{Ab}
	T2	8.68 ± 2.58^{Ca}	17.37 ± 0.95^{Ba}	20.98 ± 1.10^{Aa}
TBARS (MA mg/g)	C	0.04 ± 0.01^{B}	0.14 ± 0.01^{A}	0.16 ± 0.01^{A}
	T1	0.04 ± 0.02^{B}	0.12 ± 0.01^{A}	0.14 ± 0.01^{A}
	T2	0.04 ± 0.01^{B}	0.12 ± 0.01^{A}	0.14 ± 0.01^{A}
HADH activity (Unit /)	C	3.26 ± 0.25^{C}	5.55 ± 0.17^{Bb}	9.65 ± 0.24^{A}
	T1	3.66 ± 0.91^{B}	9.00 ± 0.10^{Aa}	9.60 ± 0.47^{A}
	T2	3.30 ± 0.23^{B}	9.02 ± 0.17^{Aa}	9.59 ± 0.45^{A}

[A,B] Means± SD with different superscript in the same row are significantly different($p<0.05$)

[a,b] Means± SD with different superscript in the same column are significantly different($p<0.05$)

[1)] C: Control, T1: Treatment 1(one cycle freeze- thaw), T2: Treatment 2(two cycles freeze-thaw)

MECHANISM OF THE PRODUCTION OF TROPONIN T FRAGMENTS DURING POSTMORTEM AGING OF PORCINE MUSCLE

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Background

It is well known that muscle is converted to meat as food during postmortem aging. Due to the increase of peptides and free amino acids during postmortem aging, meat taste is improved. During postmortem aging, a peptide APPPPAEVHEVHEEVH derived from troponin T (TnT) was found not only to contribute to improvement of taste (sour-suppressing) but also to be useful as a conditioning indicator in meat (Okumura et al., 2003). However, the details of TnT degradation and the relationship between TnT degradation and muscle tenderization remain poorly understood.

Objectives

The objectives of this study were to determine N-terminal amino acid sequences of TnT degradation fragments during postmortem aging and to clarify the involvement of calpain in the degradation.

Materials and methods

Preparation of myofibrillar proteins

In all steps of myofibrillar proteins preparation from porcine longissimus thoracis muscle (LT), samples were kept on ice. The meat samples were minced and homogenized with 0.04M Tris-HCl buffer (pH 7.4) containing 0.16M KCl. The precipitate obtained was washed and centrifuged three times in 0.16M KCl for 15min at 3,000 x g. After centrifugation, pellet was suspended in 0.16M KCl containing 5mM NaN_3. Finally, the solubilized myofibrillar proteins was filtered by nyron net to remove connective tissue.

Hydrolysis of myofibrillar proteins by m-calpain

m-Calpain from rabbit skeletal muscle was purchased from Sigma (Germany). Myofibrillar proteins were prepared from LT stored for 2 days after slaughter. Myofibrillar proteins (2.5mg) were hydrolyzed with m-calpain (125U) at 30°C for 180 min in 50mM acetate buffer (pH 6.2) containing $CaCl_2$ (5mM) and 2-mercaptoethanol (0.1%). The portion of this mixture (200 μl) was taken after 0, 10, 30, 60, and 180 min and added with 30mM Tris-HCl (pH6.8) buffer (100 μl) containing 60% glycerol, 3% SDS, 3% 2-mercaptoethanol, and 0.03% bromophenol blue to stop the reaction.

SDS-PAGE and electroblotting

All steps of SDS-PAGE and western blot analysis were performed at room temperature. The solubilized myofibrillar proteins (300 μg) were loaded onto 55mm-wide lane of SDS-PAGE gels. The 12.5% gel was run in 25mM Tris buffer containing 192mM glycine and 0.1% SDS at a constant current (14mA) for 2 hr 45 min. Prestained Precision Protein Standard (Bio-Rad, Hercules, CA) was used as the marker.

Western blot analysis

After SDS-PAGE, the gel was soaked in a transfer buffer (25mM Tris, 192mM glycine, 15% methanol, 0.05% SDS). Then, the proteins on the gel were transferred to a polyvinylidenedifluoride (PVDF) membrane (Immobilon-P_{SQ} Millpore, Bedford, MA) in the same transfer buffer. The current was set at 3mA/cm^2-membrane for 90 min. The electroblotted membrane was then blocked by blocking buffer (PBS containing 1% BSA and 0.1% Tween-20) for 30 min. After blocking, the membrane was incubated for 30 min with anti-fTnT polyclonal goat antibodies raised against a peptide corresponding to the internal region of human fTnT (Santa Cruz Biotechnology, Inc., Santa Cruz, CA). The primary antibodies were used at a 1:100 dilution in an antibody buffer (PBS containing 0.1% BSA and 0.01% Tween-20). After three washes with 0.1% Tween-20/PBS for 5 min each and being blocked with blocking buffer for 30 min, the membrane was incubated with

biotin-conjugated anti-goat secondary antibodies (Santa Cruz Biotechnology). The secondary antibodies were used at a 1:500 dilution in the antibody buffer for 30 min. After three washes, the membrane was incubated with avidin-DH and biotin-conjugated HRP (VECTORSTAIN® ABC-PO kit, Vector Laboratories, Burlingame, CA) according to the manufacturer's protocol. After three washes, the membrane was finally stained with a DAB substrate kit (Vector Laboratories).

Determination of N-terminal amino acid sequence of proteins

The electroblotted proteins on PVDF membrane were stained with Coomassie Brilliant Blue R-250 (CBB; Bio-Rad). The bands of interest were excised, and then the N-terminal amino acid sequences were analyzed by a G1000A Protein Sequencer (Hewlett Packard, Palo Alto, CA).

Results and discussion

The results of Western blot analysis revealed that multiple degradation products of TnT were generated in porcine LT during postmortem aging (Fig. 1) and by calpain hydrolysis (Fig. 2). Before aging and hydrolysis, four fTnT bands were detected molecular masses of around 37 kDa. These bands were thought to be intact TnT isoforms. These bands were decreased with aging and by calpain hydrolysis, and their degraded fragments with molecular masses of around 30 kDa increased. At 7 days postmortem, anti-fTnT-positive six bands were detected, and three of six bands were determined their N-terminal sequences with molecular masses of approximately 29, 28, and 27 kDa. The N-terminal amino acid sequences of 29, 28, and 27 kDa bands were EVHEPEEKPRPKLTAP, EKPRPKLTAPKIPEG, and APKIPEGEKVDF, respectively. After calpain hydrolysis, five anti-fTnT-positive bands were detected and three of five bands were determined their N-terminal amino acid sequences with molecular masses of approximately 30, 29, and 27 kDa. The N-terminal amino acid sequences of 30, 29, and 27 kDa bands were APPPPAEV, EVHEPEEK, and APK, respectively.

In our previous study, we have determined the amino acid sequences of porcine fTnT isoforms deduced from the DNA sequences (accession nos. AB176595- AB176602 in DDBJ/EMBL/GenBank nucleotide sequence databases). The number of amino acid residues of the fast and slow TnT isoforms were 270 (fTnT1), 265 (fTnT2), 259 (fTnT3), 249 (fTnT4), 261 (sTnT1), and 250 (sTnT2). The N-terminal sequence EVHEPEEK was found only in fTnT2 and fTnT3 (Fig. 3). As shown in Fig. 4, the cleavage sites His_{37}-Glu_{38} (a1 and b2) and Thr_{51}-Ala_{52} (a3 and b3) were cleaved after postmortem aging as well as by calpain hydrolysis. On the while, Glu_{43}-Glu_{44} (a2) was cleaved only after postmortem aging, and Glu_{21}-Ala_{22} (b1) was cleaved only by calpain hydrolysis. These results suggested that His_{37}-Glu_{38} and Thr_{51}-Ala_{52} were cleaved by calpain hydrolysis in postmortem aging, while Glu_{43}-Glu_{44} was by another proteinases hydrolysis, such as cathepsins. Recently, we found the sour-suppressing peptide APPPPAEVHEVHEEVH from postmortem aged pork (Okumura et al., 2003). From the results in the present study (Fig. 4), the sour-suppressing peptide would be produced by calpain hydrolysis at Glu_{21}-Ala_{22} and His_{37}-Glu_{38} sites. Howevere, APPPPAEVHEVHEEVH sequence was not detected from the N-terminal sequences of TnT fragments in postmortem aged porcine meat. The following thing is a probable reason; in postmortem aged pork, several proteinases, such as calpains and cathepsins, were involved in TnT degradation and, as a result, the peptide was liberated from TnT. On the while, in postmortem aged beef, the sequence APPPPAEVHEVHEEVH was determined from 32.1 kDa fragment (Muroya et al., 2004). These results indicated the ratio of proteinases might be different in porcine and bovine.

In this study, we clarirfied effect of calpain on TnT degradation. Howevere, the involvement of other proteinases such as cathepsins in the TnT degradation needs to be also clarified.

Conclusion

It is highly possible that a peptide APPPPAEVHEVHEEVH is derived from TnT by calpain during postmortem aging.

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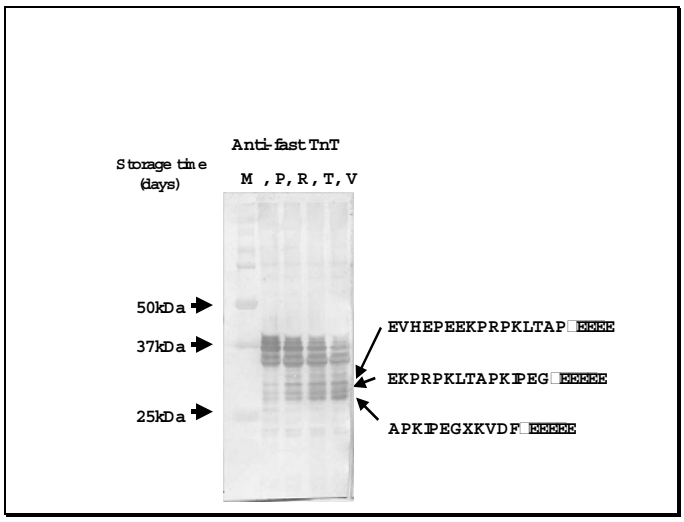

Fig. 1 Western blot analysis of TnT fragments using anti-fast TnT antibody.
Myofibrillar proteins from porcine longissimus thoracis muscle at 1, 3, 5, 7 days postmortem were analyzed.
The N-terminal amino acid sequences of the bands (indicated with arrows) were shown in the figure.
M, molecular mass markers.

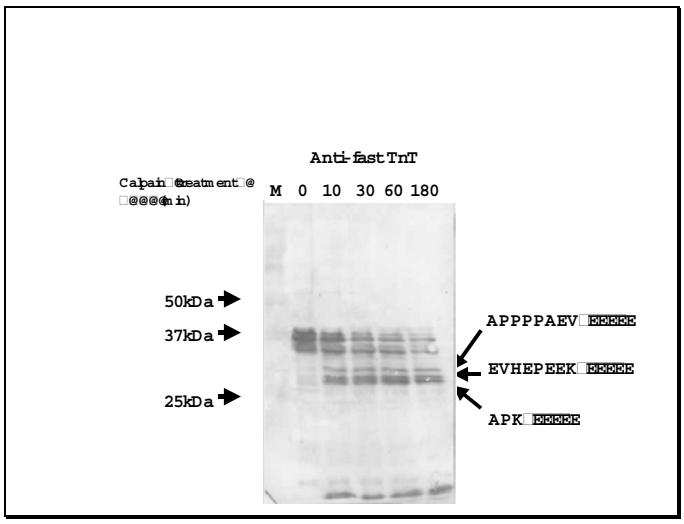

Fig. 2 Western blot analysis of TnT fragments using anti-fast TnT antibody.
Calpain hydrolysate of myofibrillar proteins from porcine longissimus thoracis muscle were analyzed.
The N-terminal amino acid sequences of the bands (indicated with arrows) were shown in the figure.
M, molecular mass markers.

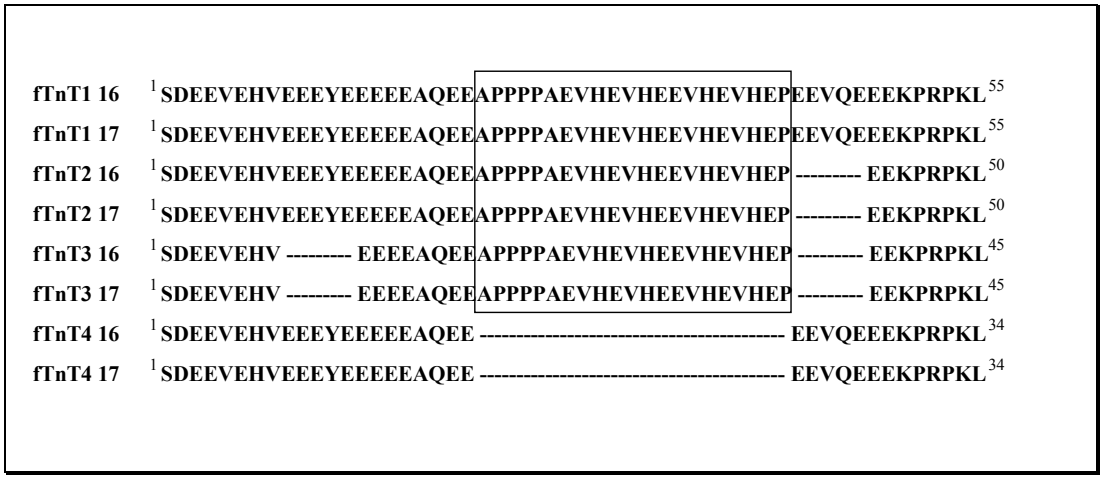

Fig. 3 The amino acid sequences of N-terminal region of eight porcine fTnT isoforms.
Sour-suppressing peptide is boxed.
--- indicates exon regions of alternative splicing.

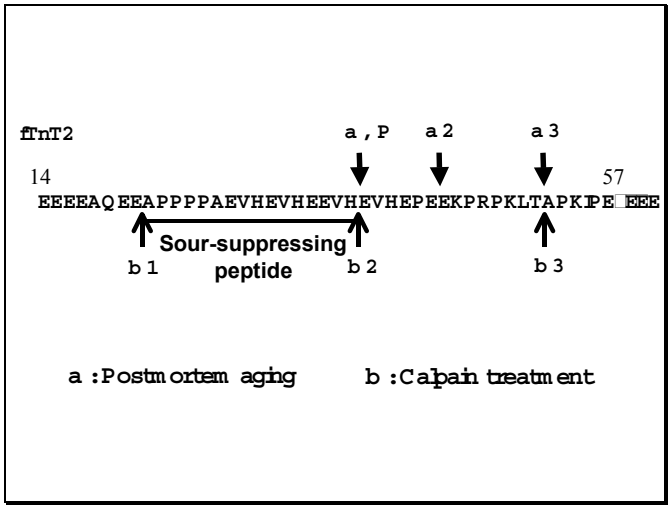

Fig. 4 The amino acid sequence of N-terminal region of porcine fast-TnT2 (fTnT2) and its cleavage sites.
a, indicates cleavage sites observed after postmortem aging.
b, indicates cleavage sites observed by calpain hydrolysis.
Sour-suppressing peptide is underlined.

EFFECT OF RN$^-$ AND CALPASTATIN (*CAST*) GENE AS RELATED TO MEAT QUALITY OF STRESS RESISTANT FATTENERS

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Background

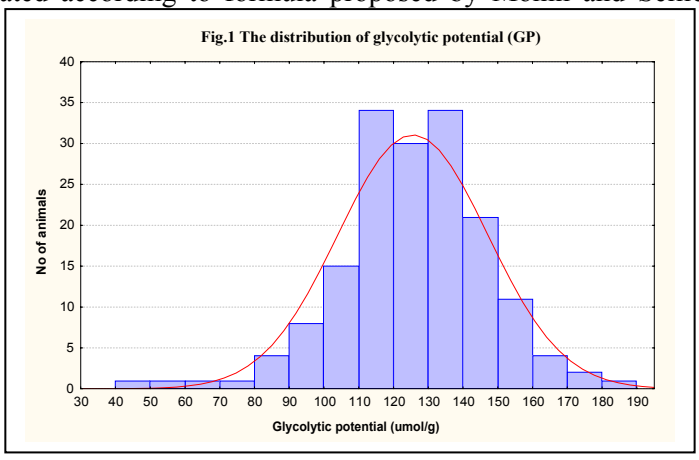
Pork quality traits are composite, being affected by major genes (RYR1 RN) and the candidates e.g. mapped on 2 chromosome calpastatine gene – an endogenous inhibitor of calpain (Koćwin-Podsiadła et al. 1995, Przybylski et al.1995, Sellier 1998, Sensky et al. 1999).

Objectives

The present study was conducted to estimate the polymorphism of calpastatin (*CAST*) gene identified with *Hinf*I, *Msp*I and *Rsa*I restriction endonucleases, and the effect of RN$^-$ gene (determined on the basis of glycolytic potential), on meat quality traits.

Materials and methods

The investigations were carried out on 169 fatteners of three genetic groups: Landrace (41), Landrace x Duroc (67) and Landrace x Yorkshire (61), obtained from F_0 animals, imported from Denmark. The animals were kept under the same environmental conditions and fed a full bath feed. The animals were slaughtered at the live weight about 110 kg (hot carcass weight 85 kg), 4-5 hours after transportation, using the electrical stunning method and recumbent bleeding out. Immediately after slaughter blood samples were collected in EDTA-coated tubes for subsequent DNA analysis for the RYR1 and *CAST* genotype. The RYR1 genotypes were established according to Fuji et al. (1991). The polymorphism of *CAST* gene was identified with *Hinf*I, *Msp*I and *Rsa*I restriction endonucleases, according to Ernst et al. (1998). At 45 min *post mortem* the samples from *Longissimus lumborum* (LL) muscle were collected into tubes with 0,5 M PCA for determination of glycogen (Darympale and Hamm, 1973) and lactate (Bergmayer, 1974). On the basis of them, the glycolytic potential (GP) was calculated according to formula proposed by Monin and Sellier (1985). The RN genotypes were identified on the basis of glycolytic potential (GP) and its bimodal distribution: rn^+rn^+ (GP≤130µmol/g) RN$^-$/? (GP>130µmol/g) (Fig.1). The pH was measured directly in the tissue of *Longissimus lumborum* muscle, using a pH-meter Master produced by Dramiński (Poland). The electrical conductivity was evaluated using a LF-Star apparatus, produced by Matthaus (Germany). The lightness (L*) of the muscle tissue was measured 24 hours *post mortem* using a Minolta CR-310 apparatus (Japan) in CIE L*a*b* colour system. The drip loss at 48, 96 and 144 hours after slaughter was evaluated in accordance with the method of Prange et al. (1977). The shear force was determined in (144h) on cooked meat slices, using an Instron 1140 apparatus with Warner-Bratzler device. The data was analysed, using a two-way analysis of variance in a non-orthogonal scheme. The significance of differences between means of groups for the investigated traits, was calculated using Tukey's test.

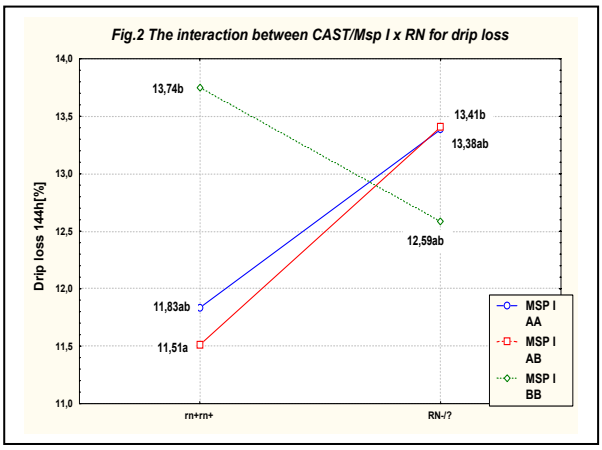


Results and discussion

The high muscle glycolytic potential (GP>130µmol/g) has an effect on pH decline (at 24, 48 and 96 hours after slaughter), and higher meat lightness [Tab.1]. The low pH_u is connected with a higher drip loss from pork meat (Kauffman et al. 1993, Bertram et al. 2000). The deeper glycogenolysis (as the effect of RN$^-$ allele), affected fluid loss from fresh meat during *post mortem* storage, giving higher (about 0,7 percent points) drip loss in 48 hours after slaughter. Josell et al. (2003) suggested, that high amount of glycogen, the most important substrate for ATP regeneration, might contribute to hinder muscle contraction in RN$^-$ carriers, while Deng et al. (2002) showed, that the low water-holding capacity of meat from RN$^-$ carriers, was caused by high degree of denaturation of proteins. The tenderness (expressed by shear force) has been shown to be related to RN gene, which is in agreement with studies by different authors (Lundstrom et al. 1994, Enfalt et al. 1997, Josell et al. 2003).

In table 1 showed the effect of calpastatin gene (regardless RN$^-$ gene) on analysed meat quality traits. The AA genotype at the *CAST/Hinf*I locus affected the initial pH, given (in comparison to animals with AB and BB genotypes) lower at about 0,15 units pH_{35} and 0,22-0,25 units pH_3. In contradiction to this experiment, in the investigations of Koćwin-Podsiadła et al. (2003), carried out on fatteners differentiated by RYR1 gene, the faster pH_{35} decline, in group of fatteners with BB genotype at *CAST/Hinf*I locus, was noted. There was no effect of GP (RN gene) on drip loss measured in 96 and 144 hours *post mortem* but the influence of *CAST/Hinf*I gene on fluid loss, was noted. It should suggest that *CAST/Hinf*I genotype is connected with a later drip loss from fresh meat during storage. The AA animals compared with AB and BB porkers at the *CAST/Hinf*I locus given about 4 pp. higher drip loss measured in 96 and 144 hours *post mortem*. During the storage from 48 to 144 hours after slaughter, a highest fluid loss has been shown from fresh meat of AA genotype porkers at the *CAST/Hinf*I locus (8, 5 and 6 pp. respectively for AA, AB and BB genotype at the *CAST/Hinf*I locus).

In contrast, the BB genotype porkers at *CAST/Rsa*I locus (compared with AA and AB animals) affected fluid loss, given about 2 pp. higher drip loss measured in 144 hours *post mortem*. During the storage from 48 to 144 hours after slaughter, a highest fluid loss has been shown from fresh meat of BB genotype porkers at the *CAST/Rsa*I locus (8, 6 and 5 pp. respectively for BB, AA and AB genotype at the *CAST/Rsa*I locus).



It should the mention that both genotypes with highest drip loss (AA at *CAST/Hinf*I and BB at *CAST/Rsa*I) had a lowest percentage share in analysed population of fatteners (8,88 and 6,51% respectively).

The AA genotype at the *CAST/Msp*I affected the glycolytic changes in *Longissimus lumborum* muscle, given about 0,05 units higher pH at 24 and 144 hours after slaughter. The effect of calpastatin gene (*CAST/Msp*I and *CAST/Rsa*I) on meat quality traits, in the investigations of Emnet et al. (2000), was not found.

In this experiment, the interaction was found between RN gene and *CAST/Msp*I for a drip loss measured in 144 hours after slaughter (Fig.2). The AA genotype at the *CAST/Msp*I locus was differed by RN gene, given 1,9 pp. lower drip loss (144h) in non-carriers of RN gene. Among non-carriers of RN$^-$ gene, were two groups related *CAST/Msp*I genotype and differed by drip loss measured in 144 hours *post mortem*. The AB animals compared with BB porkers at the *CAST/Msp*I given 2,23 pp. lower drip loss from *Longissimus lumborum* muscle.

Conclusions

Meat from RN$^-$ carriers, compared to meat from non-carriers had higher glycogen content, lower ultimate pH and higher meat lightness (L*) and drip loss (48h).

The highest fluid loss from fresh meat during storage in a group of porkers with AA genotype at the *CAST/Hinf*I and BB genotype at the *CAST/Rsa*I locus, was noted. The frequency of mentioned above genotypes with highest drip loss was lowest in analysed population and reached 8,88 and 6,51% respectively.

In this experiment, we also found the interaction between RN gene and *CAST/Msp*I for a drip loss evaluated in 144 hours after slaughter. The non-carriers of RN$^-$ gene, but with AB genotype at the *CAST/Msp*I locucs

porkers compared with BB genotype animals at the same locus of *CAST* and RN, given 2,23 pp. lower drip loss (144h after slaughter) during storage of fresh *Longissimus lumborum* muscle samples.

Acknowledgment
This work was financed by the State Committee for Scientific Research (KBN) project – No. PBZ-KBN-036/P06/04.

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Table 1. The effect of RN^- and calpastatine (*CAST*) gene on analysed meat quality traits.

	*CAST/Hinf*I				*CAST/Msp*I				*CAST/Rsa*I				rn^+rn^+	$RN^-/?$	Sign
	AA n=15	AB n=70	BB n=84	Sign	AA n=31	AB n=73	BB n=65	Sign	AA n=71	AB n=87	BB n=11	Sign	n=95	n=74	
GP [µmol/g]	124,52 ±12,21	127,13 ±25,70	126,36 ±26,22	NS	122,23 ±25,86	126,72 ±21,11	128,33 ±28,47	NS	126,59 ±27,51	127,39 ±23,72	119,10 ±16,59	NS	111,47A ±16,19	145,84B ±20,60	**
Glycogen [µmol/g]	44,65 ±8,32	46,35 ±14,87	46,06 ±13,67	NS	43,42 ±15,40	46,00 ±13,56	47,37 ±14,22	NS	47,21 ±13,96	45,71 ±13,70	41,30 ±12,79	NS	38,15A ±	56,20B ±10,50	**
Lactate [µmol/g]	35,22 ±11,02	35,09 ±10,37	34,22 ±10,62	NS	35,40 ±9,97	35,34 ±10,45	33,56 ±10,85	NS	32,83 ±9,54	35,94 ±10,75	36,51 ±13,31	NS	35,14 ±10,61	34,07 ±10,39	NS
pH_{35}	6,49a ±0,11	6,22b ±0,17	6,58b ±0,16	*	6,59 ±0,20	6,60 ±0,16	6,57 ±0,19	NS	6,59 ±0,17	6,59 ±0,18	6,52 ±0,23	NS	6,56a ±0,19	6,62b ±0,16	*
pH_3	6,01a ±0,21	6,26b ±0,21	6,23b ±0,25	*	6,19 ±0,26	6,23 ±0,24	6,23 ±0,23	NS	6,22b ±0,25	6,24b ±0,22	6,01a ±0,32	*	6,19 ±0,26	6,26 ±0,21	NS
pH_{24}	5,56 ±0,08	5,56 ±0,12	5,54 ±0,09	NS	5,61B ±0,11	5,52A ±0,10	5,55A ±0,08	**	5,55 ±0,09	5,55 ±0,12	5,58 ±0,08	NS	5,57B ±0,11	5,52A ±0,09	**
pH_{48}	5,37 ±0,06	5,41 ±0,11	5,40 ±0,08	NS	5,43 ±0,11	5,40 ±0,09	5,39 ±0,08	NS	5,40 ±0,09	5,41 ±0,10	5,41 ±0,09	NS	5,42b ±0,10	5,38a ±0,08	*
pH_{96}	5,37 ±0,08	5,39 ±0,11	5,40 ±0,09	NS	5,42 ±0,09	5,40 ±0,11	5,38 ±0,08	NS	5,39 ±0,08	5,40 ±0,11	5,42 ±0,07	NS	5,43B ±0,09	5,36A ±0,08	**
pH_{144}	5,55 ±0,09	5,49 ±0,12	5,49 ±0,10	NS	5,55b ±0,13	5,49a ±0,11	5,48a ±0,10	*	5,50 ±0,10	5,48 ±0,12	5,59 ±0,02	NS	5,52 ±0,12	5,46 ±0,09	NS
EC_{24} [mS/cm]	3,42 ±1,08	3,49 ±1,18	3,90 ±1,18	NS	3,61ab ±1,20	3,47a ±1,02	3,97b ±1,30	*	3,67 ±1,07	3,73 ±1,27	3,44 ±1,20	NS	3,79 ±1,16	3,50 ±1,19	NS
L*	53,47 ±3,03	54,69 ±3,20	54,70 ±2,86	NS	53,97 ±2,92	54,74 ±3,14	54,71 ±2,95	NS	54,38 ±2,79	54,89 ±3,27	53,54 ±2,15	NS	53,90a ±2,98	55,48b ±2,87	*
Drip loss 48h [%]	8,02 ±2,88	7,08 ±2,14	6,73 ±2,18	NS	6,95 ±2,15	7,19 ±2,49	6,80 ±2,02	NS	6,88 ±2,37	6,97 ±2,08	7,89 ±2,74	NS	6,69a ±2,18	7,38b ±2,29	*
Drip loss 96h [%]	14,06B ±4,79	10,89A ±2,54	10,79A ±2,73	**	11,42 ±3,48	11,05 ±3,28	10,99 ±2,37	NS	11,01 ±2,94	10,96 ±2,84	13,07 ±4,38	NS	11,03 ±3,13	11,18 ±2,81	NS
Drip loss 144 [%]	16,19b ±1,69	12,48a ±2,59	12,75a ±3,01	*	12,38 ±2,90	12,41 ±2,90	13,19 ±2,79	NS	13,02a ±3,10	12,34a ±2,61	15,98b ±1,63	*	12,46 ±2,88	13,04 ±2,82	NS
Shear force [N/cm^2]	45,41 ±9,94	62,36 ±15,06	56,26 ±13,26	NS	60,32 ±11,42	60,59 ±12,77	54,43 ±15,67	NS	59,97 ±14,79	55,77 ±13,71	56,11 ±17,76	NS	63,22B ±13,62	47,24A ±8,35	**

Results are given as means ± SD; **, A, B signigicant at P≤0,01; *, a, b signigicant at P≤0,05

THE INFLUENCE OF RYR1 AND CAST/MSPI GENES POLYMORPHISM AND THEIR INTERACTIONS ON SELECTED PORK MEAT QUALITY TRAITS

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Background

Post mortem proteolysis of myofibrillar proteins is associated with activity of the calpain system (-μ and -m calpain) and their inhibitor calpastatin (Sensky et al. 1999, Hao et. al 2000). Calcium channel activity is regulated by domain L of calpastatin (Hao et al. 2000). Ca^{2+} level in sceletal muscle is also regulated by *RYR1* gene. Activity of calpastatin (as endogenous inhibitor of calpain) in sceletal muscle is highly related to rate of meat tenderization and protein turnover after slaughter (Goll et al. 1998). Thus, *CAST* represents an excellent candidate gene for studying variation in pork quality (Ernst et al. 1998).

Objectives

The aim of this study is analysis of the effect of polymorphism of *RYR1*and calpastatin (*CAST*) genes for some meat quality traits taking into consideration group of meatiness. An effect of interactions between variants of *RYR1* and *CAST* gene and group of meatiness for investigated meat quality traits was also analysed.

Materials and methods

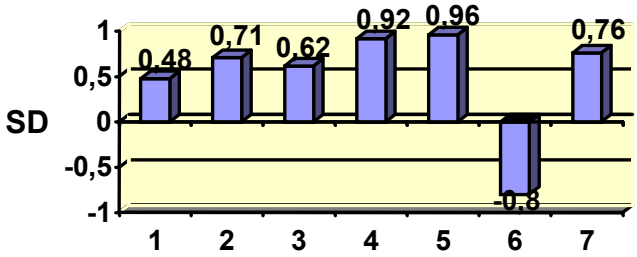
Investigations were carried out on 201 fatteners being crosses of [(Polish Large White x Polish Landrace) x (Duroc x Pietrain)] (77), [(Landrace x Yorkshire) x (Duroc x Pietrain)] (40) and [(Polish Large White x Polish Landrace) x (Hampshire x Pietrain)] (84). The animals were kept in similar environmental conditions, fed balanced mixtures and slaughtered using electrical stunning ("Inarco" system) at 4-5 hours after transportation on the distance 300 km. Immediately after slaughter blood samples were collected in EDTA-coated tubes for subsequent DNA analysis for the *RYR1* and *CAST* genotype.
Average warm carcass weight of analysed animals was 78.40±0,54 kg (mean value ± se). Carcasses belonged to three groups of meatiness: I ≤50.0; II from 50.1 to 55 and III >55 percent of meat in carcass respectively. Average meatiness of carcasses analysed population of fatteners was 51.28±0.40 %. In each group was similar number of gilts and castrates.
The following meat quality characteristics immediately after slaughter were determined: pH of *Longissimus Lumborum* (*LL*) muscle tissue (immediately in carcass - pH_{35}) and in water homogenate of muscle tissue (pH_{45}); R_1 expressed as IMP/ATP ratio at 45 min *post mortem* according to Honikel and Fischer (1977). At 24 h *post mortem* pH, meat lightness (measured with Minolta CR310 Chroma Meter in CIE L*a*b* system), water holding capacity (WHC) according to Grau and Hamm (1952) and Pohja and Niniivaara (1957) modification and losses of weight of meat in cooking process were determined. Drip loss from muscle tissue at 48 and 96 h after slaughter was determined according to Prange et al. (1977). Besides, analysis of protein, water and dry matter content in *LL* muscle tissue were determined. Meat lightness and pH_{24} in *Semimembranosus (SM)* muscle were also executed. At 45 min *post mortem* samples from *Longissimus Lumborum* muscle were collected into tubes with 0.5 M PCA for determination of glycogen (Dalrymple and Hamm 1973) and lactate (Bergmeyer, 1974). On the basis of them the glycolytic potential (GP) was calculated according to formula proposed by Monin and Sellier (1985).
The *RYR1* genotypes were established according to Fujii et al. (1991). Polymorphism of *CAST* gene was identified with *Msp*I endonuclease according to Ernst et al. (1998).
Statistical elaboration of the data was executed using three-way non-orthogonal ANOVA. Statistical model comprised: *RYR1* and *CAST* genes polymorphism, group of meatiness and their interactions:
Detailed comparison of average values of analysed groups was made using Tukey' test.

Results and discussion

In analysed population of animals a highly significant influence of *RYR1* genotype on pH value measured at 35 min *post mortem* immediately in *Longissimus lumborum* muscle (pH_{35}) as well as at 45 min *post mortem* in water homogenate of muscle tissue (pH_{45}) and on lightness of *LL* muscle ($P \leq 0.01$) was observed. The *RYR1* gene polymorphism affected also lactate level in *LL* muscle tissue, R_1 value, WHC and drip loss in both terms of measurements ($P \leq 0.05$). It should be stressed that analysed animals were of *CC* and *CT* genotype of the *RYR1* locus. Most profitable values of above mentioned parameters in stress resistant (*CC*) group of fatteners were noted.

A significant influence of *CAST* gene polymorphism identified with *Msp*I enzyme (*CAST/Msp*I) was noted for R_1 coefficient value, drip loss from muscle tissue at 48 and 96 h *post mortem* ($P \leq 0.01$) and for lactate level, WHC and protein and water content ($P \leq 0.05$).

Fig. 1. Differences between mean values of meat quality traits of animals with AA and BB genotypes at the CAST/MspI locus, expressed in SD units of the traits



1-Lactate level; 2-R_1; 3-WHC; 4-drip loss at 48h; 5-drip loss at 96h; 6-% of protein; 7-% of water in LL muscle

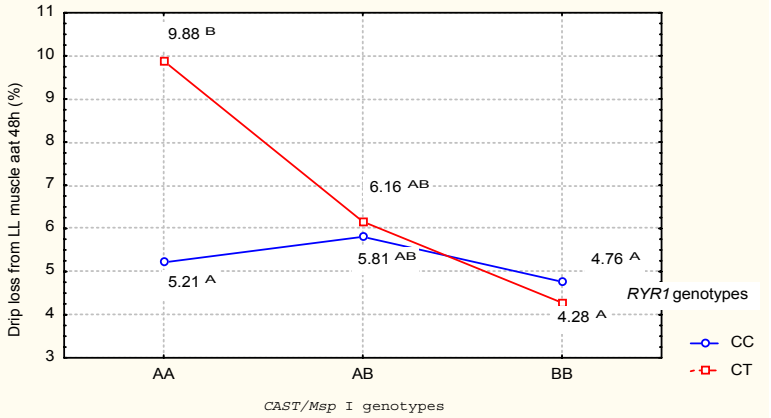
We have observed that animals with *BB* genotype at this locus were characterised by most profitable values of all these traits. High and significant influence of *CAST/Msp*I gene on drip loss from muscle tissue obtained in these investigations is especially interesting, taking into consideration important problem concerning a high variation of this trait (from 2 to 16%) signalised by several authors (Honkavaara 1997, Bertram et al. 2000, Schäfer et al. 2002).

Among analysed meat quality traits affected by *CAST* genotype, differences between average phenotypic values of *AA* and *BB* homozygotes in relation to *CAST/MspI* locus were close to 1 SD unit for drip loss at 48 h as well as at 96 h *post mortem* (0,92 and 0,96 SD respectively) (Fig. 1).

This indicates that *CAST/Msp*I genotype has an effect close to the major effect for drip loss from *LL* muscle tissue.

Statistically significant interaction between *RYR1* and *CAST/Msp*I loci was noted for: drip loss at 48 h post mortem ($P \leq 0.01$) (Fig. 2), and pH_{24} of *LL* muscle ($P \leq 0.05$) (Fig. 3). Value of drip loss at 48 h *post mortem* was differentiated between *CC* and *CT* animals at *RYR1* locus being *AA* homozygotes at *CAST/Msp*I locus. Among animals of *CT* genotype at *RYR1* locus carrying *AA* or *BB* genotypes at *CAST/Msp*I locus drip loss at 48 h *post mortem* differed significantly (9,88 and 4,28% respectively). Conducted additionally one-way ANOVA analysis showed that animals with *AA*/*CT* genotype at *CAST/MspI* and *RYR1* loci, respectively, showed significantly highest drip loss at 48 h *post mortem* (9,88 %), higher drip loss at 96 h and R_1 value, worse WHC and protein content than *BB/CT* animals, whereas meatiness of these two groups did not statistically differ.

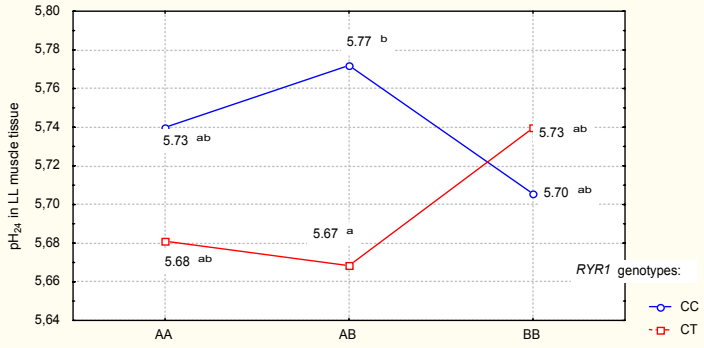
Fig. 2. Interactive effect of *CAST/Msp*I and *RYR1* genotypes for drip loss from LL muscle at 48h



Explanations: A,B mean values showed at the plot, signed by different capital letter differ significantly at P<

It is known that animals with *TT* or *CT* genotype at the *RYR1* locus show higher level of Ca^{2+} ions released from cells as a result of defective functioning of calcium channels under stress conditions. Next, proteolytic activity of calpain system (calpains and their inhibitor - calpastatin) is significantly dependent on Ca^{2+} ions level. One should suppose that different variants of calpastatin conditioned by polymorphism of *CAST* gene could have different sensibility to level of Ca^{2+} ions, and the same different activity stopping proteolytic activity of calpain. This may explain the differences in drip loss and pH values noted between fatteners with the same *RYR1* genotype but differentiated by *CAST/Msp*I genotypes.

Fig. 3. Interactive effect of *Cast/Msp* I and *RYR1* genotypes for pH_{24} in LL muscle tissue



Explanations: a, b mean values showed at the plot, signed by different small letter differ significantly at P<=0.05

Investigations carried out by Koćwin-Podsiadła et al. (2003) in similar scheme but only on (Polish Large White x Polish Landrace) x (Hampshire x Pietrain) crossbreeds showed significant interaction between *RYR1* and *CAST/Hinf*I genotypes for drip loss from *Longissimus Lumborum* muscle at 48 h *post mortem*.

Ciobanu et al. (2002) confirmed the effect of polymorphism of *CAST* gene identified with *Hpy*188I and *Pvu*II restriction enzymes on drip loss.

The polymorphisms of the *CAST* gene genotyped in this study were located in intron 7 and it is difficult to conclude their effect on calpastatin level or activity. The effect of analysed mutations on meat quality traits may be due to the linkage to any other mutation within the coding or regulatory regions of the *CAST* gene being the causal mutation.

Conclusions

In analysed fatteners' population highly significant influence of *RYR1* gene polymorphism on pH_{35}, pH_{45}, R_1 values that are basis of PSE meat classification and also on lactate level in *Longissimus Lumborum* muscle was noted.

Fatteners with *BB* genotype at the *Cast/Msp*I locus by lower lactate level (7.59 µmol/g), R_1 value (0.05), WHC (0.93 cm^2), drip loss at 48 h (3.03%) and at 96 h *post mortem* (3.36%) values, lower water content (1.31%) and also by about 0.5% higher protein content were characterized in comparison to fatteners with *AA* genotype at this locus. Relationships between polymorphism at the *CAST* locus and some meat quality traits, especially the high effect of *CAST/Msp*I polymorphism on drip loss of muscle tissue at 48 h as well as at 96 h *post mortem* (0,92 and 0,96 SD respectively) suggest that effect of the *CAST* gene is close to a major effect for this trait.

Interactions between genotypes *CAST/Msp*I and *RYR1* indicate that quality of meat influenced by *RYR1* genotype may be modifying by simultaneous influence of genotype as regards *CAST* locus. The polymorphism at the *CAST/Msp*I locus was closely related with drip loss from LL muscle tissue at 48 h among animals with *CT* genotype at the *RYR1* locus (with similar meat content in carcass) whereas this relationship among stress resistant (*CC*) animals was not confirm.

Obtained interesting results indicate that relationships between polymorphism of *CAST* gene and meat quality of pigs should be further investigated.

Acknowledgement

This work was financed by the State Committee for Scientific Researches (KBN), No. of project PBZ-KBN-036/P06/04

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POST-MORTEM PROTEOLYSIS IN FRESH PORK: ROLE OF THE LYSOSOMAL ENZYME SYSTEM AND OF THE CALPAIN SYSTEM

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Background

It is well known that the tenderness of meat increases during storage and it is generally believed to be caused by an enzymatic degradation of muscle proteins post mortem (PM). Two enzyme systems have continually been suggested as possible candidates for the proteolytic degradation PM. Several studies have suggested the lysosomal cathepsins to be involved in the tenderization process (Dutson, 1983; Etherington et al., 1987; Mikami et al., 1987; O'Halloran et al., 1997; Ouali et al., 1987). Cathepsins are able to degrade a wide spectrum of muscle proteins and have a pH optimum close to normal ultimate pH in meat which makes lysosomal enzymes good candidates for catalysing PM proteolysis and meat tenderization. In the last decade much work has implicated a major role of the calpain system for tenderization of beef and lamb. In beef the initial at-slaughter levels of the calpain system have been found to be related to the PM tenderization process and ultimate tenderness (Shackelford et al., 1991; Whipple et al., 1990; Zamora et al., 1996) and the calpain system has also been suggested to be primarily responsible for PM protein degradation and tenderness development during refrigerated storage leaving little or no importance of the lysosomal enzyme system (Hopkins and Thompson, 2002; Koohmaraie, 1996; Taylor, 2003). In pork, sparse data exists on the relationship between the initial level of enzyme activity and PM protein degradation and tenderness. However, a recent study on pork suggests that both enzyme systems could play a role (Ertbjerg et al., 1999a).

Objectives

The intentions of the present paper are to report data related to PM proteolysis in fresh pork and discuss the mechanisms of PM proteolysis and to what extent calpains and cathepsins are involved in this.

Materials and methods

Nine litters of three female pigs and three male pigs were allocated to three diet groups. Diets composition, slaughter procedure and sampling procedure were as described in Therkildsen et al. (2004). Briefly, samples for m-calpain, μ-calpain, calpastatin, cathepsin B+L, cathepsin B, β-glucuronidase and cystatin were taken 15 min after slaughter and frozen in liquid nitrogen. Temperature decline in the carcasses were measured over 24 h PM by inserting a temperature logger 10 min PM at left side LD at last rib. Samples for myofibrillar fragmentation index (MFI) were taken day 1, day 2 and day 4 PM. Calpastatin, m-calpain, μ-calpain and MFI were determined as described (Kristensen et al., 2002). Lysosomal enzyme activity and cystatin activity were done as described in Kristensen (2003). The data were tested for normal distribution using the Shapiro-Wilk test within the UNIVARIATE procedure of SAS (SAS Inst. Inc., 1999 - 2000). Variables not satisfying the requirement of a normal distribution were transformed. The effects of diet and sex were removed from all variables using the GLM procedure of SAS. Multiple linear regression models were obtained by the REG procedure of SAS using stepwise regression. A P-value of 0.15 was used to introduce and keep variables in the models.

Results

The effects of diet, sex and the interaction have consequently been removed from all data before modeling. Multiple linear regressions models were obtained using the MFI values as dependent variables and the at-slaughter activities of μ-calpain, calpastatin, cathepsin B, cystatin and the temperature decline from 60 minutes until 180 minutes PM as independent variables (Table 1). Calpastatin correlated to m-calpain ($r = 0.37$; $P = 0.009$), β-glucuronidase and cathepsin B+L to cathepsin B ($r = 0.63, 0.68$; $P = < 0.001$ & < 0.001) and μ-calpain, cathepsin B, cystatin, and temperature decline to pH45 ($R = -0.30, 0.37, 0.33, -0.50$; $P = 0.03$,

0.01, 0.02, < 0.001). To fulfill the requirement of non-covarying independent variables m-calpain, cathepsin B+L, β-glucuronidase and pH45 were excluded from the models. Modeling MFI values resulted in highly significant models explaining almost 50 % of the variation (Table 1). Calpastatin had the highest explainable power followed by cathepsin B in the models obtained for MFI values day 1 and 2 PM. At 4 days PM cathepsin B had the highest explainable power followed by calpastatin. Cystatin activity and temperature decline were included in all three models.

Table 1. Multiple linear regression models on MFI-values 1, 2 and 4 days PM.

MFI day 1		MFI day 2		MFI day 4	
Variable	Partial R^2	Variable	Partial R^2	Variable	Partial R^2
Calpastatin	0.18	Calpastatin	0.24	Cathepsin B	0.24
Cathepsin B	0.10	Cathepsin B	0.18	Calpastatin	0.15
$T_{decline}$	0.04	Cystatin	0.04	$T_{decline}$	0.04
Cystatin	0.04	$T_{decline}$	0.03	Cystatin	0.03
µ-calpain	0.03				
Model R^2	0.46		0.48		0.46
P-value	< 0.001		< 0.001		< 0.001

Discussion

The tenderization of meat is mainly caused by proteolytic degradation of muscle proteins PM. The fragmentation of myofibrils has for long been associated with tenderness development of meat (Davey and Gilbert, 1969; Møller et al., 1973) and a method to measure it has been termed MFI (Olson and Parrish, 1977). The calpain system has been suggested as the primary catalyst of the PM tenderization process in beef (Huff-Lonergan et al., 1996; Koohmaraie et al., 1986) and has also been linked to the tenderization process in pork (Ertbjerg et al., 1999a; Kristensen et al., 2002; Kristensen et al., 2003). Likewise indicators of lysosomal proteolysis PM have been linked to tenderisation in beef (Calkins et al., 1987; Ertbjerg et al., 1999b; Shackelford et al., 1991). Ouali (1992) argued that the changes in the structure of meat PM caused by proteolysis only can be explained by a synergistic action between lysosomal enzymes and the calpain system. To test this, multiple linear regression models were developed using MFI as dependent variables and the at-slaughter activity of both the calpain system and indicators of the lysosomal enzymes as independent variables (Table 1). The models obtained at all time points clearly showed that calpastatin and cathepsin B explained more of the variation in MFI collectively than each of them did in isolation, suggesting that calpastatin and cathepsin B have a co-operative action in PM proteolysis. If the calpastatin activity and the activity of cathepsin B are taken as representatives of the calpain system and of the lysosomal system, respectively, the results presented in Table 1 strongly suggest a role for both enzyme systems in PM proteolysis of pork as proposed by (Ouali, 1992).

There has been some debate regarding the involvement of the calpain system and lysosomal system in PM proteolysis and tenderization of meat, and some researchers have argued against the importance of the lysosomal system and favored the importance of the calpain system (Hopkins and Thompson, 2002; Koohmaraie, 1996; Taylor, 2003). Koohmaraie (1996) argued that no role can be assigned to the lysosomal enzymes until it is clearly confirmed that the enzymes are released from the lysosomal system during PM storage. The lysosomal proteases are encapsulated within the lysosomal system and therefore have no direct access to the myofibrillar proteins in the living animal. However, several papers report an increased activity of free lysosomal enzymes during PM storage, indicating a release from the lysosomal system after slaughter. Dutson and Lawrie (1974) observed a two fold increase in the free activity of β-glucuconidase in beef 24 hours PM which increased to three fold after 5 days storage. Ertbjerg et al. (1999b) also observed an increase of free β-glucuconidase activity and in addition an increase of the free activity of cathepsin B+L in beef during storage for 21 days. Release of cathepsin C and β-glucuconidase was also observed in lamb PM (Dutson et al., 1980). Using electron microscopy of rabbit muscle stained with anti-cathepsin D (Kubo et al., 2002) observed a gradual diffusion of cathepsin D from the lysosomes to the myofibrils during storage for 14 days. Almost all cathepsin D were released from the lysosomes and absorbed onto the myofibrils after 14 days storage. These results signify that lysosomal enzymes are released during PM storage of meat and are therefore likely to contribute to PM proteolysis. Also a significant part of the total activity of cathepsin B and cathepsin B+L can be measured in drip (Purslow et al., 2000; Kristensen, 2003). The only way cathepsin activity can be measured in drip is via a release from the lysosomal system. An other major argument against

a role for the lysosomal enzymes in muscle proteolysis during PM storage has been that no adequate explanation is provided for lack of actin and myosin degradation PM, as cathepsins can degrade myosin and actin in vitro and neither are degraded during PM storage of meat (Hopkins and Thompson, 2002; Koohmaraie, 1996; Taylor, 2003). However, degradation of both myosin and actin has recently been reported during PM storage of meat (Berge et al., 2001; Ertbjerg et al., 1999b; Lametsch et al., 2002).
The information revealed above suggest that both the lysosomal system and the calpain system significantly contribute to PM proteolysis and tenderization of meat, which is in accordance with several other studies (Dutson, 1983; O'Halloran et al., 1997; Ouali, 1992; Ouali and Valin, 1981). Ouali (1992) studied how structural changes, biochemical changes and proteolysis were affected by tenderization of beef and rabbit. The effects of tenderization were compared to the effects of incubating purified myofibrils with either lysosomal proteases or calpains. Ouali (1992) concluded that the observed changes in beef and rabbit during tenderization only can be explained by a synergistic effect of both enzyme systems. It was further suggested that the calpain system primarily is responsible for changes occurring early PM and the lysosomal system primarily responsible for changes occurring later PM. The involvement of both enzyme systems in PM proteolysis is supported by the results presented in **Table 1**. The at-slaughter activity of a representative of the lysosomal system (cathepsin B) and of the calpain system (calpastatin) were used to model the MFI values obtained from pork 1, 2 and 4 days PM. Both enzyme systems were important contributors to the three models obtained. However, calpastatin had the highest partial correlation coefficient at 1 and 2 days PM and cathepsin B the highest at 4 days PM, which fit well into the suggested time dependency of the two enzyme systems (Ouali, 1992).

Conclusions

Taken together the results presented in this paper and previous studies suggest that both the calpain system and the lysosomal enzyme system are involved in PM protein degradation in pork, and that a co-operative mode of action is a likely mechanism.

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PRESLAUGHTER HANDLING OF PIGS AND THE EFFECT ON HEART RATE, MEAT QUALITY, AND SR-CA2+ TRANSPORT

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Background

Preslaughter stress is generally thought to be of influence on meat quality parameters, mostly with a negative effect. However, the results of published experiments are not unequivocal. Genotype, transportation, lairage time, season of the year, environmental conditions and many other factors are also of effect on meat quality. One problem is to evaluate the effective level of stress on the animal. Heart rate can be used as one indication.

Although tenderness is mostly taken into account in beef investigations, it is also an essential parameter for pig meat quality. Tenderness of meat develops by the proteolytic action of the calpain/calpastatin system on different interfilament proteins, loosening the myofibrillar structure. Calpain is activated by Ca^{2+}, indicating the role of the intracellular Ca^{2+} concentration on the tenderness. The effect of Ca^{2+} is manifested by several investigations injecting Ca^{2+} (Kerth et al, 1995), marinating meat in Ca^{2+} containing solutions (Young et al., 1995) or inducing Ca^{2+} release by high pressure (Okamoto et al., 1995). Generally, a disturbed regulation of the intracellular Ca^{2+} concentration results in inferior meat quality parameters pH, drip, and colour, but on the other hand an increased Ca^{2+} concentration may activate the protease calpain, tenderising the meat. So, it seems worthwhile to investigate the relationships between Ca^{2+} transport, meat quality and tenderness.

Objectives

The objective of this study was to elucidate the impact of different kinds of stress (nose snare, electrical goad, control) just before slaughter on the development of meat quality. Especially the effect of the SR-Ca^{2+} transport in post mortem muscle samples on the development of tenderness was of interest. To estimate the reaction of the used kinds of stress on the pigs, the heart rate was determined.

Materials and methods

Animals and stress

Thirty female German Landrace pigs were used. The pigs were produced and raised in single boxes in an experimental unit of the institute up to a live weight of 105 – 115 kg. The animals of this experimental unit are free of the mutation of the calcium release channel (CRC).

The evening before slaughter the three heaviest pigs were transported to the slaughter facility of the institute. The heart rate was measured for about half an hour before slaughter by using a Polar Heart Rate Monitor.

About 5 min before the slaughter one pig was gently driven into the stunning pen and stunned electrically. The second pig was also driven into the stunning box but received an additional stress by the application of a nose snare for 5 min. The third pig was stressed for five minutes before slaughter by using an electrical goad according to D`Souza et al. (1999). Immediately following the stress just before stunning a biopsy (shot biopsy device) was taken. The stunning and the following procedures were identical for all pigs. Immediately following exsanguination a muscle sample (0 h sample) of the *longissimus* muscle between the 13th and 15th rib was removed by shot biopsy. Also, at 45 min p.m. and at 4 h p.m. samples were taken.

Sample preparation and Ca^{2+} uptake determination

About 0.5 g of muscle tissue were homogenised and the Ca^{2+} uptake of the muscle homogenate was determined as described by Küchenmeister (1999a). A part of the homogenate was stored at -70° C and later used for protein determination and different biochemical investigations. The calcium release channel was manipulated as described earlier (Küchenmeister et al., 1999a).

Meat quality measurements
Meat quality parameters of the *longissimus* muscle were measured by standard procedures (Küchenmeister et al., 1999b). The R-value as an indication for p.m. energy metabolism was determined on 45 min samples (Honickel et al., 1977). For determination of tenderness, two slices of *longissimus* muscle (13th-15th rib) were sampled 24 h p.m. One slice was used for tenderness determination on this sampling day and the second slice was stored at about 5° C for 6 days and then the tenderness was determined using a Texture Analyser with Warner-Bratzler accessories.

Results and discussion

The effect of the applied stress on the pigs is not easy to evaluate. One parameter to indicate the stress is the heart rate (HR). Figure 1 shows that the HR of the control animals (no additional stress) increased slowly in the course of the gentle movement (start about 5 min before slaughter) from the lairage box to the stunning from about 100 beats per minute (bpm) to 175 bpm. The nose snare stress started 5 min before slaughter after gently moving the pig into the stunning pan (with increasing heart rate comparable to the control animals). So, the heart rate started at about 175 bpm (comparable to Control). Surprisingly, however, the heart rate decreased in the time course of snare use down to 100 bpm, although the pigs were shrieking and pulling. This phenomenon is in agreement with earlier investigations (Geverink et al., 2002), but a plausible explanation is missing. The use of the electrical goad not only increased the HR in a short time interval up to 200 bpm, but also the pigs were running to avoid the electrical shock. So, this seems to be an effective stress.
These different levels of stress are reflected on the meat quality parameters (Tab. 1). There were no significant differences between control and nose snare pigs. However, the use of the electrical goad resulted in significant lower pH45 values and a brighter colour. Also, the drip loss and conductivity 24 h p.m. were highest in the goad group. Figure 2 shows the pH in the time course p.m. Four h p.m. the pH values were almost identical. However, the use of the goad resulted in lower values already immediately after slaughter and 45 min p.m., indicating an increased energy consumption and glycolysis p.m. by the application of the goad. This is verified by a higher R-value (IMP/ATP) 45 min. p.m.
The Ca^{2+} uptake of the sarcoplasmic reticulum (SR) of the homogenate (Tab. 2) shows generally lower values in the goad group, although not always significantly different from the other groups. The uptake 45 min p.m. was significantly lower following goad stress (independent of the state of the calcium release channel: basic or closed), but there were no differences between control and nose snare. As expected, the uptake decreased in the time course p.m. The rate of decrease was higher with basic CRC, compared to closed CRC. While the level of decrease with closed CRC indicates deterioration of SR-ATPase and SR membranes, the rate with basic CRC implies an opening effect on the CRC. A very fast and significant decrease of uptake between biopsy and 0 h samples with basic CRC was the result of applying goad stress. A reduced SR Ca^{2+} transport is supposed to increase the intracellular Ca^{2+} concentration compared to undisturbed Ca^{2+} regulation.
Our hypothesis, that a reduced Ca^{2+} uptake will be of effect on the tenderness by activation of calpains because of a higher intracellular Ca^{2+} concentration could not be verified. The tenderness, determined at 24 h p.m. samples, was not different between experimental groups (Fig. 3). This also applies for samples stored for six days before tenderness measurement. The shear values were significantly reduced after the storage of the loin chops compared to the 24 h p.m. measurements, however, the increase in tenderness was not influenced by the kind of stress. The rate of glycolysis affects the extend of the tenderisation at least in beef. A low glycolytic rate p.m. results in high shear force, whereas a high glycolytic rate leads to lower shear values (O´Halloran et al., 1997), probably related to lower or higher intracellular Ca^{2+} concentrations, respectively.

Conclusions

The immobilisation by a nose snare seems to have a very limited stress effect, indicated by decreasing HR and no significant effects on meat quality and Ca^{2+} transport. The use of an electrical goad had a major effect on all measured parameters: Heart rate, Ca^{2+} transport, meat quality, except tenderness. A reduced Ca^{2+} transport, induced by the high goad stress resulted as expected in inferior meat quality.

However, the hypothesis, that a reduced Ca^{2+} transport implies a higher intracellular Ca^{2+} concentration resulting in more tender meat could not be verified. Altogether, a stress before slaughter has to have a high level to be of significant influence on meat quality.

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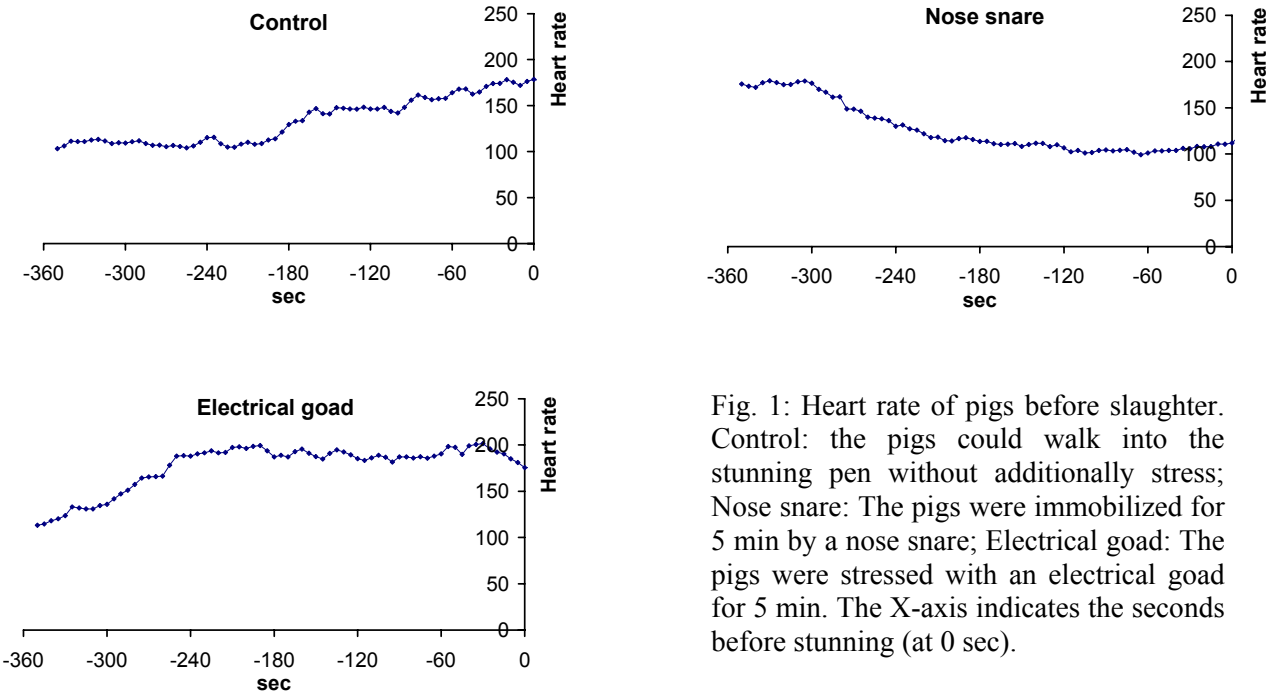

Fig. 1: Heart rate of pigs before slaughter. Control: the pigs could walk into the stunning pen without additionally stress; Nose snare: The pigs were immobilized for 5 min by a nose snare; Electrical goad: The pigs were stressed with an electrical goad for 5 min. The X-axis indicates the seconds before stunning (at 0 sec).

Tab. 1: Effect of different stress before slaughter on meat quality parameters *

	Control	Nose snare	Goad	SE
Live weight at slaughter (kg)	108.4	107.7	105.8	1.10
Lean meat (%)	55.4	53.5	54.9	0.99
pH 45	6.28 a	6.24 a	5.86 b	0.11
Conductivity 24 h p.m. (mS)	5.70 a	6.15 a,b	8.49 b	0.88
Colour (Minolta) L*	49.9 a	49.2 a	53.7 b	1.22
Drip loss (%)	4.82 a,b	4.43 b	6.83 a	0.82
R-value	0.85 a	0.86 a	0.97 b	0.03

* different letters indicate significant differences between experimental groups

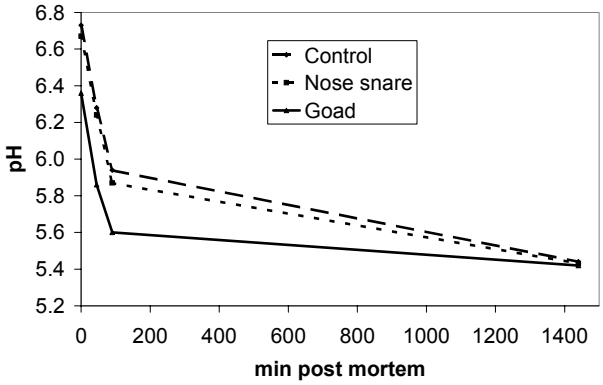

Fig. 2: Development of pH in the time course post mortem

Tab. 2: Ca^{2+} uptake rate (nM/min x homogenate protein) of *longissimus* homogenate, sampled at different intervals post mortem and immediately before stunning (shot biopsy). The calcium release channel (CRC) was closed by ryanodine treatment or basic without ryanodine treatment *

	Closed CRC				Basic CRC			
	Control	Nose snare	Goad	SE	Control	Nose snare	Goad	SE
Biopsy	114.3 A	117.2 A	120.9 A	7.9	66.3 A	59.2 A	59.6 A	5.3
0 h	127.6 A	118.4 A	113.9 A	7.5	64.2 a A	56.6 a,b A	50.2 b B	4.5
45 min	105.0 a B	104.5 a A	84.7 b B	6.1	44.1 a B	41.7 a B	31.1 b C	3.4
4 h	92.9 a C	83.3 a,b B	71.4 b C	5.5	31.5 C	26.5 C	24.7 D	2.8

* different lower case letters indicate differences between experimental groups
different upper case letters indicate differences between sampling times

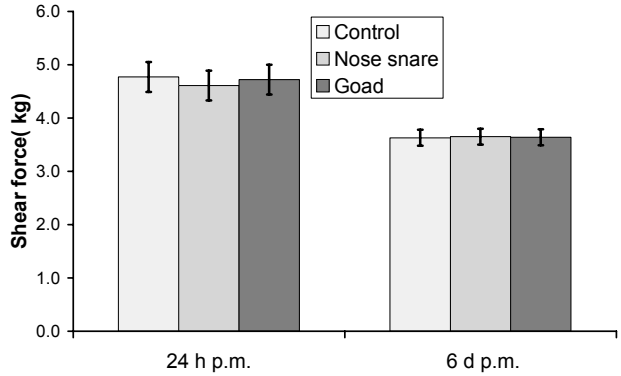

Fig. 3: Effect of stress on tenderness of *longissimus* samples, measured 24 h p.m. and following a 6 day storage

DOES THE ACTIVITY OF GLYCOGEN DEBRANCHING ENZYME LIMIT THE RATE OF THE GLYCOLYSIS?

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Background

In living muscle glycogen provides local fuel storage for short-term energy consumption. After slaughter glycogen degradation to lactate causes *post mortem* pH to decline in muscles. The rate and the extent of the pH decrease affects several meat quality traits. The complete degradation of glycogen is achieved by two enzymes: glycogen phosphorylase (phosphorylase) and glycogen debranching enzyme (GDE) (Brown and Brown, 1966). Mammalian GDE is a monomeric protein containing two independent catalytic activities: a glycan transferase (EC2.4.1.25) (transferase), and amylo-1,6-glucosidase (EC3.2.1.33) (glucosidase).

Phosphorylase catalyses the sequential phosphorolysis of the outer chains of the glycogen molecule until it reaches the fourth glucose unit from the branch point of the molecule (Walker and Whelan, 1960). Glycogen with four glucose units in every branch is called the limit dextrin state. The outer layer of limit dextrin has symmetric structure and it is converted back to an asymmetric structure by the transferase activity of GDE. This occurs by transferring a maltotriosyl group from the side chain to the main chain. The glucosidase hydrolyses the remaining glucosyl branch, producing free glucose. The debranched dextrin formed has long outer chains which are again susceptible to further degradation by phosphorylase (Brown and Brown, 1966; Nelson, Kolb and Larner, 1969; Nelson and Larner, 1970).

Yurovitzky and Milman (1975) suggested that the rate of glycogenolysis is limited by the activity of GDE. Furthermore, Immonen (2000) showed that *post mortem* glycogenolysis may stop even if there is glycogen left in the muscle and speculated that GDE may play a role in this process. However, there is very little information available about the activity of GDE in meat production animals.

Objectives

The aim of the present study was to investigate the activity of GDE in relation to temperature in porcine light *longissimus dorsi* (LD) muscle and dark *masseter* (M) muscle. If temperature affects the activity of GDE, it may influence the rate of *post mortem* glycogenolysis and glycolysis and thus the ultimate pH of meat. The method for determining the activity of GDE used in the present study measures activities of both the transferase and the glucosidase, not the individual activities of the enzyme.

Materials and methods

Muscle samples (LD and M) from 10 pigs were obtained from a commercial abattoir. The LD sample was dissected from the last rib about 35 min after stunning. The samples were frozen and stored in liquid nitrogen. The analyses were performed within two days after sampling. The activity of GDE was first determined in the muscles of four animals at temperatures 4, 15, 25, 35, 39 and 42 °C and was further determined at higher temperatures (39, 42, 50 and 60 °C) in six additional animals.

The activity of GDE was determined using the method of Nelson, Palmer and Larner (1970) with minor modifications. The method follows the change in the iodine-complex spectrum of glycogen phosphorylase limit dextrin (limit dextrin), a natural substrate for GDE. In the present study the assay solution contained only 0.1 ml 1% limit dextrin and 0.020 ml 0.5 M sodium maleate (A24979, Sigma-Aldrich). The pH of the reaction mixture was adjusted to 6.3 ± 0.05 and then incubated at 4, 15, 25, 35, 39, 42, 50 or 60 °C before the reaction was started by adding 0.08 ml meat extract. The meat extract was prepared daily, using 2.5 ml buffer which contained 0.05% $KHCO_3$ and 0.004 M EDTA (pH approx. 7.8 at 25 °C) per 1 g wet weight muscle. The mixture was homogenised (Ultra-Turrax T25, Janke and Kunkel, Germany) and centrifuged (Sorvall Instruments RC5C) 10 min, 10 °C and 30000 G and the supernatant was used in the measurements.

The reaction was stopped in a boiling-water bath followed by immersion in an ice bath. The reaction times were 1, 1.5 and 2.5 min for LD muscle and 1, 2.5 and 4.0 min for M muscle. Iodine reagent (2.6 ml) was added to the stopped reaction mixture and the absorbance (525 nm) recorded after 20 min. The iodine reagent was prepared according to Nelson et al. (1970). The pH value (6.3) and the reaction times used for determining the temperature-activity profiles of GDE in both muscles were determined in preliminary experiments. Method blanks (zero time controls) were prepared by denaturing the meat extract protein in the boiling-water bath before adding the other reagents.

The conversion of limit dextrin to glycogen was ensured from the absorption spectra between 375 - 800 nm. Samples and the method blank were diluted 5-fold with additional iodine reagent. All the absorbance and absorption spectra measurements were obtained with a Lambda 2 spectrometer (Perkin Elmer, Ueberlinger, Germany).

Phosphorylase limit dextrin was synthesised in our laboratory according to the method of Werries, Franz and Geisemeyer (1990) as it is not available commercially. Commercially available phosphorylase *a* was passed through a column of ω-aminobutyl agarose (Werries et al. 1990). The activity of purified, dried phosphorylase *a* was measured spectrophotometrically according to Bass, Brdiczka, Eyer, Hofer and Pette (1969). Purified phosphorylase a was added to 350 mg glycogen (bovine liver, G0885, Sigma-Aldrich) in 5 ml 0.05 M phosphate buffer (pH 6.8). In the present study the solution also contained 0.1 mM 5'AMP (01930, Fluga). The solution was dialysed at 37 °C against 150 ml of the same buffer. The digest was mixed with trichloroacetic acid (final concentration 10% v/v), dialysed against water and centrifuged for 10 min at 1000 G. The limit dextrin formed was precipitated with 4 volumes of ethanol, centrifuged (5 min, 1000 G), washed twice with ethanol and dried.

Statistical analysis was performed with the Statistical Analysis System version 8.02 (SAS, 1990). The mixed procedure with Bonferroni adjustment was applied when calculating the least squares means of the variables in the temperature-activity profiles of GDE. The curves were fitted using SAS/insight and the curves were plotted with Microsoft Excell 97 SR-2 (XY(scatter), polynomial trendline). The temperature values for maximum enzymatic activity were read from the curves if possible.

Results and discussion

In both muscles the activity of GDE was higher ($P < 0.001$) at the temperatures found in the carcass just after slaughter (39 °C and 42 °C) than at temperatures found during cooling (4 °C and 15 °C) (Figure 1). In M muscle significant ($P < 0.01$) difference was also shown in activity of GDE between temperatures of 39 °C and 25 °C. The temperature-activity profiles indicated that GDE was more active in light LD muscle than in dark M muscle.

In LD muscle the optimum temperature for GDE activity was 39 °C. The activity began rapidly to fall when the temperature decreased to below 35 °C, and the enzyme was practically inactive at temperatures below 15 °C. The other glycogen degrading enzyme, phosphorylase, is also most active at normal body temperatures (Cori, Cori and Green, 1943).

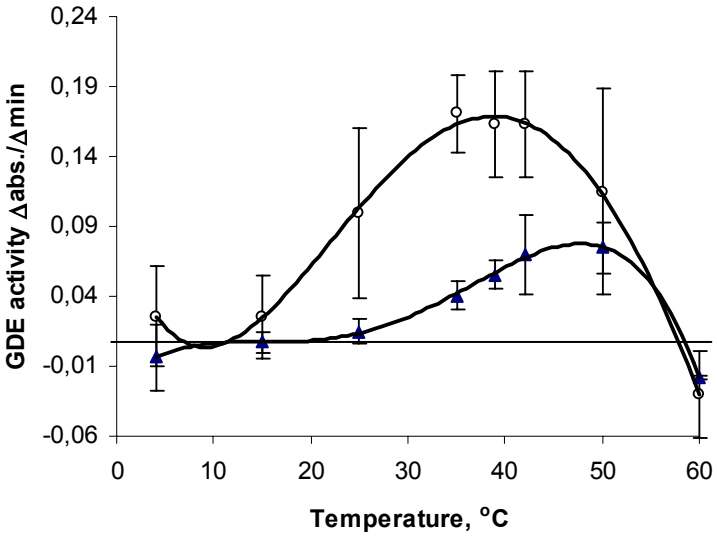

Figure 1. The activity of GDE in porcine *longissimus dorsi* (o) and *masseter* (▲) muscles in relation to temperature.

For *masseter* muscle the optimum temperature for GDE was near 50 °C and the decrease in activity began before the temperature had decreased to below the body temperature (38.5±0.65 °C; Hannon, Bossone and Wade, 1990). The decrease in activity of LD muscle GDE did not begin until the temperature had decreased to below 35 °C. Nelson and Watts (1974) have showed that the temperature optimum for rabbit muscle GDE activity is near 50 °C and that the activity sharply decreases when the temperature decreases to 20 °C which is consistent with the results of the present study. It seems that the activity of GDE does not block rapid glycolysis and pH decrease when the temperature is high since the enzyme remains activity even at temperatures above 45 °C. This may be important in pale soft and exudative (PSE) meat, where the pH decreases rapidly at high temperatures. Rapid cooling could decrease GDE activity and thus the rate of glycolysis, so reducing the formation of PSE meat.

We assume that the decrease in the activity of GDE caused by the temperature decrease also takes place in carcasses after slaughter. During the normal chilling procedure used in Finland, the core temperature of porcine LD muscle decreases to below 35 °C in about 1 h *post mortem*. At that time the pH has decreased to about 6.3 and the glycogen (assumed concentration at the time of slaughter has been normal or high) is still in a state which is susceptible for the degradation of phosphorylase. The temperature decrease of the muscles goes on due to cooling of the carcass and, therefore, when the activity of GDE is needed to continue glycogenolysis and glycolysis, its activity is not maximal. Thus, the decrease in the activity of GDE may delay the rate of glycogenolysis and glycolysis *post mortem*.

In dark M muscle, the decrease in the activity of GDE and in the rate of glycogenolysis was faster than in the light LD muscle. This is supported by the findings that M muscle temperature decreases faster and that even a slight decrease in temperature significantly reduces the activity of GDE. Mélendez-Hevia et al. (1993) have estimated that at most 34.6% of glycogen molecule is directly susceptible to the degradation of phosphorylase. We estimated that this amount is enough to cause pH to decrease from 7.0 to about 6 in dark porcine M muscle *post mortem*. After that the activity of GDE is needed so that glycogenolysis can continue. However, the ultimate pH of M muscle does not usually decrease to below 6, which may be due to the temperature decrease in M muscle during cooling which inhibits the activity of GDE and leads to delayed glycogenolysis and thus to high ultimate pH. It is not known in which state the glycogen molecules are, in other words, how many glucose units are in the outer layer of the glycogen molecule at the moment of slaughter. Also is unclear, are all the glycogen molecules within a muscle in the same state.

Beecher, Briskey and Hoekstra (1965) showed that *post mortem* glycolysis is faster at 37 °C than at 4 °C both in light and dark parts of porcine *semitendinosus* muscle. Their results showed that the glycolytic and glycogenolytic enzymes remained active at low temperatures but were slower. The present study showed that

the activity of GDE was very slow at low temperatures (below 15° C). Beecher et al. (1965) found no significant differences between the ultimate pH values of the light parts of *semitendinosus* muscle held at 37 °C or at 4 °C, while the ultimate pH of the dark part of the *semitendinosus* muscle was significantly lower in muscles held at 37 °C than at 4 °C. This is also consistent with our results where the light LD muscle GDE also showed activity at temperatures below 25 °C but the dark M muscle enzyme did not. Thus, the decrease in the activity of GDE due to temperature decrease may stop the glycolysis earlier in dark M muscle than in light LD muscle.

Conclusions

It can be concluded that GDE is more active in porcine light LD muscle than in dark M muscle. In both muscles, the *post mortem* decrease in temperature strongly reduces the activity of GDE, which may in turn regulate the rate of glycolysis. The eventual impact of activity of GDE on PSE pork and cold shortening in beef warrants further study.

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EFFECTS OF DIETARY VITAMIN E AND VITAMIN C SUPPLEMENTATION ON LEVEL OF ALPHA –TOCOPHEROL AND L-ASCORBIC ACID IN MUSCLE AND ON THE ANTIOXIDATIVE STATUS AND MEAT QUALITY OF PIGS

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Background

Vitamin E and C are primary antioxidants in biological systems and break the chain of lipid peroxidation. Many studies suggest that vitamin C and vitamin E act synergistically (Gay 1998). Previous studies evaluating the efficiency of relatively high levels of vitamin have been inconsistent in producing a growth or feed efficiency response. By Mahan et al. (1994) was used a stable source of vitamin C (magnesium-L-ascorbyl-2-phosphate) in pig feeding experiment. Another stable source of vitamin C (L-ascorbyl-2-polyphosphate, Rovimix® Stay-C® 25, Roche) was used by de Rodas et al. (1998) and Sahin et al. (2002). Data from pig experiment introduced by Kremer et al. (1999) suggest that vitamin C supplementation before slaughter can improve parameters of meat quality.

Objectives

The objective of this study was to evaluate further the effects of vitamins E and C supplementation on level of α–tocopherol and ascorbic acid and lipid peroxidation status in fresh and chill-stored meat and on some quality parameters

Materials and methods

Thirty Slovak White Meaty pigs were used in this experiment. Control group (n = 10) and two experimental groups were homozygotes, negative on malignant hyperthermia (defined by DNA based test). Control group was fed with a diet supplemented with basal level α–tocopherol (Table 1). Experimental groups received a supplemental (Table 1) level α–tocopherol (500 mg/kg), (group E, n = 10) and a supplemental level α – tocopherol (500 mg/kg) and ascorbic acid (200 mg/kg), (group EC, n = 10) for 30 days before slaughter. Vitamin E (ROVIMIX® E-50 SD, stable source of vitamin E in feed) and vitamin C (ROVIMIX® STAY-C® 35) were provided by a commercial company (Roche, Germany). Animals were slaughtered at average live weight of 110 kg. The sample of longissimus dorsi (part lumborum, LD) muscle was used immediately (24 h) and the remaining samples were wrapped in aluminium film and stored in a refrigerator at 4°C for 5 days. The concentration of vitamin E (α–tocopherol) of the samples (fresh, cooked, frozen) was measured by HPLC and vitamin C (ascorbic acid) with 2,4-dinitrophenylhydrazin as a color reagent was estimated. Lipid oxidation in samples (fresh and 5 days chill-stored) and the stability of the skeletal muscle (fresh samples) lipids against stimulated (by Fe^{2+}/ascorbate) lipid peroxidation were assessed by the 2-thiobarbituric acid method (TBARS) and expressed in terms of malondyaldehyde (MDA, mg/kg tissue) as described earlier (Lahucky et al., 2001). The pH value of the carcass (m. longissimus – between 13th and 14th rib) 45 min post mortem, electrical conductivity 3 h, color by Miniscan 24 h, total water, protein and intramuscular fat were also measured (Lahucky et al., 2001) . Drip loss analyses were made according to Honikel (1998). Shear force was determined in cooked samples (internal temperature 80°C, used also for further analyses) with Warner-Bratzler (W-B) apparatus. Statistical analyses were calculated as mean values and standard deviations and differences were evaluated by t-test.

Results and discussion

The supplementation of vitamin E (α–tocopheryl acetate) to pigs increased about 2 folds the α–tocopherol levels (Fig 1) of fresh (24 h) and 5 days chill-stored meat. The levels of α–tocopherol in longissimus dorsi muscle are higher or comparable with previously reported results (Honikel et al., 1998, Lahucky et al., 2000, 2001). Dietary supplementation of vitamin C increased fresh meat vitamin C concentrations (Fig 2) and in some extense in stored meat ($P<0.05$). The values of the moisture, crude protein and intramuscular fat (Table 2) were not influenced by dietary treatments. Improvement in pH value ($P=0.06$) after vitamin C and vitamin E supplementation (group E + C, Table 3) can support data from one experiment (Kremer et al.,

1999). They suggest that adding sodium oxalate or vitamin C to final meal given to pigs before slaughter resulted in higher early post-mortem pH, but further studies on glycolysis and glycogen metabolism would be useful as vitamin C is known as precursor of oxalic acid and sodium oxalate inhibits a key glycolytic enzyme, pyruvate kinase.

A tendency of improving drip loss (lower value) in longissimus dorsi in 24 h post mortem of normal on malignant hyperthermia pigs supplemented with vitamin E (Table 3) and significant lower ($P<0.05$) value in pigs supplemented with vitamin E and vitamin C (group E + C) were received. It seems that adding a high level of vitamin E and/or vitamin C to the diet will reduce drip loss in some situations, but perhaps not in all situations and genetic background (occurrence of mutation on ryanodine receptor gene, malignant hyperthermia status) of experimental pigs could influence the results as was also discussed earlier (Lahucky et al., 2000). Dietary levels of vitamin E (group E) and vitamin E and vitamin C (group E + C) did not substantially affect the development of lipid oxidation (Fig 3) in the fresh meat (24 h), but significant differences ($P<0.05$) we mainly received in chill-stored (5 days) between control vs. supplemented pigs (group E and group EC). Whereas the MDA of the control were increasing during 30 min of incubation, the increase was significantly ($P<0.05$) lower in supplemented groups. Significant differences ($P<0.05$) we also received between vitamin E vs. vitamin E + C group (Fig 4).

Conclusions

Dietary supplementation of vitamin E (500 mg α–tocopheryl acetate/kg feed) and vitamin C (200 mg/kg feed) to grow-finishing pigs increases the concentrations of α–tocopherol and ascorbic acid in meat (longissimus dorsi). Supplementation vitamin E and vitamin C improves meat quality parameters (drip loss, pH), but results can be influenced by genetic background of animals (occurrence of mutation on ryanodine receptor gene, malignant hyperthermia status). Lipid oxidation measured as TBARS (MDA) and antioxidative capacity (Fe^{2+}/ascorbate induced) of meat can be positively influenced by supplementation of vitamin E to grow-finishing pigs.

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Table 1. Composition and nutritive value of diet

Item	%	Item	Control	Vitamin E+C
Wheat	24.0	Organic matter (%)	82.15	82.15
Barley	40.0	Crude protein (%)	17.42	17.42
Oat	10.0	Crude fat (%)	2.79	2.79
Soybean meal	12.0	Crude fibre (%)	4.51	4.51
Wheat meal	4.0	N-free extract (%)	57.43	57.43
Lucerne meal	3.0	Ash (%)	5.63	5.63
Meat and bone meal	2.0	Metabolisable energy (MJ)	12.38	12.38
Fish meal	1.0	Lysine (%)	0.91	0.91
Mineral supplement	3.0	Vitamin A (m.j.)	5 400.00	5 500.00
Fodder salt	0.4	α-tocopherol – added (mg)	-	500.00
Biofactor supplement	0.6	- analysed (mg)	33.60	515.00
		Vitamin C – added (mg)	-	200.00
		- analysed (mg)	90.30	189.20

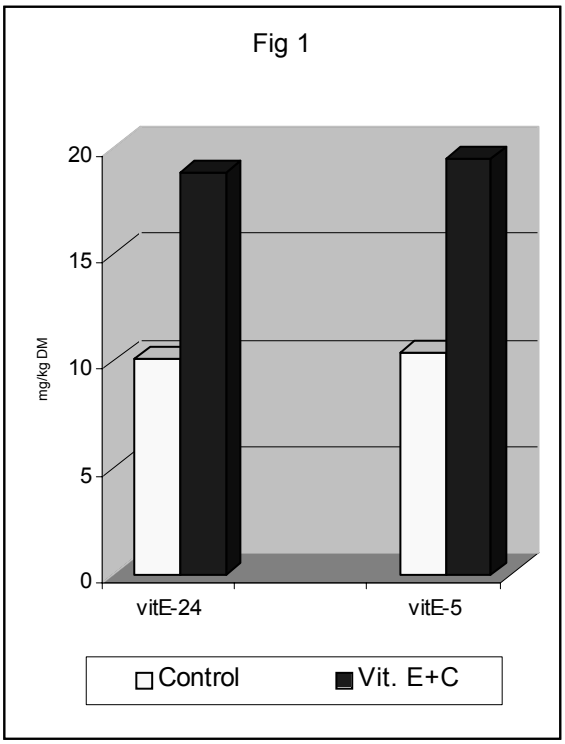
Table 2. Chemical composition of muscle *longissimus dorsi*

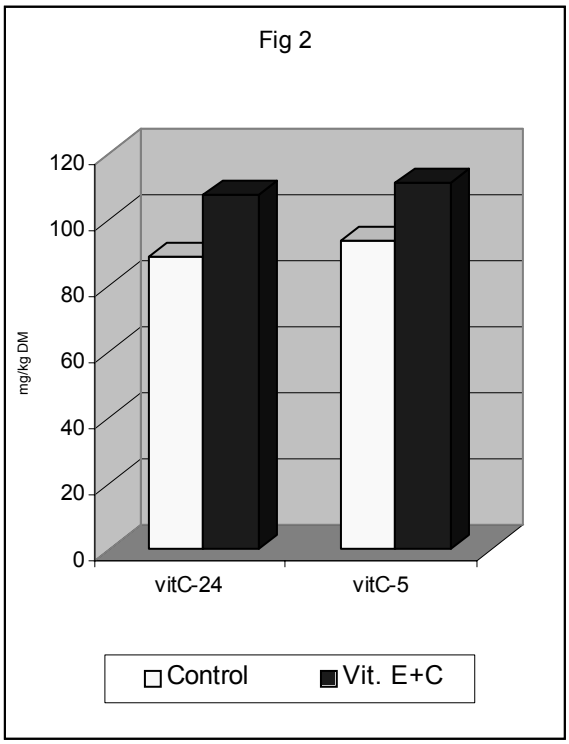
Item	Control		Vitamin E		Vitamin E+C		Significance
	mean	S.D.	mean	S.D.	mean	S.D.	
Total water, %	74.23	0.70	73.82	0.84	73.93	0.75	-
Total proteins, %	22.41	0.41	22.53	0.50	22.46	0.89	-
Intramuscular fat, %	2.76	0.76	2.81	0.94	2.81	0.81	-

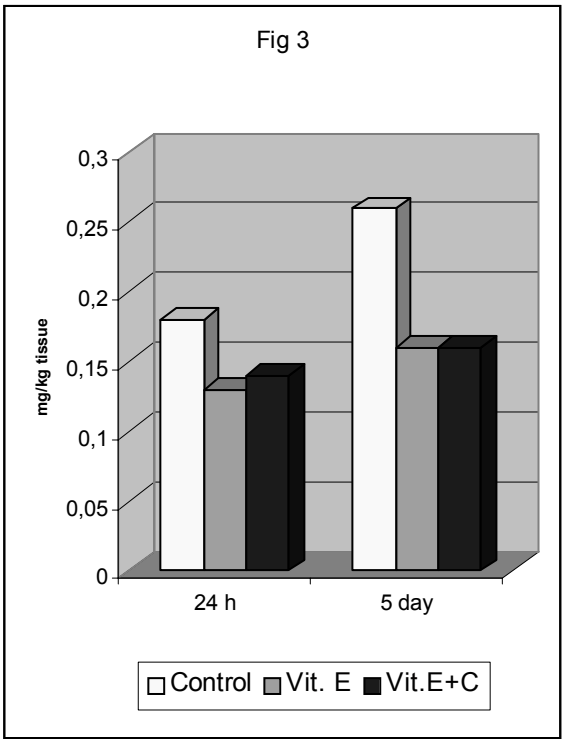
Table 3. Pork quality (m. *longissimus dorsi*)

Trait	Time	Control		Vitamin E		Vitamin E+C		Significance
		mean	S.D.	mean	S.D.	mean	S.D.	
pH	45 min	6.27	0.22	6.38	0.19	6.45	0.26	-
El. conductivity, μS	3 h	4.06	1.01	3.67	1.22	3.93	1.12	-
Colour (L)	24 h	48.67	3.64	48.58	2.36	48.60	2.14	-
Free water, %	24 h	37.84	2.95	36.73	3.33	36.41	3.25	-
Drip loss, %	24 h	4.86	1.03	4.12	1.05	4.05	0.88	*
Colour (L)	5 day	51.63	2.82	51.69	3.75	50.84	2.66	-
Free water, %	5 day	35.87	2.90	34.26	2.94	33.75	2.86	-
Shear force, kg	5 day	4.09	1.15	4.82	0.71	4.66	0.66	-

*P<0.05







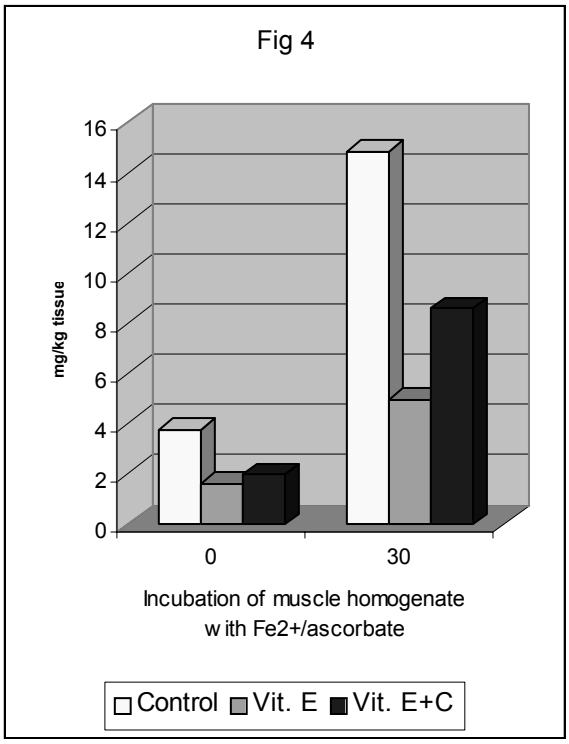


Figure 1. Content of α-tocopherol in muscle
vitE-24 = fresh meat 24 h
vitE-5 = chill-stored meat 5 days

Figure 2. Content of ascorbic acid in muscle
vitC-24, 5,

Figure 3. Level of thiobarbituric acid reactive substances (TBARS, MDA) in muscle

Figure 4. Antioxidant stability of muscle (incubation of muscle homogenate with Fe^{2+}/ascorbate)

PORK QUALITY AS RELATED TO HALOTHANE GENOTYPE AND SLAUGHTER CONDITIONS IN A BELGIAN STUDY

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Background

Pork quality is dependent on various genetic and environmental factors from whose halothane genotype and pre-slaughter factors such as fasting time, transport, abattoir lairage are recognized of great importance for final meat quality. However, most of our present knowledge is based on studies investigating the influence of a single or at the most two factors (Rosenvold and Andersen, 2003). It is therefore necessary to understand how different production or slaughter factors influence pork quality in actual practical conditions. This approach could subsequently be used in the control of the quality of pork products.

Objectives

The objectives of this study were to evaluate technological and organoleptic properties of pork meat representative of different Belgian production systems and to determine the contribution of significant factors to meat quality variability, in particular the halothane genotype, fasting time, lairage time and slaughtering plant.

Materials and methods

A total of 521 pigs were used in five Belgian commercial slaughtering plants over a 1 ½ year period. 79% of pigs belonged to four different quality production systems. The remaining 21% were randomly sampled in standard production. In the slaughterline, pH_1 and electrical conductivity (PQM_1) were measured 45 minutes *post mortem* in the *longissimus dorsi* muscle at the level of the two last thoracic vertebrae. The weight of 'white offals' (entire intestinal tractus) was measured with a hanging electronic scale (Kern HCB 20K50). Hot carcass weight of all pigs was determined just before chilling. One 2.5 cm thick cut of the *longissimus dorsi* muscle was removed 24 hours *post mortem* in order to measure the ultimate pH (pHu), ultimate electrical conductivity (PQMu), water holding capacity (WHC), color (CIE $L^*a^*b^*$) and tenderness (WBPSF). The pH was measured by using an inserting combined pH electrode (Ingold ref 104063123) on a Knick 913 pH-meter. Electrical conductivity was assessed with a Pork Quality Meter (Intek). The Labscan II device (Hunterlab) was used to objectively measure CIE L^* (brightness) and a^* and b^* (color) parameters. The water holding capacity was estimated as drip loss and cooking loss. Drip loss was assessed as the percentage weight loss after 4 days storage in a plastic bag at 2°C ; cooking loss was measured as the percentage weight loss after cooking in an open plastic bag in a waterbath during 50 min at 75°C. Warner-Bratzler Peak Shear Force (WBPSF) was determined with a Lloyd LR5K universal testing machine perpendicular to the muscle fibre direction on ten 1.25 cm diameter cores obtained from heated cuts. Lairage time, calculated from the time between animals arrival at the abattoir and stunning was divided into five intervals (≤ 1, 1-2, 2-3, 3-4, >4 hours). In order to assess the fasting time, the weight of white offals was expressed as a percentage of hot carcass weight. The slaughtering plants differ in several aspects : slaughterline speed, the mean number of pigs slaughtered per week, handling treatment, stunning systems. Halothane genotype was determined with a DNA test using a polymerase chain reaction technique according to the method described by Nakajima *et al.* (1996). The data were analyzed using Statistical Analysis System (SAS Institute inc., Cary, NC, USA, 1999). Several models were adjusted to the data using General Linear Models (GLM) SAS procedure to estimate the influence of halothane genotypes -homozygote negative stress (CC), heterozygote negative stress (CT) and homozygote positive stress (TT)-, lairage time (≤ 1, 1-2, 2-3, 3-4, >4 hours) and slaughterhouses (1, 2, 3, 4, 5) on the variability of pH_1, PQM_1, pHu, PQMu, drip loss, cooking loss, CIE $L^*a^*b^*$ and WBPSF. The relative weight of white offals on hot carcass was included in the models as covariate. Least Squares Means (LSM) were computed for significant effect in the models and compared pairwise by the Student's t-test.

Results and discussion

Table 1 shows the average results for the total population. A large variation for the results of pH_1, drip loss and brightness was observed, indicating the presence of pale, soft, exudative (PSE) meat. Missing data for some parameters reduce the number of samples introduced in statistical models.

The proportion of variance explained by the GLM models (R^2) and the significance level of the effects in the models are summarized in Table 2. For all parameters -except for the pHu, cooking loss and the WBPSF- a moderate to great part of variation was explained by the model (R^2 = 0.22-0.56). Similar determination coefficients (R^2 = 0.04-0.59) were reported by Casteels *et al.* (1995) when they studied the influence of halothane genotypes, stunning method and slaughter weight on the variability of meat quality using GLM models. The halothane genotype effect was highly significant ($p<0.001$) on most meat quality traits : pH_1, PQM_1, PQMu and color parameters (CIE $L^*a^*b^*$) but not significant on pHu and cooking loss. The lairage time had a highly significant influence on PQMu, CIE b^*, drip loss but for the other parameters it had negligible or no influence. There was a highly significant effect of the slaughtering plant on pH_1, PQM_1, PQMu and color parameters (CIE $L^*a^*b^*$) and a significant influence ($p<0.01$) on the variability of drip loss and cooking loss. By contrast, the fasting time had no effect on most meat quality properties except on drip ($p<0.01$) and cooking loss ($p<0.001$). These results are in accordance with those of De Smet *et al.* (1996) whose found no effect of feed withdrawal on most meat quality traits. As indicated by the regression coefficient, an increase of offals/hot carcass weight seems to be related to an increase of drip or cooking loss.

The halothane genotype Least Squares Means and their standard errors (SE) for all parameters are listed in Table 3. Meat quality was negatively affected by the presence of the halothane gene. Drip loss differed significantly between genotypes with the TT pigs having the highest drip loss (6.8%) while that of the CC (5.5%) was the lowest and that of CT intermediate (6.1%). Cooking loss was also significantly higher in TT pigs indicating lower water holding capacity. Significant differences between CC and TT genotypes ($P< 0.05$) were observed in terms of pH_1 (6.12 *vs* 5.69), PQM_1 (4.9 *vs* 6.4) and PQMu (9.7 *vs* 12.7). The CIE L^* and b^* values (56.9% and 16.6) were significantly higher in homozygous stress positive animals (TT) comparatively to CC (55.0% and 15.8) or CT pigs (54.6% and 15.4). The higher L^* value of the TT genotypes indicated paler meat. The WBPSF was the lowest for CT pigs with no significant difference observed between the CC and TT genotypes.

The abattoir Least Squares Means and their standard errors for all parameters are listed in Table 4. The slaughterhouse 1 had lower pH_1, pHu, higher PQM_1 and CIE L^* values compared with slaughterhouses 2, 3 ,4 and 5. Cooking loss, drip loss and PQMu means were the highest for the meat originating from slaughterhouse 5. Although it would be necessary to determine the reasons for abattoirs differences, the small and old structure of the abattoir 1 with no training of the staff could partially explain the results.

Conclusions

From these results, it can be concluded that the halothane genotype is the most important factor determining organoleptic and technological properties of pork meat. A significant proportion of TT stress-susceptible pigs which can develop PSE meat was found in this Belgian study. Additional efforts to reduce halothane gene in pig population have to be realized to improve meat quality. Slaughterhouse and lairage time are significant factors influencing quality attributes of pork. The influence of the fasting time (assessed by offals/hot carcass weight) was low except on drip and cooking loss.

Further research is needed to evaluate the interaction terms slaughterhouse x genotype and lairage time x genotype and to identify slaughtering factors which could explain slaughterhouse differences in terms of meat quality.

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This work is part of a project supported by the Direction Générale de l'Agriculture, Direction de la Qualité des Produits, Région Wallonne, Belgium.

Table 1. Number, mean, standard deviation, minimum and maximum of the measured parameters

Parameter	Number	Mean	Standard Deviation	Minimum	Maximum
pH_1	497	6.00	0.35	5.30	6.85
pHu	403	5.40	0.10	5.10	5.95
PQM_1 (mS/cm)	418	5.1	2.4	2.8	22.4
PQMu (mS/cm)	372	10.9	3.4	2.8	19.2
CIE L∗(%)	512	54.6	4.4	41.6	66.4
CIE a∗	512	6.3	2.0	0.9	12.4
CIE b∗	512	15.5	1.8	10.5	20.1
Drip loss (%)	396	6.5	2.0	1.2	11.4
Cooking loss (%)	490	30.2	2.5	20.2	39.2
WBPSF (N)	490	37.5	7.8	21.0	71.7

Table 2 : Proportion of variation explained by the GLM models (R^2), the significance level (Pr > F) of the influencing factors and the regression variable of the 'white offals'/hot carcass weight on the parameter in the case of significance.

Parameter	R^2	Genotype Pr > F	Lairage time Pr > F	Slaughterhouse Pr > F	Offals / hot carcass weight	
					Pr > F	Regres./%
pH_1	0.56	0.0001^{***}	0.0272^{*}	0.0001^{***}	0.3822	-
pHu	0.16	0.2117	0.1164	0.0489^{*}	0.1648	-
PQM_1	0.34	0.0001^{***}	0.8760	0.0001^{***}	0.6925	-
PQMu	0.33	0.0001^{***}	0.0001^{***}	0.0001^{***}	0.5816	-
CIE L∗(%)	0.46	0.0001^{***}	0.0111^{*}	0.0001^{***}	0.1384	-
CIE a∗	0.26	0.0001^{***}	0.0138^{*}	0.0001^{***}	0.7278	-
CIE b∗	0.35	0.0001^{***}	0.0001^{***}	0.0001^{***}	0.7448	-
Drip loss (%)	0.22	0.0224^{*}	0.0001^{***}	0.0080^{**}	0.0016^{**}	0.27
Cooking loss (%)	0.17	0.0519	0.0132^{*}	0.0017^{**}	0.0004^{***}	0.33
WBPSF (N)	0.11	0.0012^{**}	0.4696	0.2406	0.2047	-

Table 3 : Halothane genotype least squares means ± SE of meat quality traits

Parameter	CC			CT			TT		
	Number	Means	SE	Number	Means	SE	Number	Means	SE
pH_1	57	6.12b	0.04	210	6.03b	0.02	87	5.69a	0.03
pHu	52	5.44a	0.02	190	5.42a	0.01	49	5.43a	0.02
PQM_1 (mS/cm)	50	4.9a	0.3	185	4.5a	0.2	70	6.4b	0.3
PQMu (mS/cm)	50	9.7a	0.6	175	12.5b	0.4	47	12.7b	0.6
CIE L∗(%)	59	55.0a	0.5	214	54.6a	0.3	87	56.9b	0.5
CIE a∗	59	6.4b	0.3	214	5.6a	0.2	87	6.9b	0.2
CIE b∗	59	15.8a	0.2	214	15.4a	0.1	87	16.6b	0.2
Drip loss (%)	52	5.5a	0.4	173	6.1ab	0.3	49	6.8b	0.4
Cooking loss (%)	59	30.5ab	0.4	205	30.2a	0.2	74	31.1b	0.3
WBPSF (N)	59	38.6b	1.3	205	35.8a	0.8	74	40.3b	1.1

Results followed by the same letter are not significantly different ($p<0.05$)

Table 4 : Slaughterhouse least squares means ± SE of meat quality traits

Parameter	Slaughterhouse 1			Slaughterhouse 2			Slaughterhouse 3			Slaughterhouse 4			Slaughterhouse 5		
	n	Means	SE	n	Means	SE	n	Means	SE	n	Means	SE	n	Means	SE
pH_1	78	5.71a	0.04	153	6.11c	0.03	71	6.13c	0.04	27	5.90b	0.05	25	5.90abc	0.11
pHu	79	5.41a	0.02	155	5.46b	0.02	-	-	-	27	5.41a	0.02	30	5.44ab	0.05
PQM_1 (mS/cm)	55	7.3b	0.4	137	5.1a	0.3	57	4.5a	0.3	27	4.7a	0.4	29	4.7a	0.8
PQMu (mS/cm)	73	9.4a	0.6	142	8.2a	0.5	-	-	-	27	13.5b	0.6	30	15.5b	1.4
CIE L∗(%)	79	59.3c	0.6	153	53.0a	0.5	71	52.8a	0.5	27	55.9b	0.7	30	56.3abc	1.5
CIE a∗	79	7.0c	0.3	153	5.9b	0.2	71	5.8b	0.3	27	4.7a	0.4	30	8.1c	0.8
CIE b∗	79	16.2b	0.3	153	14.7a	0.2	71	15.1a	0.2	27	15.0a	0.3	30	18.7c	0.7
Drip loss (%)	79	5.4a	0.4	138	5.0a	0.3	-	-	-	27	5.8a	0.4	30	8.4b	0.9
Cooking loss (%)	79	30.0b	0.4	155	29.1a	0.3	47	30.0b	0.4	27	30.8b	0.5	30	33.1c	0.1
WBPSF (N)	79	37.2ab	1.4	155	35.9a	1.1	47	37.7ab	1.4	27	40.2b	1.6	30	40.0ab	3.6

Results followed by the same letter are not significantly different ($p<0.05$)

GIANT FIBRES IN BROILER CHICKENS

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Background

Giant fibres can be described as being generally rounded in shape, bigger than the normal fibres around them, the shape of which can also be modified by them, and usually slightly separated from the surrounding fibres and localized at the borders of muscle fascicles. This is a great simplification of the problem as proven by the fact that their real origin and meaning has been the subject of discussion for over 30 years and still there is no clear answer to the question.

The issue of giant fibres was first dealt with by Cassens et al. in 1969. These authors focused their attention mainly on the frequency and on the histochemical characteristics of such fibres in the muscles of adult and growing pigs. Considering their particular staining pattern following histochemical evaluation as well as the fact that they were more frequent in muscles taken from stress-sensible pigs, these authors reached the conclusion that giant fibres may represent a pathological muscle modification. In later years other authors (Handel et al., 1986) focused their attention on giant fibres also observing them with the electron microscope which revealed, among other things, a reduced quantity of sarcoplasmic reticulum and an irregular disposition of the miofilaments inside the myofibres. This last point, as well as the lack of the characteristic myofibrillar banding pattern seen on examination of longitudinal sections, might be explained by supercontraction. These authors concluded that giant fibres might be due to a defect in single developing muscle fibres and not to degenerative changes within the muscle. A recent study by Fazarinc et al. (2002) deals with the influence of the halothane gene on the percentage of giant fibres. These authors reached the conclusion that giant fibres are glycolitic fibres which, due to the accumulation of intracellular lactate during post-mortem glycolisis, become swollen. Their appearance may also be facilitated by stress or stress susceptibility.

All of the above articles deal with pigs, but this is by no means the only animal where giant fibres can be found, as a matter of fact they have been observed in muscles of various species such as cattle and poultry. In this regard there is a paper (Remignon et al., 2000) concerning the moment, post-mortem, in which giant fibres appear in turkey muscles. These authors only found giant fibres in post-rigor (24h post-mortem) and not in pre-rigor (3 minutes post-mortem) muscles and therefore hypothesized that they are due to alterations in the developing muscle fibres that lead to structural and metabolic anomalies that give origin to their appearance during the biochemical events typical of rigor mortis.

Objectives

The purpose of this study is to evaluate the number as well as the histological and histochemical characteristics of giant fibres in various chicken muscles since literature concerning this topic is quite scarse. Furthermore the post-mortem time of appearance of giant fibres will be taken into consideration in order to compare their behaviour in chicken to that found by Remignon et al. (2000) in turkeys.

Materials and methods

A total of 11 male Ross broiler chickens were collected at the slaughterhouse just after plucking (10 minutes port-mortem) and muscle samples, parallel to fibre direction, were immediately taken from the left half of the carcasses, rolled in talcum powder, wrapped in aluminum foil, labelled, frozen and stored in liquid nitrogen until analyses were performed. The carcasses were then placed in portable coolers and taken to the laboratory where they were stored at 4°C until 24h post-mortem, when samples of the same muscles where taken from the right half of the carcasses, prepared as described above and stored in liquid nitrogen. Four muscles known, from literature and from previous studies, to have a different fibre distribution were considered: 1. *m. Pectoralis major*; 2. *m. Semimembranosus*; 3. *m.Ileotibialis lateralis*; 4. *m. Femorotibialis medius* (samples of this muscle were only taken from 5 animals). Serial cross-sections were obtained in a cryostat at -20°C and stained with hematoxylin-eosin (8µm thick) for histological evaluations and using

periodic acid schiff (PAS) (10 μm thick) for evaluation of the glycogen content. Serial, 8 μm thick, cross-sections were also processed for myofibrillar ATPase activity after acid and alkaline pre-incubation (Padykula and Herman, 1955; Guth & Samaha, 1969) and for succinate dehydrogenase (SDH) activity (Nachlas et al., 1957) or for the combination of the two (Solomon and Dunn, 1988) depending on the muscle considered. Myofibres were classified, according to the terminology introduced by Ashmore and Doerr (1971), as types βR, αR, and αW. βR fibres are stable after acid pre-incubation, labile after alkaline pre-incubation and SDH positive; both αR and αW fibres are labile after acid pre-incubation and stable after alkaline pre-incubation but while αR fibres are SDH positive, αW fibres are SDH negative.
Images were acquired and analysed, in order to determine percentages and cross sectional areas, using an image analysis system (analySIS, Soft Imaging System) implemented on a workstation equipped with a graphic card linked to a video camera placed on the microscope (Olympus BX51). Measurements were determined on about 200 fibres, from a random field in each muscle sample section stained with hematoxylin-eosin. Giant fibre percentages were calculated considering about 600 fibres from 3 random fields in order to have a better estimate of their number.
Data were statistically evaluated using Student's T test.

Results and discussion

The results concerning muscle fibre distribution of the different muscles are reported in table 1. Muscle *Pectoralis major* and muscle *Ileotibialis lateralis* are composed of mostly glycolitic fibres that have the same distribution throughout the muscle, while m. *Semimembranosus* and m. *Femorotibialis medius* are made up of various parts with a different fibre type distribution. This explains why the results are not as homogeneous for these last two muscles as they are for the first two, perhaps even a very small variation in the sampling site can determine variations in the fibre distribution.
Giant fibres did not all react in exactly the same way when they were subjected to histochemical evaluation. However, we can say that they could be classified as α type fibres as their oxidative capacity is variable yet never extremely strong, and they are more positive than α type fibres when processed for myofibrillar ATPase activity both after alkaline and after acid pre-incubation. This result is in contrast with that obtained by Chiang et al. (1995) who classified giant fibres in chicken mostly as αR and βR. Giant fibres are also always negative after PAS staining both 10 minutes and 24 hours after slaughter.
The percentage of giant fibres found in the muscle samples, 10 minutes and 24 hours post-mortem, is reported in table 2. The tables indicate that while the number of giant fibres is similar in all four muscles 10 minutes post-mortem, 24 hours post-mortem giant fibres are much more numerous in m. *Ileotibialis lateralis* and especially in m. *Pectoralis major*. The latter muscles show a significant variation in giant fibre percentage between the two sampling times ($p<0,01$) and it is interesting to notice that m. *Pectoralis major*, which has the greatest variation for this parameter, is also the muscle with the biggest difference in glycogen content between the two sampling times (table 3). This could imply that there is a link between the appearance of giant fibres and the biochemical events involving glycogen and glycolitic enzymes that take place post-mortem. This could also explain why there are usually more giant fibres in "white" muscles as seen in this experiment as well as in previous work on poultry (Chiang et al., 1995). Nonetheless, all the fibres composing these muscles are in the same condition, so it is not clear why only some of them become giant fibres. As Severini et al. (1997) suggested in a study concerning the presence of giant fibres in normal and PSE pig muscles, this could be due to a latent or very limited myopathy that could itself be accentuated by stressful conditions or by stress-susceptibility, thus explaining the higher number of these fibres sometimes seen in PSE pork. Remignon et al. (2000) in their study on turkey muscles found no giant fibres in pre-rigor muscles, while in the present study some were found. This could be due either to a species difference (chicken versus turkey) or to the small difference in sampling time (10 minutes versus 3 minutes post mortem).
Contrary to their name giant fibres are not always bigger than normal fibres. Nonetheless, on average, the mean cross sectional area of giant fibres is bigger than that of any other fibre type (tables 4 and 5).
While observing muscle samples coloured with hematoxylin-eosin, it was possible to notice that some of them contained fibres with a lower staining intensity. The evaluation and comparison of the fields containing such fibres for all of the enzymatic activities considered, proved that these fibres were always negative or only slightly positive, thus their nature and classification was not clear. These fibres resemble those found by Severini et al. (1998) in PSE pork muscle.

Conclusions

Giant fibres were more common in "white" muscles and had staining patterns similar to α type fibres. Some giant fibres also had some of the alterations usually seen in pathological muscle fibres such as an apparent hypercontraction which could explain the higher staining intensity they sometimes show. It could be said that the origin of giant fibres is connected to the biochemical events that take place during post-mortem glycolitic metabolism. There must be a reason though, that explains why only some of the fibres composing a muscle become giant fibres. This could be due to an alteration occurring in the live animal.

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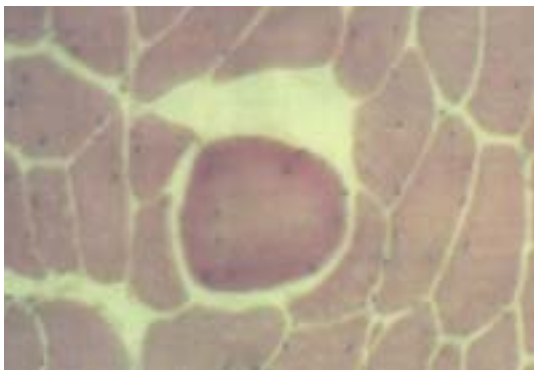

Figure 1: Giant fibre coloured with E.E.

Table 1: Mean muscle fibre distribution

	P.major	I.lateralis		Semimembranosus			F.medius		
	αW	αW	αR	αW	αR	βR	αW	αR	βR
Mean	100	71,5	28,5	62,8	33,9	3,3	58,8	40,3	0,9
St.Dev.	0	9,0	9,0	9,9	8,0	3,8	13,7	11,9	2,0

Table 2: Percentage of giant fibres 10 minutes and 24 hours post-mortem

SAMPLE	P.major		I.lateralis		Semimembranosus		F.medius	
	10 min	24 hours	10 min	24 hours	10 min	24 hours	10 min	24 hours
1	0,7	5,1	4,4	2,8	1,2	2,1		
2	0,6	3,6	1,8	4,2	1,1	3,6		
3	0,2	4,1	0,6	2,3	0,4	1,1		
4	1,0	5,1	3,8	3,8	4,5	3,4		
5	1,8	12,5	1,7	8,2	1,3	7,2		
6	0,1	7,2	0,7	2,5	0,5	3,0		
7	0,3	7,1	2,4	7,1	0,6	1,0	0,2	0,5
8	0,2	7,0	1,9	2,2	1,3	0,7	0,2	0,8
9	0,2	12,7	1,8	6,7	0,0	0,5	0,6	0,8
10	0,1	11,8	0,8	5,9	0,3	0,2	1,4	0,5
11	0,5	15,9	1,0	2,9	0,2	0,5	0,5	0,4
Mean	**0,5**	**8,4**	**1,9**	**4,4**	**1,0**	**2,1**	**0,6**	**0,6**
St.Dev.	**0,5**	**4,1**	**1,2**	**2,2**	**1,2**	**2,1**	**0,5**	**0,2**

Table 3: Amount of glycogen

Sample	P.major			I.lateralis			Semimembranosus			F.medius		
	10 min		24 h	10 min		24 h	10 min		24 h	10 min		24 h
	% positive fibres		% positive fibres	% positive fibres		% positive fibres	% positive fibres		% positive fibres	% positive fibres		% positive fibres
1	42,1	+	0	26,8	-/+	0	50,3	+/-	0			
2	66,9	+	0	49,6	+	0	5,4	+	0			
3	70,8	+/-	0	61,7	+/-	0	52,3	+/-	0			
4	79,8	+	0	15,4	+	0	23,1	+/-	0			
5	88,1	+	0	64,3	+	0	71,0	+	0			
6	87,6	++	0	62,8	+	0	70,8	+	0			
7	95,2	++	0	49,1	+	0	25,3	+/-	0	51,5	+	0
8	94,1	++	0	61,8	++	0	31,1	+	0	90,7	+	0
9	89,7	++	0	4,0	+/-	0	0,6	+/-	0	4,5	+/-	0
10	84,5	+	0	38,3	+	0	1,4	+/-	0	29,5	+/-	0
11	90,7	++	0	23,0	+	0	1,7	+	0	3,8	+/-	0

+ and – signs stand for high or low staining intensity

Table 4: Giant fibre area (μm^2)

	P.major	I.lateralis	Semimembranosus	F.medius
Mean area	5530,6	5872,3	6241,9	5478,4
St.Dev.	1342,7	1422,3	2006,2	2350,9

Table 5: Normal fibre area (μm^2)

	P.major	I.lateralis		Semimembranosus			F.medius		
	αW	αW	αR	αW	αR	βR	αW	αR	βR
Mean	3486,4	3523,0	2304,2	3231,8	2954,3	2128,0	3516,6	2251,8	1184,0
St.Dev.	1307,3	1023,5	652,9	423,1	299,5	576,9	1072,2	705,6	234,9

HOW DOES LACTATE ENHANCEMENT IMPROVE BEEF COLOR STABILITY?

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Background

In both raw and cooked meat products, lactate limits discoloration by minimizing red color changes during storage and display (Brewer *et al*. 1991; Papadopoulos *et al.* 1991; Prestat *et al*. 2002; Lawrence *et al.*, 2003). However, the mechanism by which lactate improves muscle color stability is unknown.

How does lactate improve muscle color stability?
We speculate that lactate's ability to stabilize muscle color is linked to regeneration of nicotinamide adenine dinucleotide (NADH), which is necessary for metmyoglobin reduction; a reaction that promotes color stability. We believe this link between lactate and NADH regeneration is lactate dehydrogenase (LDH; Figure 1). From a meat quality standpoint, LDH may be an "overlooked" endogenous enzyme that has potential to influence color life.

Metmyoglobin reduction
Oxidation of myoglobin is inevitable; thus, maximizing metmyoglobin reduction (gain of electrons) is essential for color stability. It is widely accepted that NADH donates electrons to metmyoglobin via both enzymatic and nonenzymatic pathways (Brown and Snyder 1969; Hagler *et al*. 1979; Livingston *et al*. 1985; Renerre and Labas 1987; Stewart *et al*. 1965). However, the location of the NADH pool that provides electrons to postmortem metmyoglobin reduction is unknown (Renerre and Labas 1987).

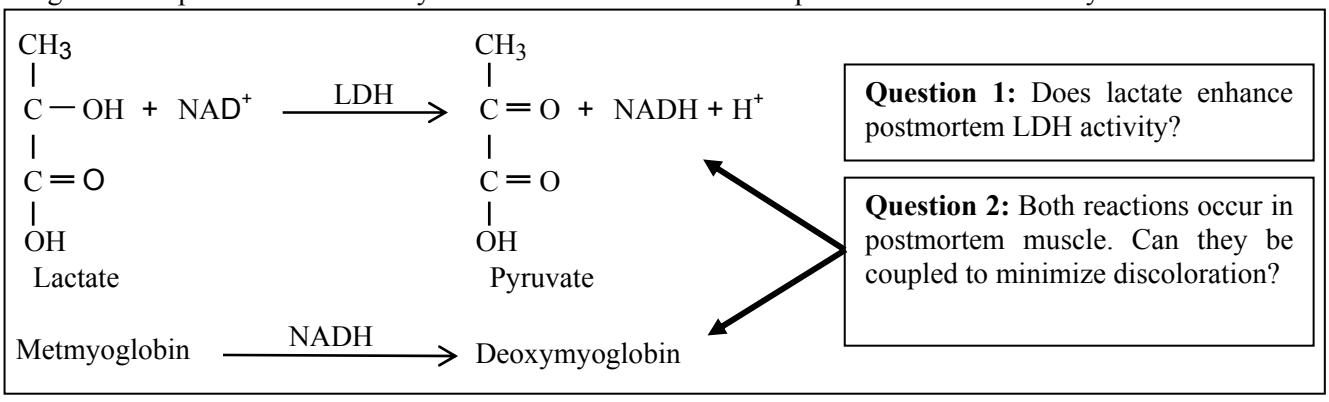
The NADH pool
Stewart *et al*. (1965) provided early speculation regarding replenishment of the NADH pool by suggesting that LDH catalyzes the production of reduced pyridine nucleotides from lactate and diphosphopyridine. These authors believed this mechanism was feasible because both lactate and LDH are abundant in postmortem muscle. However, to date, this mechanism has not been evaluated or proven.

Objectives

Hypothesis: Enhancing beef with lactate increases LDH activity, which consequently improves meat color stability by promoting metmyoglobin reduction (Figure 1).

Objective: Evaluate if enhancement of beef *longissimus* with potassium lactate increases postmortem lactate dehydrogenase and metmyoglobin reducing activity.

Figure 1: Proposed mechanism by which lactate enhancement improves beef color stability.



Materials and methods

Experiment 1: Whole loin enhancement
Five boneless beef strip loins (5 days postmortem) were divided into four equal sections. Each section within a loin was assigned randomly to one of four treatments (1 = no enhancement; 2 = enahancement with water; 3 = 1.5% potassium lactate in the finished product; 4 = 2.3% potassium lactate in the finished product). Strip loin sections were pumped at 10% of their green weight using a multineedle injector. Pumped loin sections were cut into 2.54 cm-thick steaks, overwrapped in polyvinyl chloride (PVC), and displayed for 7 days at 1ºC in open-top display cases under 1614 lux of continuous fluorescent light (3000 K). Lactate dehydrogenase activity was measured on day 1, day 3, and day 7 of display. The experimental design was a split plot where the whole plot design structure was a completely randomized block (n = 5) with a one-way treatment structure consisting of 4 treatments. Within the whole plot, loins served as blocks, and each of four loin sections were the experimental units to which enhancement treatments were applied. Steaks within a loin section (subplot experimental units) were assigned randomly to 1 of 3 display times.

Experiment 2: Model system enhancement
Eight randomly selected beef *longissimus* steaks from different animals were used in the model system. Each steak was divided into three equally sized pieces. To each piece within a steak, one of three treatments was assigned randomly (1 = nonenhanced; 2 = nonenhanced plus LDH inhibitor; and 3 = potassium lactate). Pieces assigned to treatment 3 (lactate) were injected with a syringe to a concentration of 2% lactate in the final product. Oxalate (LDH inhibitor in treatment 2) was added during quantification of LDH activity (described below). Steaks were overwrapped in PVC and stored at 4°C for 7 days. Lactate dehydrogenase activity and metmyoglobin reducing activity were measured after day 1 and day 7 of storage. The experimental design was a randomized complete block (steaks were blocks) with repeated measures. For both experiments, data were analyzed using SAS and significance is represented at $P < 0.05$.

LDH activity
Lactate dehydrogenase activity was measured using a spectrophotometric technique (Wahlefeld 1987). From each loin, a 2.0 g sample was homogenized with 0.01 M potassium buffer (pH 7.2). Homogenates were centrifuged for 30 minutes at 14,600 x *g* (4°C). In brief, β-Nicotinamide-adenine dinucleotide, Tris buffer, and L-Lactate were added to 0.1 mL of homogenized muscle supernatant. After 5 minutes of incubation at 30°C, LDH activity was measured using absorbance at 339 nm, which indicates the production of NADH via lactate oxidation (NAD reduction increases absorbance at 339 nm; L-Lactate + NAD^+ → Pyruvate + NADH + H^+). To determine activity, absorbance at 339 nm was measured at 30-second intervals for 2.5 minutes. Activity in U/L was calculated as: change in absorbance ($\Delta A/\Delta t$) x 4.21 x 10^3. For those samples assigned to treatment 2 (LDH inhibitor), oxalate was added after centrifugation to the supernatant. All assays were performed in duplicate and averaged for statistical analysis.

Metmyoglobin reducing activity
Step 1: Samples (3 x 2 x 1.27 cm^3) were submerged in 0.3% sodium nitrite. After 20 minutes, samples were blotted dry, immediately vacuum packaged, and reflectance from 400 – 700 nm was measured. Step 2: Samples were incubated at 30ºC and reflectance was measured after 2 hours. Percent metmyoglobin was determined according to AMSA (1991) and metmyoglobin reducing activity (MRA) was claculated as: (Observed decrease in metmyoglobin during incubation ÷ initial metmyoglobin concentration) x 100.

Results and discussion

Experiment 1: Whole loin enhancement
Enhancing loins with potassium lactate tended to increase LDH activity (Table 1). On day 7, both 1.5% and 2.3% lactate resulted in signifcantly more LDH activity than enhancement with only water. Using 2.3% lactate significantly increased lactate dehydrogenase activity compared to nonenhanced control loins at the end of display (day 7). Lactate dehydrogenase activity increased during display. Neither treatment influenced muscle pH (Control samples = 5.8 and lactate enhanced samples = 5.9).

Experiment 2: Model system enhancement
Compared to nonenhanced controls at the end of storage (day 7), adding 2.0% potassium lactate to beef *longissimus* significantly improved LDH and metmyoglobin reducing ability (Tables 2 and 3). However,

there was no significant difference between nonenhanced steaks and lactate-enhanced steaks early in storage (day 1). Thus, as storage time increased, the ability of potassium lactate to promote LDH and MRA activity also increased. Conversely, there was no significant change in LDH activity during storage for nonenhanced steaks. As expected, oxalate minimized LDH activity. Muscle pH was not influenced by treatment (pH = 5.9).

Lactate dehydrogenase activity in muscle has been used as an indicator of endpoint temperatures in meat products (Collins *et al*. 1991; Keeton and Morris 1996; Stadler *et al*. 1991 & 1997). However, food-safety researchers commonly measure pyruvate reduction rather than lactate oxidation, which was measured in our experiment. Nevertheless, previous work support our findings that LDH remains active postmortem.

Meat color stability is vital for maximizing consumer purchasing. Product inconsistency and central packaging have driven beef purveyors to utilize enhancement technology aimed at improving shelf life and product quality. In addition to water, salt, and phosphate, beef processors have a vested interest in color-stabilizing, antimicrobial, and palatability-enhancing ingredients such as lactate. Determining mechanisms present in postmortem muscle that support myoglobin reduction may be beneficial for developing future enhancement technologies that improve the shelf life of muscle based food products.

Conclusions

There is a lack of knowledge regarding the mechanism of lactate-induced color stability. The role of metmyoglobin reduction and NADH in meat color stability has been well documented. However, mechanisms in postmortem muscle involved in replenishing the NADH pool have received little attention. It is possible that lactate dehydrogenase is an endogenous enzyme involved in postmortem NADH production.

We conclude that enhancing beef *longissimus* with lactate can influence both postmortem LDH activity and metmyoglobin reducing ability. We speculate that LDH converted postmortem-injected lactate to pyruvate and NADH, which replenished the reducing equivalent pool of postmortem muscle and chemically reduced metmyoglobin (increased MRA). This project suggests that lactate has potential to not only benefit beef processors through improved shelf life, but also to benefit researchers by providing a better understanding of postmortem replenishment of the NADH pool.

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Table 1: Effects of enhancing beef strip loins with potassium lactate on lactate dehydrogenase activity during display at 1ºC.

Display time (days)	Nonenhanced control	Enhanced with water	1.5% Potassium lactate	2.3% Potassium lactate
1	342.6a	320.2a	379.4ab	407.6b
3	352.3ab	313.4a	358.9ab	386.6b
7	816.7ab	770.9a	858.3bc	916.7c
Average[d]	503.8b	468.2a	532.2bc	570.3c

[abc]Least square means (activity determined via NADH production and expressed as U/L) within a row with a different letter differ ($P < 0.05$).

[d]Least square means averaged across display times.

Table 2: Effects of potassium lactate on beef *longissimus* lactate dehydrogenase activity during storage.

Storage time at 4ºC	Nonenhanced control	2.0% Potassium lactate	Oxalate*
1 days	146.6ay	156.6ay	100.8by
7 days	190.0by	223.4az	120.0cy

[abc]Treatment effects: least square means within a row with a different letter differ ($P < 0.05$).
[yz]Storage time effects: least square means within a column with a different letter differ ($P < 0.05$).
**Longissimus* was enhanced with lactate and oxalate was added during quantification of LDH activity. Activity determined via NADH production and expressed as U/L.

Table 3: Effects of potassium lactate on beef *longissimus* metmyoglobin reducing activity* during storage.

Storage time at 4ºC	Nonenhanced control	2.0% Potassium lactate
1 days	88.5a	85.2a
7 days	51.3c	70.8b

[ab]Least square means with a different letter differ ($P < 0.05$).
*Metmyoglobin reducing activity (MRA) was calculated as: (Observed decrease in metmyoglobin during incubation ÷ initial metmyoglobin concentration) x 100.

EFFECT OF TRANSPORT TIME AND SEASON ON ASPECTS OF RABBIT MEAT QUALITY[1]

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Background

The events involved in the transport chain, and other related factors, may induce stress in rabbits (Jolley, 1990) and can affect aspects of meat quality (Masoero et al., 1992). Little is known about the effect of transport on rabbit meat texture or colour. Ultimate meat pH is the most commonly measured parameter in studies that consider ante-mortem effects on meat quality. However, while it is clear that even travelling short distances can reduce live weight (shrinkage), decrease glycogen reserves and increase meat temperature (Jolley, 1990), this is not always reflected in changes in ultimate pH. There may be no effect when transport is only a slight stress and the animals are in good condition. The relationship between initial muscle glycogen content and ultimate pH is only linear at very low levels of glycogen. Thus, they are not lowered enough to have a substantial effect on the ultimate pH, especially when the animals can recover during lairage. Changes in meat texture and colour with respect to ante-mortem stress have been considered, but normally the effect of transport time has been mixed with other confounding factors (e.g., breed, production system, nutrition, etc.). In Spain, transport of commercial rabbits to the abattoir is relatively short, typically less than 3 hours. Recently, a report by the Scientific Committee on Animal Health and Animal Welfare has proposed important limitations on transport time and the European Parliament has suggested decreasing all journeys for all species to less than nine hours. Although these measures may improve animal welfare, it remains unclear whether imposing them on a commercial level will also improve meat quality. This fact is even more important in commercial rabbits where little information is available.

Objectives

The aim of this study was to analyze whether transport times of up to 7 hours can have a significant effect on instrumental meat quality traits in rabbits. Due to the characteristics of the Spanish climate, with very hot summers and cold winters, replicates were performed in two seasons. We also considered the effect of position on the truck as a factor which could affect meat quality.

Materials and methods

We analysed the instrumental quality of meat from 156 rabbits that were transported by road, together with other non-target animals, for 1h or 7h in winter and summer with three replicates each. The average temperature during transport was recorded every 5 minutes with a Testo thermometer at the level of the study animals. The average temperatures were 11ºC ± 3 and 28ºC ± 2 in winter and summer, respectively. Three main effects were considered: transport time, season and position on the truck (top, middle or bottom) in a multi floor cage rolling stand (MFRS). For each season and journey time, 36 animals were selected (12 in each position). The stocking density during the transport was 360 cm^2 per animal. The cage size was 57 x 57 x 25 cm, in MFRS & Sañudo, 2001). For texture meat analyses, longissimnus dorsis muscles were vacuum packaged and frozen at –18ºC. The compression and Warner-Bratzler (WB) analyses were performed on sample slices as in Campo et al. (2000) with an Instron with 12 cages each. The total capacity of the truck was 2400 rabbits per trip. The rabbits were slaughtered after 3 h of lairage in the same MFRS. Average carcass weight was 1192.50 g (±121). Carcasses were chilled under commercial conditions at 0ºC for 24 h. The meat pH was measured at 24 h post-mortem on the lumbar region with a Crison 507 electrode. Sixteen additional rabbits were taken randomly to study the proportion of muscle, bone and fat of the carcasses by dissection in the lab. The longissimus dorsi was removed from both sides and the right side was sliced into three steaks for instrumental analysis. Water holding capacity (WHC) was measured 24 h after slaughter and expressed as press juice using the Grau and Hamm method (Cañeque 4301. Briefly, thawed steaks (internal

[1] Funded by CICYT Ministry of Science and Technology of Spain. Project AGL 2002-01346 COTRANS.
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temperature 17-19ºC) were cut transversally to be studied either as raw or cooked meat. Texture of the raw meat was analysed using a modified compression device that avoids transversal elongation of the samples. The stress was assessed at 20% (P20), 40% (P40), 60% (P60) and 80% (P80) of the maximum compression (MS). Meat was vacuum packaged prior to cooking in a water bath at 75ºC until the internal temperature reached 70ºC. Samples (1 cm^2 cross-section) were cut with muscle fibres parallel to the longitudinal axis of the sample. Maximum load (ML) and toughness (TO) were assessed in heated meat using a Warner Bratzler device, shearing until breaking the samples (10 mm wide and 10 mm thick). The sample gauge was 10 mm, gauge length 30 mm, load cell 100 kg (minimum load level 0.001 kg), crosshead speed 150 mm /min (high extension limit 30 mm) and sampling rate 20 points/second. Meat colour was measured in the reflectance spectrum every 10 nm from 400 to 700 nm using a Minolta (2002) reflectometer-colorimeter. Measurements were taken from the surface of a slice of meat from each animal transported. Slices were freshly cut at 24 h post-mortem and measured after 24 h blooming. Each slice was placed at 4ºC on individual plastic foam trays and wrapped with an O_2 permeable film without touching the sample. The L*a*b* values were taken using the standard illuminant D65 and 10º standard observer for all measurements. The data were analysed using the least square methodology of the GLM procedure of SAS (SAS, 1988), fitting a three-way model including the fixed effect of transport time (2 levels), season (2 levels) and position on the stand cages (3 levels) plus the interaction effect.

Results and discussion

The average percentage composition of the left middle carcass was 75.31 (±2.7) muscle, 4.62 (±1.09) fat and 16.39 (±1.8) bone (n=16 animals). The significance of the main effects is presented in Table 1. The effect of position on the truck was not significant. Season and journey time had significant effects on several instrumental meat quality parameters. The interaction between time and season was only significant for Warner Bratzler variables.

The least square means for all the instrumental variables are presented in terms of season and journey time in Table 2. The parameters fell within the range of good quality meat. Similar results were found by Trocino et al. (2002) and Xicato et al. (1994) in their studies on rabbit transport. Transport time did not affect pH24, WHC or Warner Bratzler texture parameters. All the texture parameters evaluated by compression were significantly affected by journey time ($p \leq 0.001$). In general, the values were higher after short journeys. Transport time slightly affected colour parameters, and a* was significantly higher after long journeys ($p \leq 0.05$), especially in winter. Other studies (Jolley, 1990; Masoero et al. 1990 and Dal Bosco et al., 1997) have found that pH increase after longer journeys.

Season had a significant effect on all the response variables, with the exception of compression at 80% and maximum stress. The pH 24 values were significantly higher in winter than in summer, but always below 6. The same situation was observed for WHC. Some authors have found that transport affects WHC (Trocino et al., 2002; Jolley, 1990). Nevertheless this parameter could be affected by the treatment applied (Ouhayoun & Dalle Zotte, 1996). All texture variables analyzed by Warner Bratzler were higher in winter than in summer. The same situation was true for 20% compression. The colour parameters were all affected by season. Redness was higher in winter than in summer but the opposite occurred for yellowness. Lightness varied less, but the difference was still significant ($p \leq 0.05$) between long journeys, increasing for summer trips. There was a significant interaction between journey time and season for texture traits evaluated by Warner Bratzler (Figure 1). According to our results, instrumental meat quality could be affected by the multifactor stressors involved in the transport process. Values for meat colour were similar to Trocino et al. (2002), but higher than Jolley, 1990. Dal Bosco et al. (1997) found that meat from animals from short journeys had significantly higher L*.

Few studies have considered the effect of transport on instrumental measurements of meat quality in commercial rabbits. Under the controlled commercial conditions of this work, transport time of up to seven hours significantly affected the instrumental quality of rabbit meat as measured by a modified compression device, which reflects the mechanical resistance of the myofibrillar structure (P20) and connective tissue strength (P80). No differences were found in ultimate pH between transport groups, which conditioned the lack of variation in other variables such as WHC and other texture parameters assessed by Warner Bratzler shear device and the slight effect of redness.

Season affected almost all the variables, but it was not completely independent from the effect of transport time. Even when the effect of journey time was not significant (for WB variables), there was a significant interaction between transport time and season, with the worst texture values after short journeys in winter

and long journeys in summer. Independently of journey time, season did not have an effect on meat quality. In summary, transport time had an effect on instrumental meat quality in terms of compression and a slight effect on colour. Within the range of transport times analysed, there were no significant changes in pH, which is the main parameter used to judge meat quality on an industrial level. Finally, position within the multi floor cage stand did not affect instrumental meat quality.

Conclusions

The general conclusion of this study is that, even under optimum commercial conditions, rabbit meat quality could be affected by the multiple stressors involved in the transport process. Transport affected several measures of meat quality and this effect depended on the season of the year.

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Table 1. Summary table of the significance of the main effects and their interaction for instrumental meat quality parameters.

Response Variable	Main Effects in the Full Model						
	Season	**T**ime	**P**osition	**S*T**	**S*P**	**P*S**	**S*T*P**
pH24	***	NS	NS	NS	NS	NS	NS
Water holding capacity	***	NS	NS	NS	NS	NS	NS
Warner-Bratzler							
Shear force (Kgf)	***	NS	NS	***	NS	NS	NS
Toughness (Kgf/cm2)	***	NS	NS	***	NS	NS	NS
Compression							
C20 (20%)	***	***	NS	NS	NS	NS	NS
C80 (80%)	NS	*	NS	NS	NS	NS	NS
Maximum Stress (N/cm2)	NS	***	NS	NS	NS	NS	NS
Colour							
L* (lightness)	*	NS	NS	*	NS	NS	NS
a* (redness)	***	*	NS	NS	NS	NS	NS
b* (yellowness)	***	NS	NS	NS	NS	NS	NS

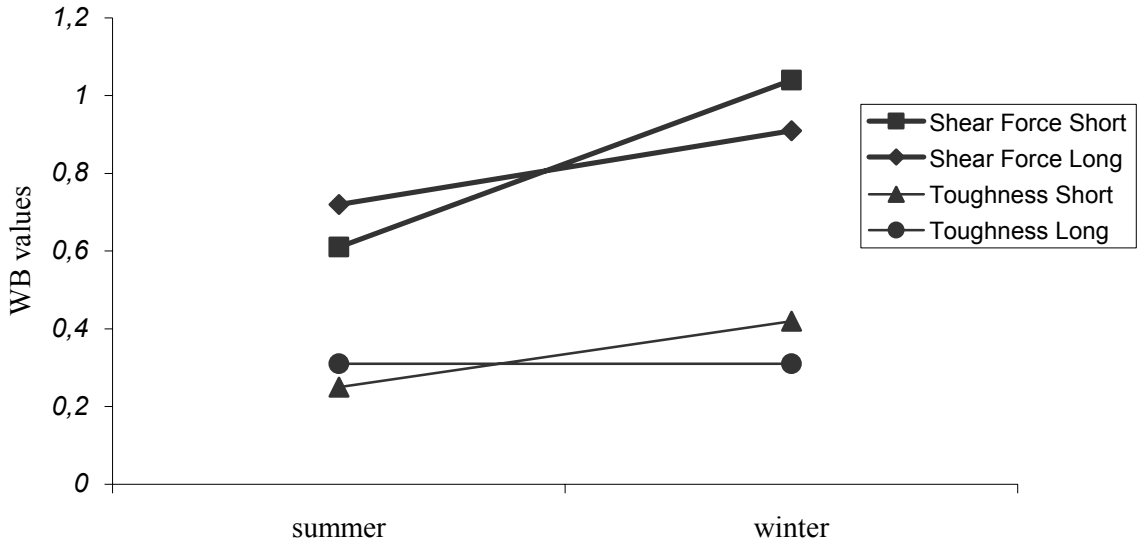
The levels of significance were * $p<0.05$ ** $p<0.01$ *** $p<0.001$. **S**eason refers to summer or winter. **T**ime: journey time (1 hours or 7 hours). **P**osition: position in the multi floor cage rolling stand (top, middle or bottom cages) during transport.

Table 2. Least square means (±S.E.) of instrumental meat quality parameters in summer and winter and after two different transport times (1 h or 7 h).

	Summer (1)		Winter (2)	
Response Variable	1 h	7 h	1 h	7 h
pH24	5.75±0.02a	5.77±0.01a	5.97±0.03b	5.90±0.02b
Water holding capacity	12.61±0.45a	12.12±0.44a	14.93±0.45b	14.57±0.42b
Warner-Bratzler				
Shear force (Kgf)	0.61±0.04a	0.72±0.04a	1.04±0.03b	0.91±0.04c
Toughness (Kgf/cm^2)	0.25±0.01a	0.31±0.02b	0.42±0.02c	0.31±0.02b
Compression				
P20 (20%)	10.65±0.34a	9.69±0.33b	12.32±0.34c	11.39±0.35a
P80 (80%)	16.66±0.56a	17.01±0.57a	17.36±0.52a	14.92±0.51b
Maximum stress (N/cm^2)	21.90±0.62a	20.61±0.0.61a	24.02±0.63b	20.74±0.60a
Colour				
L* (lightness)	58.46±0.34ab	59.36±0.36b	58.44±0.33ab	57.95±0.31a
a* (redness)	2.34±0.21a	2.49±0.20a	3.45±0.22b	4.19±0.26c
b* (yellowness)	4.09±0.25a	4.18±0.27a	2.92±0.20b	3.18±0.22b

Different letters in the same row indicate significant differences ($p \leq 0.05$)

Figure 1. Interaction effect between transport time (short or long) and season (winter or summer) for the Warner Bratzler texture traits.



TRANSPORT RELATED BRUISING IN CATTLE

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Introduction

The bruising of cattle is used as an indicator for animal welfare problems during pre-slaughter handling and can reduce meat quality (1). The aim of this study was to describe the prevalence of carcass bruising in cattle observed at *post-mortem* meat inspection in a Norwegian abattoir and to assess risk factors.

Materials and methods

The study was carried out in a commercial abattoir processing approximately 20,000 cattle a year. The drivers recorded transport parameters. Carcass bruising were assessed by two official veterinarians at *post-mortem* meat inspection. Bruises were categorised according to size as small (diameter < 4 cm), medium (diameter 4 - 10 cm) or extensive (diameter > 10 cm). Only bruises adjacent to *Tuber coxae*, *Trochanter major* or *Tuber ischii* were recorded. Data on carcass conformation, fat cover and weight were obtained from the abattoir.

Carcass bruising was set as continuous response variable, and the regressor variables included both categorical and continuous variables. Descriptive statistics were performed, and ANOVA was used for hypothesis testing of risk factors. An ANOVA model was used for each bruise site and size.

Statistical analysis

The data were analysed using one-way cross tabs procedure for bruising, descriptive statistics and analysis of variance (ANOVA) procedure for hypothesis testing of the risk factors. Carcass bruising was set as response variable, and transport risk factors were set as regressors. The following model was used (2):

$$Y_i = \beta_0 + \beta_1 + ... + \beta_j + \varepsilon$$

Where i = p number of bovine carcass bruisings, j = q number of transport risk factors

No interactions were included in the model.

The model was analysed using SYSTAT Version 10 © SPSS Inc. 2000 Standard Version, Statistics – GLM – Estimate Model. Carcass bruising was set as continuous response variables, and the regressor variables both included categorical and continuous variables.

Results and discussion

Data from 84 transports comprising 1252 cattle were obtained during a period from May to August 2003. All animals were transported direct from the farms, and included The predominant breed in the region is Norwegian dairy cattle. Transport times were generally short (Table 1).

Table 1. Transport time

Transport time (hours)	
Minimum	0.25
Maximum	7.0
Mean	2.35
Median	2.0

The overall prevalence of carcasses with bruises ≥ 4 cm was 36.2 %. Table 2 summarises the recorded bruises.

Table 2. Prevalence (%) of bruises in cattle (n=1252) according to size and localisation

Bruise diameter (cm)	< 4			4 – 10			> 10		
Site of bruising	None	Unilat.	Bilat.	None	Unilat.	Bilat.	None	Unilat.	Bilat.
Tuber ischii	11.4	16.7	71.9	80.4	15.0	4.6	88.3	8.1	3.6
Trochanter major	3.7	13.4	82.9	87.3	10.3	2.4	94.5	4.4	1.1
Tuber coxae	2.1	10.9	87.0	89.9	8.8	1.3	96.7	3.0	0.3

The ANOVA identified animal category, driver, increasing transport time, increasing number of herds mixed in vehicle pen, ascending order of loading and increasing area (m^2) per animal as the primary risk factors for carcass bruising. Details from the ANOVA analysis (Table 3).

Table 3. Effects of risk factors on bruising (univariable analyses)

Bruise site		*Tuber ischii*			*Trochanter major*			*Tuber coxae*		
Risk factor	Bruise size (cm)	< 4	4 - 10	> 10	< 4	4 - 10	> 10	< 4	4 - 10	> 10
Category (steer, heifer, cow, calf)		***	***	*	*	NS	NS	***	***	NS
Driver ID		*	NS	NS	NS	**	NS	NS	NS	**
Transport time		*	NS	*	NS	NS	NS	NS	NS	**
No of herds mixed in vehicle pen		NS	NS	**	*	NS	NS	NS	NS	NS
Order of loading		NS	NS	NS	NS	NS	NS	NS	*	**
Area per animal (m^2)		NS	NS	NS	NS	NS	NS	*	NS	**
Registrar		NS	NS	NS	NS	NS	NS	NS	NS	**
Number of stops for loading		*	NS	NS	*	NS	NS	NS	NS	NS
Number of animals in vehicle pen		NS	NS	NS	NS	NS	NS	NS	NS	*
Carcass weight (kg)		NS	NS	NS	NS	NS	NS	NS	NS	NS
Carcass conformation (EUROP)		NS	NS	NS	NS	NS	NS	NS	NS	NS
Carcass fat cover (EUROP)		NS	NS	NS	NS	NS	NS	NS	NS	NS
Loose or tied		NS	NS	NS	NS	NS	NS	NS	NS	NS
Vehicle pen (front, middle or rear)		NS	NS	NS	NS	NS	NS	NS	NS	NS
Placement in pen if tied (front or rear)		NS	NS	NS	NS	NS	NS	NS	NS	NS
Area per 100 kg carcass weight (m^2)		NS	NS	NS	NS	NS	NS	NS	NS	NS

*P-value 0.01 – 0.05, **P-value 0.001 – 0.01, *** P-value < 0.001, NS (non-significant) P-value > 0,05

The low prevalence of bruising found in this study contrasts earlier findings from the UK. Jarvis and others (3) found that 97 % of carcasses observed were bruised, and that cattle from markets had more bruises than cattle transported direct from farms. Weeks and others (4) found that in cattle transported direct to abattoir 53.7 % of observed carcasses were bruised, while in cattle from markets 71.0 % were bruised. The difference in prevalence of bruising found in this study and other studies can partly be explained by differences in bruise recording system and type of lairage. All cattle in this study were lairaged in individual pens, which eliminates mounting behaviour. Cows have a higher risk for bruising than other categories of cattle. The same observation has been made in previous works by Weeks and others (4) and Wythes and others (5). This may be due to poorer muscle conformation,

reduced ability to cope with movements of the vehicle during transport, or a combination. The predominant housing system for dairy cows in the region is tie stalls, while steers mainly are kept loose in pens. Holleben and others (6) found higher bruising scores in cattle from tied housing. Transport time is found to be a risk factor for bruising, indicating that transport time is relevant in terms of animal welfare. Ascending order of loading is also identified as a risk factor, despite that transport time is shorter for cattle loaded in the end of the journey. In previous work performed by Tarrant and others (1988) stress was found to increase with pen localisation towards the tail of the truck (7). The truck design is probably of importance in this matter and should receive further investigation. The driver is showed to influence the risk of bruising. This could be explained by the fact that driving skills varies between drivers. Careful braking and cornering is important for the animals in order to avoid loss of balance (7).

Conclusion

The overall prevalence of bruising ≥ 4 cm was 36.2 %. The primary risk factors for bruising were found to be category, driver ID, transport time and number of animals mixed in vehicle pen.

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ANALYSIS OF POST MORTEM PRODUCTS OF PROTEOLYSIS IN PORCINE TCA SOLUBLE EXTRACTS AND THEIR ASSOCIATION WITH TENDERNESS

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Background

Proteolytic degradation of myofibrillar proteins has been shown to contribute to *post mortem* tenderisation with the rate and extent of proteolysis being associated with meat tenderness (Troy and Tarrant., 1987; Sentandreu et al. 2002, & Sawdy et al., 2004). Analysis of the products of proteolysis has held much interest in terms of developing diagnostic tests for meat tenderness. In the past a lot of focus has been directed to the study of products of *post mortem* proteolysis in the myofibrillar fraction of muscle. In more recent years, however, the analysis of more soluble proteolytic fragments has held much interest (Nakai et al., 1995; Mullen et al., 1998; Stoeva et al., 2000). One dimensional gel electrophoresis (1DGE) allows visualising products on the basis of molecular weight while two dimensional gel electrophoresis (2DGE) separates proteins according to their isoelectric point and molecular weight. In this manner 2DGE provides a very high resolution separation. Application of these techniques may add further definition to the pattern of proteolysis over the *post mortem* ageing process and its association with pork tenderness.

Objectives

The objective was to apply 1-DGE and 2-DGE to track *post mortem* proteolytic events and their associations with tenderness, in Trichloroacetic acid (TCA) soluble extracts from porcine *M.longissimus dorsi*.

Materials and methods

Forty pigs were slaughtered and carcasses treated conventionally in a commercial abbatoir. A sample was excised from the *M.longissimus dorsi* (striploin, LD) at 1d, 3d and 7d *post mortem*. These samples were snap frozen in liquid nitrogen and stored at –80^{o}C. The LD was excised at 1d postmortem and chops were taken for analysis of eating quality. Tenderness was measured by Warner Bratzler shear force (Shackelford et al., 1991). Compositional analysis was conducted to determine any variation in intramuscular fat, protein and moisture levels. Sarcomere lengths were determined according to the laser diffraction method (Cross *et al.,* 1980). Other quality measurements included drip loss (Honikel and Hamm, 1994), cook loss, pH rate of decline and temperature profile up to 1d postmortem.

Based on the quality analysis, carcasses which displayed extreme values of tenderness were selected while controlling for extreme values of sarcomere length and intramuscular fat. Samples were alloted to three catagories, namely tender (Class I), tough becoming tender (Class II) and tough (Class III). A representative sample from each category was selected and TCA soluble extracts were prepared for both 1DGE and 2DGE analysis. 2-D gels were produced in triplicate for each sample, according to the method described in Morzel et al. (2004) with slight modifications.
Spots were detected and quantified by the image analysis software PDQuest. Spots of interest, i.e. those more associated with the "tender" class, were defined as spots that were either specific to the class I sample, or spots that were over-expressed at least three-fold in the class I (compared to class III) on day 7.

Results and discussion

Initially 1DGE analysis was conducted on extracts (1d, 7d, 14d) from each of the classes where bands of various molecular weights were seen to increase and decrease over the ageing period (Figure 1). In the tender sample increases were observed in lower molecular weight bands (Areas 3 & 4) which were not so obvious in the tough sample. These bands may be the product of proteolysis of myofibrillar or sarcoplasmic proteins over the postmortem ageing period. Two other areas of interest were noted (Areas 1 & 2). The tough sample appeared to have a higher level of the higher molecular weight band in Area 1, compared to the

tender sample. In addition in Area 3 the bands seem to appear sooner in the tough sample. Each lane received similar protein loading, however in some instances some of the changes observed may be due to larger proteins being held back in the gel matrix. However, repeated analysis of these gels have revealed similar banding patterns.

A representative 2DGE image is presented in Figure 2. Approximately 120-160 spots were successfully detected and quantified on all gels. Spots specific to class I are squared in blue, whereas spots over-expressed in class I are squared in red. Spots are designated by their unique number attributed by PDQuest. As an example, figure 3 shows the quantity (ppm) of spot 2504 in the three samples, as affected by *post mortem* time. It is very likely that most of those potential biomarkers are protein fragments, generated by *post mortem* proteolysis.

Both 1DGE and 2DGE will be repeated on further samples. Comparisons between gels will be invaluable in the interpretation of 1D gels. Proteins of interest in both gel formats are being identified by MALDI-ToF Mass Spectroscopsy.

Conclusions

Changes in banding protein patterns were evident in 1DGE gels. 2DGE electrophoresis allowed us to propose a list of potential biomarkers of tenderness in pork meat. In order to confirm their relevance, these need to be identified by MALDI-ToF Mass Spectroscopy and quantified in a larger set of samples.

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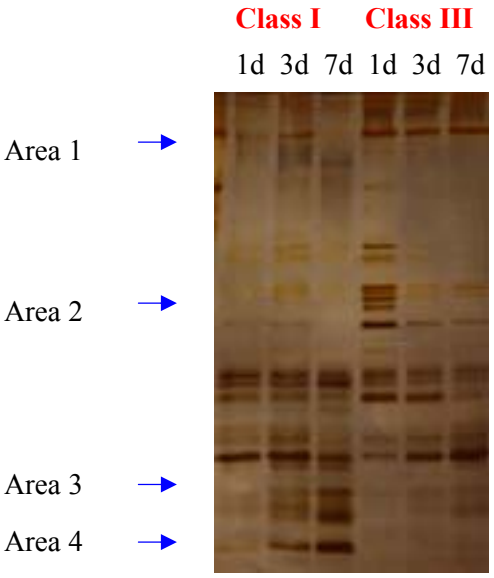

Figure 1. 1-D SDS PAGE of Class I and III TCA soluble protein extracts aged to 1d, 3d and 7d *postmortem*. Blue arrows mark areas of interest.

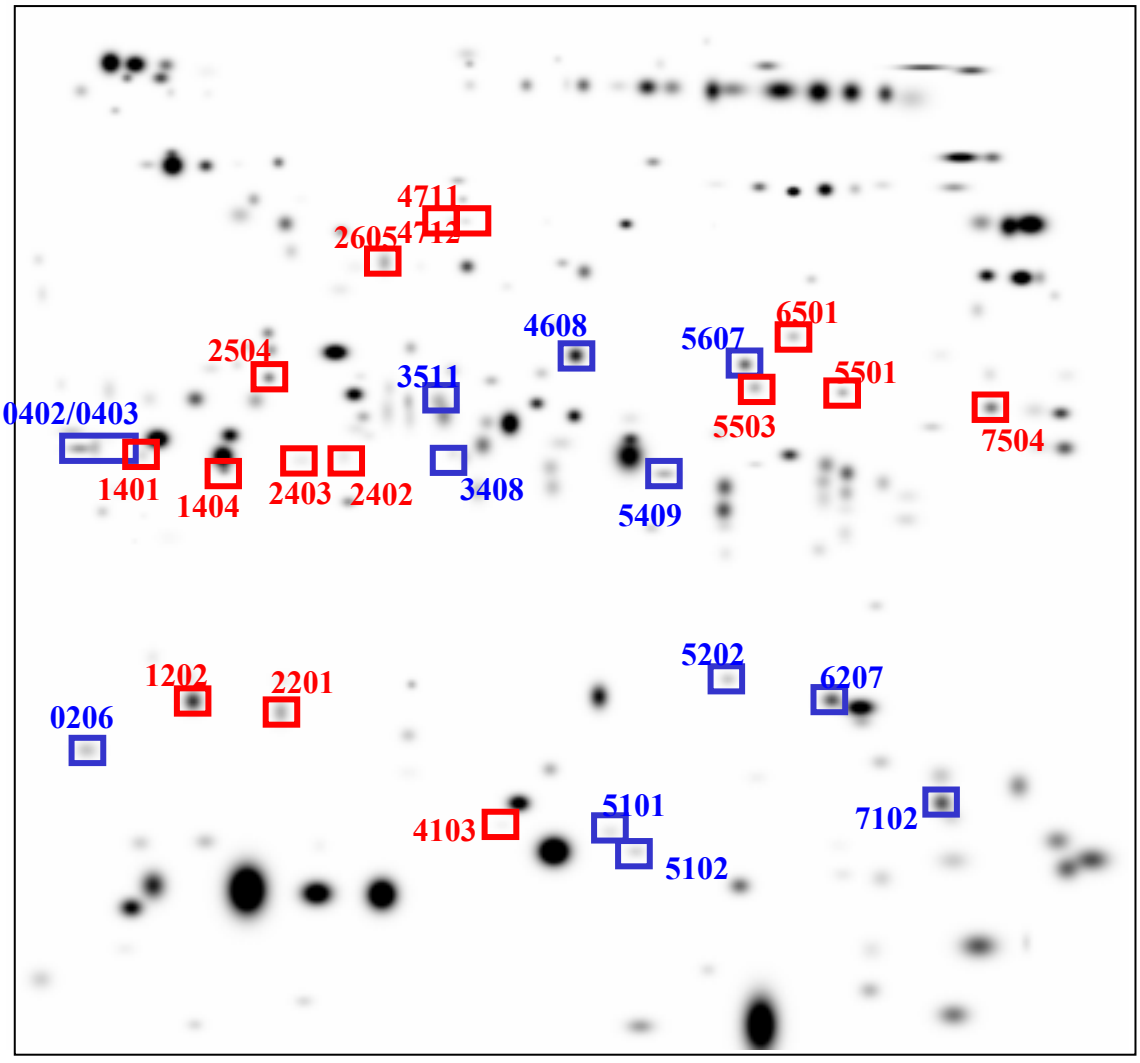

Figure 2. 2DGE: Synthetic representation TCA-soluble proteins, separated according to their pI (3-10 left to right) and MW (top to bottom). Potential biomarkers of pork meat tenderness are squared

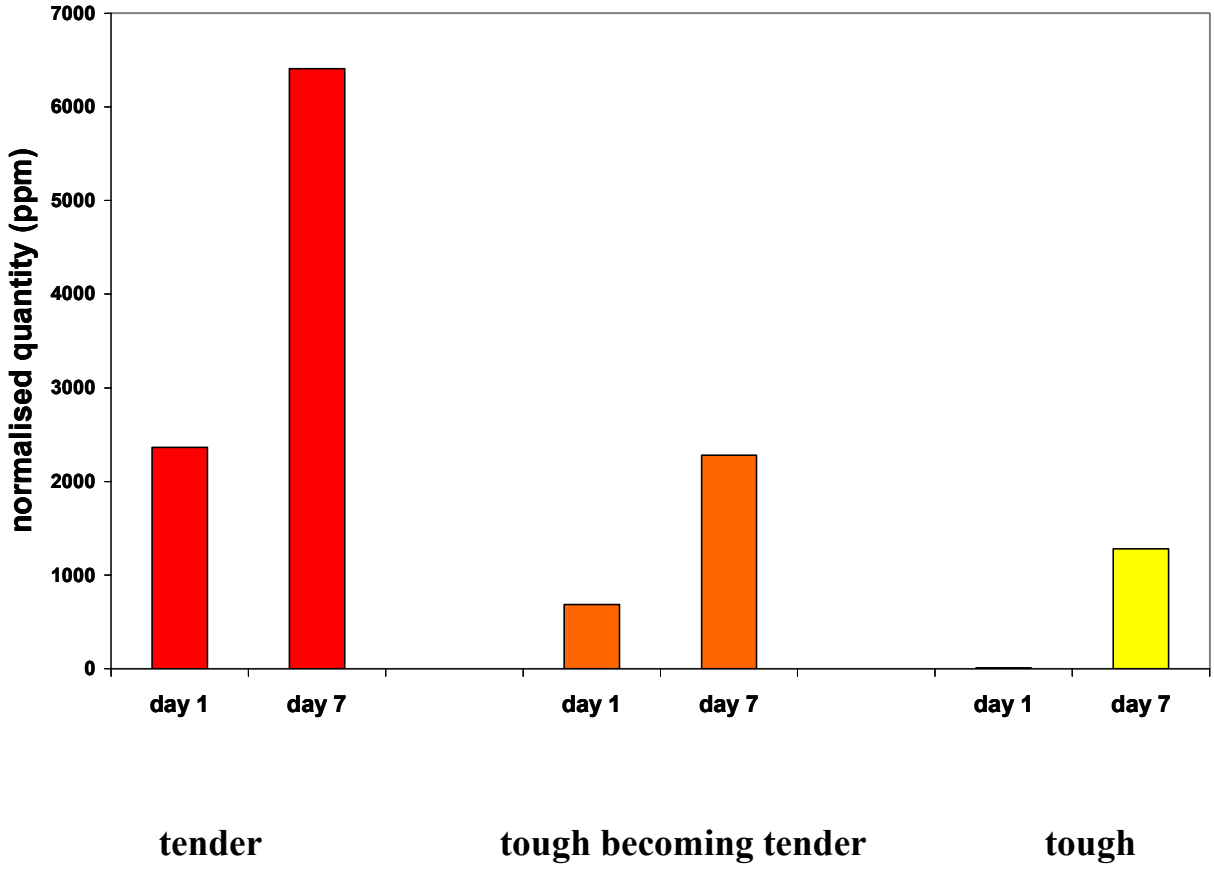

Figure 3. Normalised quantity (ppm) of spot 2504 in the three samples, as affected by *post mortem* time

DIFFERENCE IN DEGRADATION OF TROPONIN-T ISOFORMS AMONG BOVINE MUSCLES DURING POSTMORTEM AGING

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Background

The beef tenderization during postmortem aging, largely affected by protein degradation, differs among the muscles (Olson *et al.*, 1976; Koohmaraie *et al.*, 1988). Though the activities of Ca^{2+}-dependent protease (calpains) and the inhibitor (calpastatin) vary among different type muscles, the ratio of calpain/calpastatin does not seem to be different among the muscles examined (Olson *et al.*, 1977; Koohmaraie *et al.*, 1988; Whipple and Koohmaraie, 1992). Each muscle has specific composition of four muscle fiber types which are determined by expression pattern of myofibrillar contractile protein isoforms, such as myosin heavy chains (MyHCs) (Schiaffino and Reggiani, 1996). The extent and rate of postmortem degradation of myofibrillar proteins are presumptively affected by muscle fiber type. Olson *et al.* (1976) and Whipple and Koohmaraie (1992) reported that degradation of myofibril proteins occurs faster in bovine white (faster type) muscles (longissimus, biceps femoris, semitendinosus, or gluteus medius) than in the red (slower type) muscles (psoas major or supraspinatus). In a detail investigation of pork aging by Christensen *et al.* (2004), the extent and rate of troponin T (TnT) and desmin degradation did not depend solely on muscle fiber type determined by MyHC ATPase histochemistry. Different type muscles have different isoform composition of myofibrillar contractile proteins. The degradation pattern may be changed by the type of the isoforms, because the cleavage pattern of the isoform proteins are thought to differ, depending on the molecular structures. Actually, four recombinant isoforms of rabbit fast type TnT (fTnT) are degraded by calpain at different rates in vitro (Gomes *et al.*, 2001). Furthermore, we found that fast and slow type TnT isoforms are differently degraded in postmortem bovine LT muscle (Muroya *et al.*, 2004).

Objectives

The purpose of this study is to clarify the relationship among protein degradation patterns of muscles and the isoforms types of the protein substrates. To this end, we investigated the difference in degradation pattern of TnT isoforms among muscles with different isoform composition during beef aging.

Materials and methods

The lingual (TN), masseter (MS), diaphragm (DP), longissimus thoracis (LT) muscles for 0 d postmortem samples were excised from a Japanese black steer within 1 hr after slaughter. After overnight hanging of the carcass at 2°C, the muscle blocks were excised from the carcass. At 1, 5, and 14 d postmortem, the muscle samples were prepared from the central part of the block bagged during aging at 2°C. For western blotting analysis, myofibrils were extracted from the muscles by 0.1 M potassium phosphate buffer (pH 7.4) with 0.6 M KCl. The myofibrils were applied to SDS-PAGE using 12.5% or 15% gel, followed by transfer to polyvinylidenefluoride membrane. In the western blotting, the TnT bands were detected by using commercially-available anti fast- or slow-type polyclonal antibodies for TnT (Santa Cruz Biotechnology, Santa Cruz, CA).

Results and discussion

According to our previous analysis investigating MyHC and TnT isoform expression in bovine skeletal muscles by RT-PCR, MS and DP expressed slower type MyHC and TnT isoforms, while TN and LT expressed the faster type isoforms (Muroya *et al.*, 2002; Muroya *et al.*, 2003). According to the MyHC isoform expression pattern, TN is characterised to be slower than LT. Especially, MS exclusively expressed the slow type isoform mRNAs, which agrees with the present results of isoform-specific TnT western blotting (Figs. 1 and 2).

Eight fast- and two slow-type TnT (sTnT) isoform mRNAs were detected by RT-PCR, and the distribution of the isoforms varies among the muscles (Muroya *et al.*, 2003). The fTnT isoforms are classified into four types in SDS-PAGE by the amino acid numbers, or approximate molecular weight. The four fTnT isoform types were detected in bovine TN, MS, DP, and LT muscles, with different distribution among the muscles (Fig. 1). The DP and TN muscles expressed two sTnT isoforms, while MS and LT muscles expressed only a higher molecular weight one (Fig. 2), as we detected by RT-PCR previously.

The degradation pattern of LT muscle revealed that fTnT and sTnT isoforms are degraded differently from each other (Figs. 1 and 2). On the other hand, even though two high molecular weight fTnT and one sTnT isoforms in DP and TN muscles were the same as those expressed in LT, all of the isoforms in DP and TN did not appear to be degraded. The sTnT isoform in MS muscle appeared to be slightly degraded.

The results revealed that postmortem TnT degradation in beef varies depending on the muscle type. The degradation was observed only in LT muscle that has faster contractile properties than MS, DP, and TN muscles, suggesting that TnT proteins are degraded more easily in faster muscles than in slower muscles. This is consistent with the previous results reported by Olson *et al.* (1976) and Whipple and Koohmaraie (1992). There might be differences in activity of calpain or calpastatin and in some metabolic effect among the muscles examined. Though fTnT and sTnT isoforms are differently degraded, the difference in the rate and extent of TnT degradation among fTnT or sTnT isoforms during postmortem aging in beef is still unknown.

Conclusions

The results revealed that postmortem TnT degradation in beef differs depending on the muscle type. The degradation was observed only in LT muscle that has faster contractile properties than MS, DP, and TN muscles, suggesting that TnT proteins are degraded more easily in faster muscles than in slower muscles.

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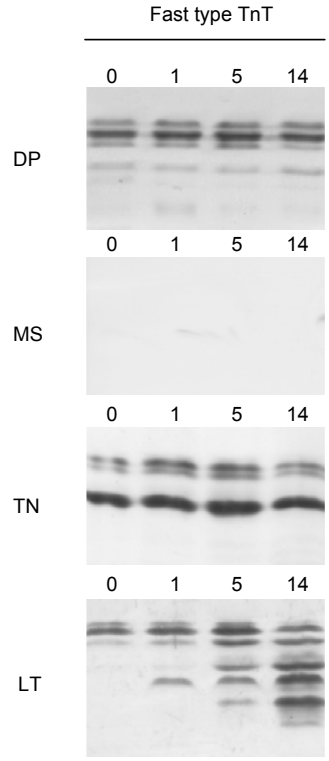

Fig. 1. Postmortem degradation pattern of fast type troponin T isoforms. Numbers indicate day postmortem. TN: lingual muscles, MS: masseter, DP: diaphragm, LT: longissimus thoracis.

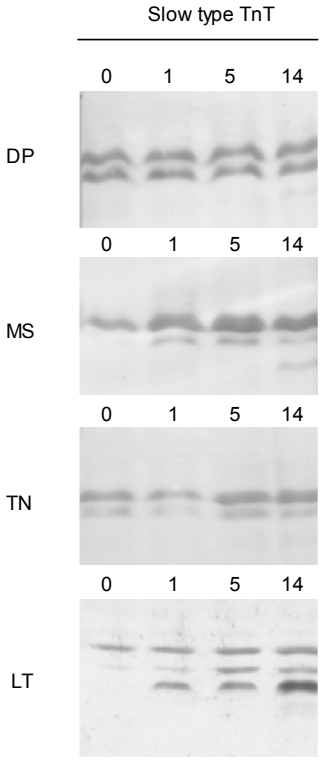

Fig. 2. Postmortem degradation pattern of slow type troponin T isoforms. Numbers indicate day postmortem. TN: lingual muscles, MS: masseter, DP: diaphragm, LT: longissimus thoracis.

SPECIES-SPECIFIC DIFFERENCE IN INDUCING PIG ADIPOSE CONVERSION FROM THAT OF MOUSE

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Background

Subcutaneous adipose tissue in pigs represents a major source of both cost inefficiency and consumer concerns. Numerous investigations have been carried out to regulate the carcass fat for the meat quality traits through both nutritional controls and selective breeding. However, there are some difficulties to monitor adipose development in vivo studies, due to the complicated interactions of all kinds of neural and hormonal signals involved in adipocyte metabolism. Therefore, investigators have established in vitro cell culture systems as useful tools for studying adipose development.

Much of the knowledge about cellular and molecular events accompanying adipose conversion in recent years has been based on murine preadipocyte cell lines. Since no cell lines from porcine adipose tissue have been established so far, understanding the control of adipocyte differentiation in pigs is limited in either the approach of using these mouse cell lines or otherwise, primary culture systems. As a result, it can always come into question whether the data from mouse cell lines and pig primary stromal-vascular cells with cellular heterogeneity in its population, can represent the characteristics of pig adipocytes.

Objectives

We have established a preadipocyte clonal line from porcine subcutaneous tissue (PSPA) for the study of pig adipose development (Nakajima et al., 2003). The present study was designed to demonstrate whether there are species specificity in adipose conversion between mouse and pigs, by comparing the most widely studied mouse 3T3-L1 cell line and PSPA cells.

Materials and methods

Cell culture: A clonal porcine subcutaneous preadipocyte (PSPA) cell line was maintained in the preadipocyte condition by cultivation in Dulbecco's modified Eagle's medium (DMEM, 1 g/L glucose) supplemented with 10% fetal bovine serum, 1000 IU/ml penicillin, and 1 mg/ml streptomycin. Cells were passaged every 4 days, with the density of inoculation kept constant (1×10^4cells/cm^2).

For experiments, cells were plated at 2.1×10^4cells/cm^2 to obtain confluency within 3 days. After reaching confluence (day 0), adipose conversion was induced in high-glucose (4.5 g/L) DMEM containing 10% FBS, 5 μg/ml insulin, 0.25 μM dexamethasone, 33 μM biotin, 17 μM pantothenate, 5 mM octanoate. The medium was changed every other day and the cells were allowed to differentiate for 10 more days.

The mouse embryo 3T3-L1 cells were plated at a density of 0.5×10^4cells/cm^2 in growth medium for 3 days. At confluence, the medium was then shifted to high-glucose DMEM supplemented with 10% FBS, 0.5 mM of 1-methyl-3-isobutylxanthine (MIX), and 0.25 μM dexamethasone, to induce differentiation. Forty-eight hours later, this medium was replaced with 10% FBS high-glucose DMEM containing 5 μg/ml insulin for the remaining 8 days.

Triglyceride Assay: Triglyceride (TG) in the cell lysate was extracted with chloroform-methanol and quantified enzymatically using a Triglyceride G Test Wako Kit.

Extraction of mRNA and RT-PCR analysis: Messenger RNAs were isolated from PSPA cells using the QuickPrep Micro mRNA Purification Kit and then reverse transcribed using the First-Strand cDNA Synthesis Kit. The synthesized cDNA was amplified with AmpliTaq Gold by PCR using paired forward and reverse primers with the ribosomal protein L7 (RPL7) as the internal control (Venuti et al., 1995). Primer sequences were as follows: peroxisome proliferator-activated receptor (PPAR) γ2 (GGTGAAACTCTGGGA GATTCTCTTA, GGCTCTTCGTGAGGTTTGTTGTACAG); PPARγ1 (CCTTAAACGAAGAGTCATCTT TTAGCG, GGCTCTTCGTGAGGTTTGTTGTACAG); CCAAT element-binding protein α (C/EBPα, AAG TCGGTGGACAAGAACAGCAACGAGTA, ATTGTCACTGGTCAGCTCCAGCACCTT); adenovirus E2 promoter-binding factor 1 (E2F1, TGGACCTGGAAACTGACCATCAGTACCT, TCTTGGACTTCTTGGC

AATGAGCTGGATG); proliferating cell number antigen (PCNA, CTGGTGAATTTGCACGTATATGCCG AG, AGGGGTACATCTGCAGACATACTGAGTGT); thymidine kinase (TK, TCGGACCCATGTTCTCG GGAAAAAGT, ACTCCACGATGTCAGGGAAAAACTG); lipoprotein lipase (LPL, CATAGCAGCAAA ACCTTTGTGGTGATCC, TTGGTCAGACTTCCTGCAATGCCAGCA); adipocyte-specific fatty acid binding protein (aP2, TTTGCTACCAGGAAAGTGGCTGGCAT, GCAGTGACACCATTCATGACACAT TCC); stearoyl CoA desaturase 1 (SCD1, ACCGTGCCCACCACAAGTTTTCAGAAA, GCTCCAAGTGA AACCAGGATATTCTC); hormone-senstive lipase (HSL, CGCAGTGTGTCTGAAGCAGCACTGGC, AT GACCGAGTCGTCCAGCATGGGGTC); RPL7 (GCAGAACCCAAATTGGCGTTTGTCATCAG, GATG ATGCCGTATTTACCAAGAGATCGAGC).

Western blot analysis: Both nuclear proteins and cytoplasmic proteins were prepared by using the CelLytic NuCLEAR Extraction Kit. Twenty-five micrograms of nuclear protein and 50 μg of cytoplasmic protein were separated by electrophoresis through SDS-polyacrylamide 12.5% gels. After they were electrotransferred onto nitrocellulose membranes, blotting membranes were incubated with primary polyclonal antibodies specific to PPARγ, C/EBPα, PCNA, E2F1, and glycerol-3-phosphate dehydrogenase (GPDH). Secondary antibodies were horseradish perocidase-conjugated anti-rabbit, anti-mouse or anti-goat antibodies. Antigen-antibody complexes were visualized by the ECL detection system.

Results and discussion

When confluent PSPA cells were stimulated with insulin, dexamethasone biotin, pantothenate, and octanoate, growth was arrested, and the cells exhibited a marked increase in lipogenesis. However, adipose conversion was not induced upon exposure of PSPA cells to a standard hormonal mixture of 3T3-L1 cells (Student et al., 1980), and their cell numbers increased as did the preadipocytes in growth medium (Fig.1A). Also, some differences in cell behavior were observed between PSPA cells and 3T3-L1 cells (Fig.1A and B).

To investigate whether every component of the PSPA medium was equally essential to the induction of adipocytes, PSPA cells were exposed to differentiation media each of which was lacking one agent. Under these culture conditions, the absence of either octanoate or dexamethasone from the medium resulted in a marked decrease in lipid accumulation (data not shown). Furthermore, octanoate was the only factor able to induce growth arrest. Octanoate supplementation to 3T3-L1 medium dramatically improved TG accumulation of PSPA cells while accompanying growth arrest (data not shown). These data strongly suggested a correlation between the growth-inhibiting effect of octanoate and terminal differentiation. Additionally, based on the result of a dexamethasone-depleted medium in which the cell numbers were maintained as normal adipocytes, it appears that dexamethasone regulates PSPA preadipocyte differentiation through a different induction pathway from octanoate.

The expression of a number of adipogenic genes and proteins was studied by RT-PCR and Western blot analysis. Expression patterns of E2F1, PCNA and TK were consistent with the results indicated so far by means of cell numbers, and expression patterns of LPL, aP2, SCD1 and HSL consistent with TG content. The most interesting result was that the expression of those master regulators of adipocyte gene transcription, PPARγ2 and C/EBPα. The absence of octanoate from the differentiation medium expressed only PPARγ2 but not C/EBPα, and in contrast, removal of dexamethasone resulted in expression of C/EBPα but not PPARγ2 (Fig.2).

Conclusions

Species specificity in adipose conversion between mouse and pig preadipocytes has been shown as the responsiveness to inducers was not equal between these two. Hormonal cocktail of 3T3-L1 cells failed to induce adipose conversion of PSPA cells and kept on proliferating. Growth arrest by octanoate was required for PSPA cells to enter terminal differentiation. Our results can probably provide an answer as to why researchers often prefer serum-free conditions in porcine primary cultures instead of serum-containing medium (Hentges and Hausman, 1989; Surywan and Hu, 1993; Gerfault et al., 1999). This was because none of the agents added according to mouse reports were sufficient to make preadipocytes cease dividing, whereas serum-free medium readily did so. We propose that mouse is not suitable for the study of porcine adipose development.

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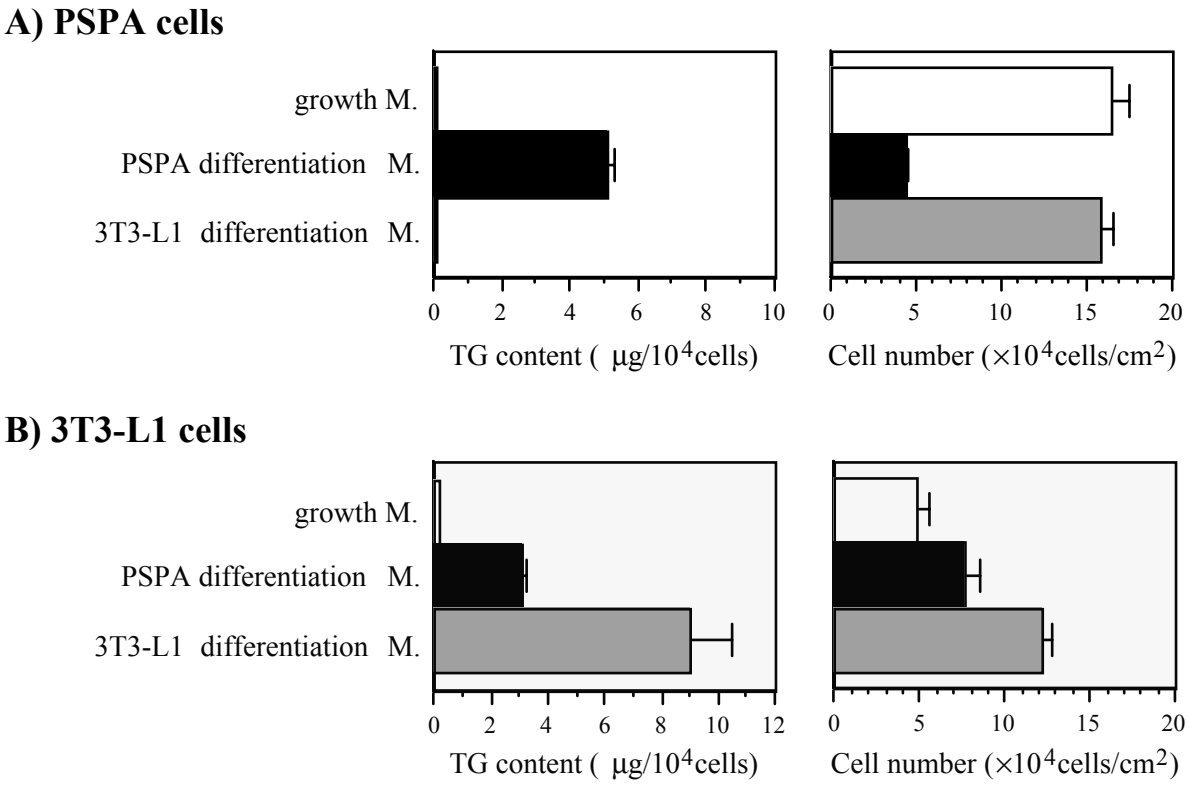

Fig. 1. TG accumulation and cell number of (A) PSPA cells and (B) 3T3-L1.

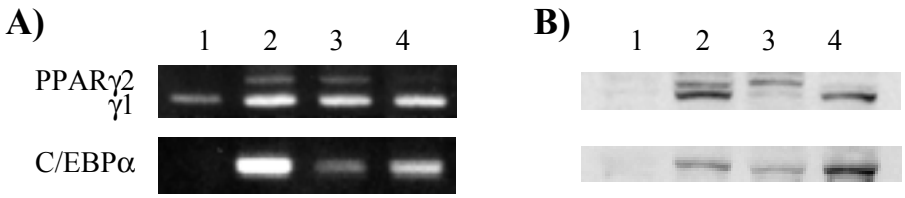

Fig. 2. Effect of octanoate and dexamethasone on the induction of adipocyte-specific transcription factors. (A) RT-PCR and (B) Western blot analysis. 1: growth medium, 2: PSPA differentiation medium, 3: octanoate-depleted medium, 4: dexamethasone-depleted medium.

INTERMUSCULAR VARIATION IN DEGRADATION OF HIGH MOLECULAR WEIGHT PROTEINS IN BOVINE MUSCLES DURING POSTMORTEM AGING

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Background

Beef tenderisation during postmortem aging differs among muscles in an individual carcass (Olson et al., 1976; Koohmaraie et al., 1988; Ilian et al., 2001). Although the sequence of postmortem metabolic events that indicate final meat tenderness remains unexplained, research has implicated the involvement of protein degradation. High molecular weight proteins, such as titin (□3,000 kDa) and nebulin (□800 kDa), are unique in size and play important roles in maintaining muscle structure, thus there is potential for degradation of these proteins to impact on meat tenderisation (Taylor et al., 1995; Huff-Lonergan et al., 1996).

The activities of Ca^{2+}-dependent proteases (calpains) and the inhibitor (calpastatin) are suggested to play an important role in protein degradation (Koohmaraie et al., 1988; Whipple and Koohmaraie, 1992; Ilian et al., 2001). Recently, the novel calpain 3 (94 kDa) isoform has been of particular interest as not only it is expressed almost exclusively in skeletal muscle, at least two binding sites for this protease are located on the titin molecule (Sorimachi et al., 1995). The expression of calpains 1, 2, and 3, and calpastatin have been shown to vary between muscles of different fibre type (Ilian et al., 2001; Jones et al., 1999). Furthermore, muscle fibre type has been suggested to have an effect on the extent and rate of postmortem degradation. Skeletal muscles can be characterized into fast and slow muscles according to the distribution of four muscle fibre types, which contract at different rates according to the expression pattern of myofibrillar protein isoforms, such as myosin heavy chains (Schiaffino and Reggiani, 1996). The degradation of myofibril proteins has been reported to occur faster in bovine white (faster) muscles, such as longissimus thoracis (LT) and semitendinosus (ST), than in red (slower) muscles, such as psoas major (PM) (Olson et al., 1976). There appears to be a complex relationship between protein degradation, muscle fibre type, and the calpain system, and their ultimate effect on meat tenderness.

Objectives

The objective of this study was to determine the effect of degradation of high molecular weight proteins on the rate of tenderisation among four bovine muscles during postmortem aging.

Materials and methods

The diaphragm (DP), psoas major (PM), longissimus thoracis (LT), and semitendinosus (ST) muscles for 0 d postmortem samples were excised from a Japanese Black steer within 1 h after slaughter. After overnight hanging of the carcass at 2˚C, the muscle blocks were excised from the carcass, bagged and aged at 2˚C. At 1, 3, 7, and 14 d postmortem, muscle samples were prepared for immediate Warner Bratzler shear force measurement (kg/cm^2), along with electrophoresis samples that were frozen at -40˚C until analysis.

For SDS-PAGE analysis, crude myofibrils were extracted from the muscles by 0.1 M potassium phosphate buffer (pH 6.8) with 0.6 M KCl and protease inhibitor cocktail (SIGMA, Saint Louis, Missouri), and analysed on the same day to avoid degradation. Myofibrils were applied to SDS-PAGE using a 3% gel without stacking gel (ATTO, Tokyo, Japan), and run at room temperature for 1 h at a constant current of 10 mA, followed by 1 h at a constant current of 20 mA. Protein bands were detected by fluorescent stain using SYPRO Ruby gel stain (Bio-rad, Hercules, CA) for 16 h overnight.

Results and discussion

There was intermuscular variation in the rate of tenderisation, as shown in Figure 1. Shear force measurement showed a faster rate of tenderisation in LT compared to ST, with PM and DP being intermediate. Although published data could not be found on the tenderisation of the diaphragm, the trend in

the rate of tenderisation of LT being higher than ST and PM is consistent with previous research (Koohmaraie et al., 1988; Ilian et al., 2001).

Figure 2 shows the degradation pattern of titin and nebulin among the four muscles. The 1,200 kDa degradation product of titin is evident in Figure 2, however T1 and T2 could not be clearly distinguished, therefore the intermuscular variation in degradation of titin could not be observed in this experiment. There was a difference in degradation of nebulin among the muscles, as intact nebulin was absent by 3 d of postmortem aging in LT compared to by 7 d in ST. The degradation of nebulin in PM and DP was observed after 1 d postmortem.

Degradation of two unidentified high molecular weight proteins was evident soon after death in this study, as can be seen in Figure 3 (band *x* and band *y*). Band *x* was present at 0 d in LT and ST muscle, and was absent by 3 d in LT and by 7 d in ST, which is a similar degradation pattern to that of nebulin in the LT and ST muscles. Band *x* was not detected in PM or DP muscles. The detection of the degradation of band *x* was due to the highly sensitive fluorescent staining, as compared to Coomassie brilliant blue which has been conventionally used in previous studies (Fritz et al., 1993; Taylor et al., 1995; Huff-Lonergan et al., 1996). Band *y* appears to be filamin, which was shown to degrade to a 240 kDa fragment (Huff-Lonergan et al., 1996). In this experiment, band *y* degradation was observed to occur from 3 d in LT, and from 7 d in ST and PM, however band *y* was not degraded in DP. In addition, a degradation fragment migrating slower than band *x* was observed from 7 d of postmortem aging in PM, with trace amounts evident also from 7 d in LT, but was not detected in ST or DP, as evident in Figure 3. This fragment may be a product of titin degradation (Taylor et al., 1995).

The degradation of myofibril proteins has been reported to occur faster in bovine white (faster) muscles, such as LT and ST, than in red (slower) muscles, such as PM (Olson et al., 1976). However, in this study, the protein degradation patterns and meat tenderisation of LT and ST were different. LT exhibited a higher postmortem tenderisation rate, and in addition, the degradation of nebulin, band *x*, and band *y* was faster in the LT muscle compared to ST. The lower tenderisation rate in ST may have been the result of relatively slower degradation of nebulin, band *x*, and band *y*. These differences may be due to intermuscular variation in the expression of calpains and calpastatin. According to Ilian et al (2001), the higher relative tenderisation rate in LT compared to ST (in ovine) and in LT compared to PM (in bovine) was due to a higher level of expression of calpain 3 in LT. These results suggest that, other than muscle fibre type, the relative proportion of calpains and calpastatin in different muscles may also influence meat tenderisation.

The diaphragm is classified as a slower type muscle (Muroya et al., 2002), and has been shown to express slow type isoforms which have different degradation patterns to fast type isoforms (unpublished data). Nebulin degradation was observed in the diaphragm, however band *x* was not present and there was no degradation of band *y*. The data suggests that the degradation of myofibrillar proteins differs among the isoform types, due to the different susceptibility to proteolysis. Thus, the difference in tenderisation rate between the diaphragm and LT may indicate that muscles with different isoform composition are tenderised at different rates.

Conclusions

The results revealed that postmortem degradation of high molecular weight proteins varies among bovine muscles of the same carcass. There appears to be a relationship between the degradation of high molecular weight proteins and meat tenderisation, with the possibility of other influencial factors such as different expression levels of calpains and calpastatin among the muscles.

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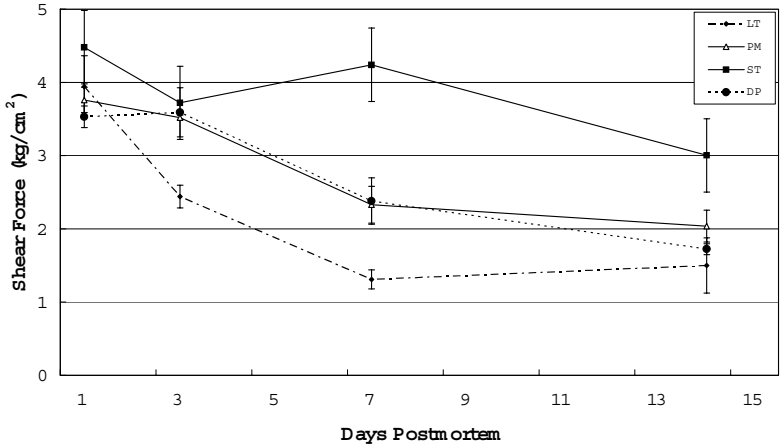

Figure 1. Shear force (kg/cm^2) of bovine longissimus (LT), semitendinosus (ST), psoas major (PM) and diaphragm (DP) at 1, 3, 7 and 14 days postmortem.

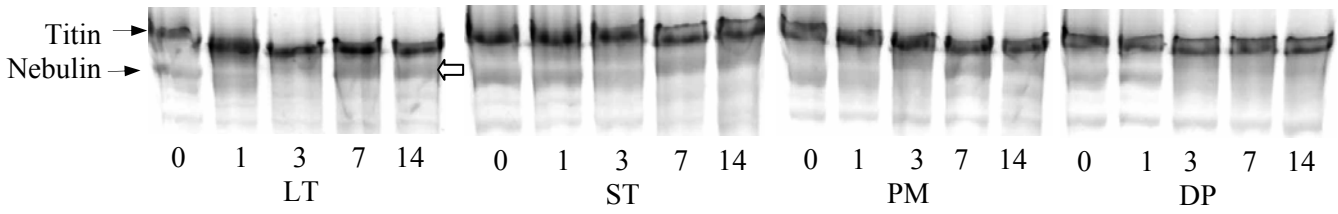

Figure 2. Postmortem degradation pattern of titin and nebulin. Numbers indicate days postmortem. LT: longissimus thoracis, ST: semitendinosus, PM: psoas major, DP: diaphragm. Open arrow designates the position of the titin 1,200 kDa fragment.

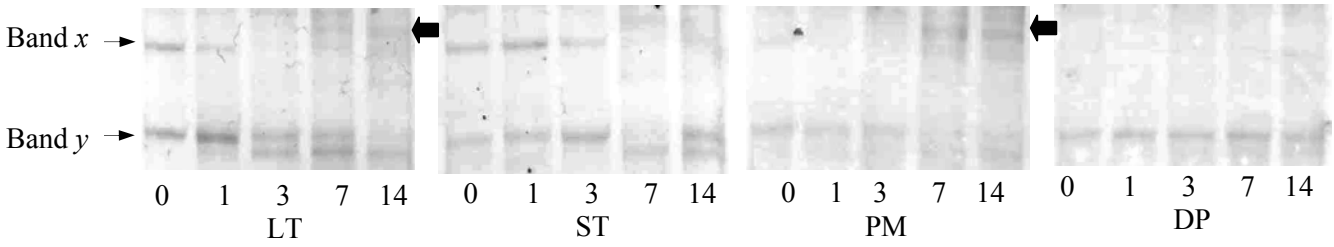

Figure 3. Postmortem degradation pattern of band *x* and band *y*. Numbers indicate days postmortem. LT: longissimus thoracis, ST: semitendinosus, PM: psoas major, DP: diaphragm. Closed arrow indicates degradation fragment, migrating slower than band *x*.

EFFECT OF TRANSPORT, LAIRAGE, AND PRESLAUGHTER STRESS ON PORK MEAT QUALITY

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Background

Preslaughter handling of pigs, including stockman interaction (loading, unloading, driving animals), transport, and lairage, has been designed to meet the pork industry's requirements rather than the pig's needs. Various preslaughter handling such as transport (Warris and Bevis, 1986), lairage (Brown et al, 1999), and preslaughter stress (Hambrecht et al., 2004a) can trigger a stress response and may have negative consequences for pork quality aspects such as color, water-holding capacity, and other sensory characteristics. Warris (1998) and Gispert et al. (2000) found that pigs transported for long periods of time are more likely to form dark-firm-dry pork because of exhaustion and fatigue. Bradshaw et al. (1996) found that short (≤1h) compared with long transports were more detrimental because the pigs did not recover from the stress of loading and could not adapt to the conditions of transport. The effect of lairage duration depends on factors such as environmental temperature and mixing. Aaslyng and Gade (2001) found that a lairage time of less than one hour does not allow for sufficient resting and may negatively impact both animal welfare and meat quality. However, extended lairage times of six hours and more may increase carcass damage and stress caused by fighting and prolonged periods without feed (Warriss, 1998). The negative factors associated with transport, lairage, and preslaughter stress increase the chances of economic losses to producers and packers.

Objectives

The objective of this experiment was to investigate to what extent various important preslaughter handling factors such as transport conditions, lairage duration and stress level immediately before slaughter affect muscle energy, blood-based stress indicators, and ultimate pork quality.

Materials and methods

All pigs were commercial halothane-free progeny of the Hypor pig breeding company. Pigs (n = 384) were assigned to one of eight treatments in a 2 x 2 x 2 factorial arrangement, with two types of transport [short (50 min) and smooth or long (3 h) and rough], two lairage durations [long (3 h; considered as optimal) or short (<45 min; considered as sub-optimal)] and two stress levels administered immediately before slaughter (minimal or high). Pigs were penned 12 pigs per pen at the production site. For each treatment, groups of four random pigs were removed (four pigs taken from each of 3 pens to form groups of 12) from their home pens on the production site and randomly allocated to either minimal or high preslaughter stress and either short and smooth or long and rough transport. Long and short lairage alternated between consecutive weeks. Transport duration and preslaugher stress levels were varied within the same slaughter day. Eight groups of 48 pigs were processed over a series of weeks at a commercial plant. Pigs were electrically stunned in a fully automated, head-to-heart stunning system. Blood samples were collected in a heparinized tube at exanguination for cortisol and lactate determination. A 1-g muscle sample was taken at 135 min postmortem from the longissimus muscle (LM) at the last rib and frozen in liquid nitrogen for determination of the glycolitic potential, a good approximation of in vivo muscle glycogen. At 30 min, 3 h and 24 h postmortem temperature and pH were measured in the LM adjacent to the third lumbar vertebra**.** At 23 h postmortem loins were harvested for final meat quality measurements. L* (lightness), a* (redness), and b*(yellowness) color values were assessed in the LM on a freshly cut surface after a 10 minute blooming period with a Minolta Portable Chroma Meter (Model CR 210**).** Electical conductivity was measured using the LF-Star. Water-holding capacity was measured by pressing a filter paper on a freshly cut surface and measuring the amount of absorbed moisture after 10 sec. Additionally, drip loss was determined as percentage of weight lost from a LM slice after 1, 3, and 7 d of storage lying face down on a metal grid in a closed plastic container. Data were analyzed by the mixed-model procedure (PROC MIXED) of SAS. Tests of multiple comparisons of least squares means were adjusted according to the TUKEY-KRAMER method to ensure the

overall significance level of $P < 0.05$. The model applied included the fixed effects of transport conditions, lairage duration, and stressor level, as well as their 2-way interactions, and the random effect of slaughter day nested within lairage.

Results and discussion

Stress indicators in blood

Results are presented in Table 1 and 2. High preslaughter stress increased ($P < 0.05$) plasma lactate and cortisol concentrations. The increase in plasma lactate concentrations seemed to be larger after both long vs. short transport (transport x stress interaction; $P < 0.05$) and after the short vs. long lairage (lairage x stress interaction; $P < 0.05$). Cortisol levels were increased after short transport only when followed by short lairage (transport x lairage interaction; $P < 0.05$). Lairage is meant to serve as a period of recovery for animals after transport. The larger increases in plasma cortisol and lactate in response to high stress indicate that both the long transport and the short lairage treatment were unfavorable and made the pigs more susceptible to subsequent stress. Moreover, the increase in cortisol concentration after short transport in combination with short lairage was probably due to a lack of recovery time in lairage from the stressful experience of loading, transport, and unloading. Results of the present expirement are in agreement with studies that showed that rough vs. smooth transport (Bradshaw et al., 1996), short vs. long lairage (Perez et al., 2002) and high vs. minimal stress (Hambrecht et al., 2004a) caused stress in pigs as indicated by elevated blood-based stress indicators.

Muscle glycolitic potential and pork quality

Long transport increased ($P < 0.05$) LM glycolitic potential with a concomitantly increased muscle lactate production. This is in disagreement with Leheska et al. (2003) who found a significant muscle energy depletion in response to longer transports and indicates that the transport treatment in the present study was physically not exhausting. Lairage had no effect on muscle energy stores whereas high preslaughter stress increased ($P < 0.05$) LM lactate production but overall decreased ($P < 0.05$) muscle glycolitic potential and muscle glycogen.

Transport and lairage did not affect pH at 30 min postmortem. At 3 h postmortem, the slightly decreased muscle glycogen and glycolitic potential after short compared with long lairage ($P < 0.05$) were reflected in a higher pH. The pH values seemed to be more increased when short lairage was combined with long transport (transport x lairage interaction; $P < 0.05$). Ultimate pH, however, was not affected by transport conditions or lairage duration. Similar to cortisol concentrations, LM temperature at 40 min was increased ($P < 0.05$) for the short transport group only when followed by short lairage. In agreement with the increased rate of lactate production, high stress decreased ($P < 0.05$) muscle pH at 30 min and 3 h postmortem. Corresponding to the lower glycolitic potentials, ultimate pH was increased ($P < 0.05$) in the high stress group. However, ultimate pH was still within the normal range of 5.3 to 5.7 (Briskey, 1964). At 3 h postmortem, high stress resulted in an increased LM temperature, but this increase was larger after long lairage (lairage x stress interaction; $P < 0.05$). This finding is difficult to explain since a similar effect was not found at 40 min postmortem. However, it is possible that long lairage allowed the pigs to relax before stress shock occured, or toward the end of long lairage pigs began to fight (resulting in an increased body temperature) and combined with the high stress, fueled the increased in muscle temperature.

High preslaughter stress increased ($P < 0.05$) filter paper moisture uptake, electrical conductivity and drip loss throughout the entire 7 day storage period. This is in agreement with the elevated muscle temperature and lower pH values that are known to be related to inferior pork quality (Cassens et al., 1963; Bowker et al., 2000). Moreover, other experiments studying the effect of high preslaughter stress on pork quality confirm the detrimental effects of stress on pork quality (van der Wal et al., 1999; Hambrecht et al., 2004a). However, despite the influence of preslaughter stress on postmortem pH, lightness and redness were not affected ($P < 0.05$). Long lairage resulted in an increased ($P < 0.05$) paleness (i.e. high L* values) in loins.

Conclusions

Preslaughter stress exerted the largest influence on animal welfare and ultimate pork quality. Therefore, it is imperative that slaughterhouse employees are educated with regard to proper, low stress animal handling procedures. Furthermore, slaughter plant design should be considered as an important tool in improving pork quality and animal welfare.

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Table 1. Effect of transport (T), lairage (L), and preslaughter stress (S) on stress indicators, muscle metabolites, pH and temperature as well as water-holding capacity and color in the longissimus muscle[a].

	Transport		Lairage		Stress			P-Value		
Item	Short	Long	Long	Short	Min.	High	Pooled SE	T	L	S
No. Animals	174	184	179	179	176	181	-	-	-	-
Blood										
Cortisol, ng/mL	67.5	65.5	61.4	71.7	55.1	77.9	4.28	0.434	0.201	0.001
Lactate, mmol/L	23.8	24.1	24.4	23.5	17.1	30.9	0.61	0.617	0.401	0.001
Muscle/meat										
Glycolitic potential, µmol/g	129.4	132.5	132.2	129.7	134.1	127.8	1.45	0.060	0.304	0.001
Lactate, µmol/g	70.2	74.3	72.0	72.6	58.1	86.4	1.08	0.008	0.736	0.001
Glycogen, µmol/g	29.6	29.1	30.1	28.6	38.0	20.7	0.77	0.621	0.155	0.001
pH										
30 min	6.51	6.46	6.49	6.48	6.64	6.33	0.027	0.079	0.740	0.001

Table 1. Continued

	Transport		Lairage		Stress			P-Value		
Item	Short	Long	Long	Short	Min.	High	Pooled SE	T	L	S
No. Animals	174	184	179	179	176	181	-	-	-	-
pH										
3 h	6.10	6.05	6.01	6.14	6.23	5.92	0.050	0.094	0.202	0.001
24 h (ultimate)	5.55	5.55	5.54	5.57	5.52	5.58	0.019	0.883	0.482	0.001
Temperature										
40 min	39.4	39.1	39.0	39.4	38.6	39.8	0.27	0.039	0.427	0.001
3 h	21.2	21.3	21.2	21.3	20.6	21.9	0.34	0.760	0.967	0.001
Electrical conductivity	8.0	8.5	7.9	8.6	6.3	10.2	0.64	0.083	0.536	0.001
Filter-paper moisture	67	65	67	65	53	80	3.6	0.437	0.727	0.001
Drip loss, %										
Day 1	1.96	2.01	1.89	2.08	1.25	2.73	0.12	0.757	0.331	0.001
Day 3	4.34	4.44	4.27	4.52	3.32	5.46	0.27	0.593	0.598	0.001
Day 7	6.19	6.30	6.01	6.48	5.21	7.27	0.24	0.575	0.277	0.001
L* value	53.9	53.7	54.3	53.3	53.9	53.7	0.23	0.529	0.029	0.494
a* value	19.4	19.1	19.4	19.1	19.2	19.3	0.11	0.008	0.141	0.411
b* value	5.5	5.3	5.5	5.2	5.5	5.2	0.10	0.018	0.100	0.001

[a]Data in the table are presented as least squares means.

Table 2. Significant (P < 0.05) two-way interactions between transport (T), lairage (L), and stress (S) [a].

	Transport × Lairage				Transport × Stress				Lairage × Stress			
	ST[b]		LT[c]		ST[b]		LT[c]		LL[d]		SL[e]	
Blood	LL[d]	SL[e]	LL[d]	SL[e]	MS[f]	HS[g]	MS[f]	HS[g]	MS[f]	HS[g]	MS[f]	HS[g]
Cortisol ng/mL	58.8^{xy}	76.1^{y}	63.9^{xy}	67.2^{x}	-	-	-	-	-	-	-	-
Lactate mmol/mL	-	-	-	-	17.6^{x}	30.1^{y}	16.5^{x}	31.6^{y}	18.4^{x}	30.4^{y}	15.8^{x}	31.3^{y}
pH												
3 hours	6.07^{xy}	6.12^{y}	5.96^{x}	6.15^{y}	-	-	-	-	-	-	-	-
Temp.[h]												
40-min	39.0^{x}	39.7^{y}	39.1^{xy}	39.2^{xy}	-	-	-	-	-	-	-	-
3-h	-	-	-	-	-	-	-	-	20.3^{wx}	22.2^{yz}	20.8^{wy}	21.7^{xz}

[a]Data in the table are presented as least square means
[b]ST = short transport
[c]LT = long transport
[d]LL = long lairage
[e]SL = short lairage
[f]MS = minimal stress
[g]HS = high stress
[h]Temp .= Temperature measured in degrees celcius (Cº)
[wxyz]Least squares means, within a two-way interaction, lacking a common superscript letter differ (P < 0.05).

MEAT TENDERIZATION BY ACIDIC LISOSOMAL PROTEINASES ASSOCIATED WITH GENOTYPE IN BEEF

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Background

Tenderness is one of the most important quality traits of beef for consumers. It is well known that meat tenderizes during storage, mainly due to the breakdown of key myofibrillar proteins, however the physicochemical and biochemical mechanisms involved in this process are still unclear. The calcium-dependent calpain system has been indicated as the major responsible for tenderness development in the early *post-mortem* periods (Koohmaraie, 1994). Nevertheless, lysosomal cathepsins may be involved in later tenderization processes, when pH conditions are favourable for acidic enzymes (Christensen et al., 2004). Ouali (1992) suggested that meat tenderization may result from the synergistic action of both calpains and cathepsins.

Objectives

The aim of the present study was to analyze the evolution of instrumental toughness and cathepsin B, B+L, D and H activities during later *post-mortem* storage of beef from different genotypes. Cytosolic and lysosomal enzymatic activities were separately considered, in order to obtain information of the differential contribution of both compartments to tenderization processes.

Materials and methods

Twenty yearling bulls of two local breeds from northern Spain, Asturiana de los Valles (AV) and Asturiana de la Montaña (AM) were studied. Animals of the AV breed were homozygous (*mh*/*mh*), heterozygous (*mh*/+) or normal (+/+) for muscular hypertrophy gene. Bulls were fattened by feeding concentrate meal and barley straw *ad libitum* and were slaughtered at a live weight around 500 kg.
At 24h *post-mortem* the ultimate pH (pH24) of the *Longissimus dorsi* muscle (LD) was measured at the 5th rib level. The muscle was sliced, vacuum packed, aged at 4º C and stored at -20ºC for further analysis. Samples for toughness and enzymatic analyses were aged 7, 14 and 21 days (except samples of double-muscled (*mh*/*mh*) AV bulls which were aged 3, 7 and 14 days). Water holding capacity was estimated with two different methods: drip loss at 48h *post-mortem* (Honikel, 1998) and expressible juice (EJ) at 7 days ageing (Sierra, 1973). Warner-Bratzler (WB) shear force (kg) was measured on cooked meat in an Instron 1011 equipment. Enzyme assays were performed on lysosomal and cytosolic extracts, obtained according to Béchet et al. (1986). The cysteine proteinases B (EC3.4.22.1), B+L (L; EC.3.4.22.15) and H (EC.3.4.22.16) were assayed fluorimetrically (Barret, 1980, modified by Schreus et al., 1995) using different specific substrates. The aspartate proteinase cathepsin D (EC3.4.23.5) was assayed spectrophotometrically (Takahashi & Tang, 1981) with modifications (Schreus et al., 1995). Protein concentration was determined as described by Bradford (1976). Enzyme activities were expressed as enzymatic units/mg of protein.
An ANOVA was used to analyze the effect of genotype on the physico-chemical and enzymatic variables. The effect of ageing period on each genotype was analyzed by ANOVA with ageing time and animal as fixed factors. Differences between means were test by LSD procedure. Principal component analysis was made to describe the relationships between physico-chemical and enzymatic variables at 7 and 14 days of ageing. All the statistical analyses were performed using the SPSS programme 11.5 (2002).

Results and discussion

The genotype affected to the ultimate pH ($p<0.05$), which was significantly lower in meat of AV bulls with muscular hypertrophy (*mh*/*mh* or *mh*/+) than in AM bulls. This could be result of the higher proportion of fast-twitch fibers and the higher glycolytic metabolism in the LD muscle of double-muscled animals (Oliván

et al., 2004). Genotype affected also significantly to the drip loss of meat at 48h *post-mortem* (p<0,001), with meat of *mh*/*mh* AV bulls showing higher juice losses than any other genotype.

Table 1. Effect of genotype on pH24 and water holding capacity of meat.

	GENOTYPE				
	AV (*mh*/*mh*)	**AV (*mh*/+)**	**AV (+/+)**	**AM(+/+)**	**Sign.**
N	5	5	5	5	
pH24	5.48 a	5.53 a	5.58 ab	5.67 b	*
Drip loss (%)	2.68 a	1.18 b	1.49 b	0.81 b	***
EJ (%)	21.72 a	20.75 ab	20.35 ab	19.15 b	NS

Means in the same row followed by different letters are significantly different (p<0.05).

Figure 1(a) shows the tenderization pattern of the different genotypes during the ageing process. Meat of AV bulls homozygous (*mh*/*mh*) or heterozygous (*mh*/+) for muscular hypertrophy showed a higher tenderization rate than AV (+/+) or AM (+/+) bulls. This produced significant differences among genotypes in the WB shear force after long storage (14 and 21 days, Table 2), when meat of AM breed showed the highest toughness. This higher tenderization rate of meat from double-muscled bulls may be related with the lower ultimate pH recorded at 24h *post-mortem* and its positive influence on the activity of acidic enzymes, like cathepsins.

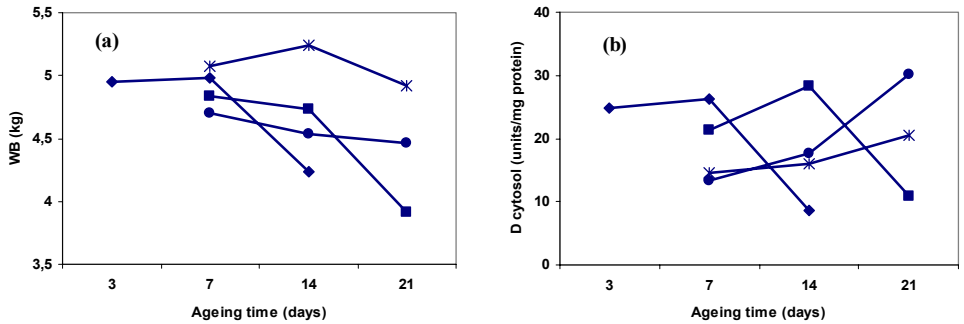

Figure 1. Time course changes in (a) WB shear force and (b) D cathepsin activity in cytosol extracts of the different genotypes (♦: *mh*/*mh* AV; ■: *mh*/+ AV, ●: +/+ AV; *: AM).

The evolution of the activity of cathepsins showed a similar pattern in lysosome and cytosolic domains, since they are complementary processes: the release of lysosomal proteases to cytosol and consistent breakdown of proteins by them produces an increase of endosomal vesicles, which interact to lysosomes giving them new substrates to act on (Alberts et al., 2002). In general enzyme activity levels were higher in cytosolic than in lysosomal extracts in all periods (Table 2), which could mean that the leakage of enzymes from lysosomes started in an early stage. The activity of cathepsins B, B+L, D and H at 7 days *post-mortem* was higher in meat of AV bulls with muscular hypertrophy (*mh*/*mh*, *mh*/+) than in other genotypes (AV(+/+), AM) in both extracts, but only reached significant differences in cytosolic space (for H cathepsin also in lysosomes). These data contribute to the hypothesis of faster tenderization rate of meat from double-muscled bulls, probably due to the lower pH conditions detected on the LD at early *post-mortem* periods, which activate processes of cellular membrane breakdown and enhance the release of the lysosomal proteases to the cytosol, as proposed by Berge et al. (2001). At later ageing the activity of most cathepsins (B, B+L and H) decreased both in lysosomal and cytosolic compartiments, probably due to the drop of availability of specific substrates, being this decrease more pronounced in double-muscled bulls than in other genotypes.

Cathepsin D showed the highest activity in both extracts and cathepsin H the lowest. That increase of cathepsin D along *post-mortem* ageing seems to be consequence of the absence of an endogenous inhibitor in muscular tissue specific for this enzyme. Rather, the role of cathepsin D as regulator of cystein-proteases could be responsible of the low activity of B, L and H cathepsins observed (Lenarcic et al., 1991), although it has been described that cystatin activity in the LD muscle is low (Koohmarie et al., 1988).

The activity of the D isoform in the cytosol showed different pattern in double-muscled (*mh*/*mh* and *mh*/+) and normal (AV(+/+) and AM) bulls (Fig. 1b). In animals with muscular hypertrophy the activity of cathepsin D was high at early and medium storage times (3-7 or 7-14 days, respectively) and showed a strong decrease after long storage (14 and 21 days respectively). However, in AV (+/+) and AM bulls there was a continuous increase of cathepsin D activity in the cytosol along the ageing period studied, which could

indicate an important implication of cathepsin D at the end of the tenderization process, that seems to be reached at longer ageing periods than those studied for these genotypes.
Figure 2 shows the relationship between the different parameters obtained through principal component (PC) analysis. Two groups of variables were clearly distinguished on the first PC. The first group included juice losses (drip loss and EJ) and high activity of all cathepsins (H, D, B and B+L) in the cytosol and in lysosomes at 7 days *post-mortem* This means that meat with high proteolytic activity at 7 days ageing exhibited higher juice losses, as a consequence of miofibrilar structure destabilization due to destruction of sarcomeres and fragmentation of actin-miosin complex. These variables were negatively correlated with the other group, composed by toughness (WB) at 14 days *post-mortem* and high enzyme activities in lysosomes (B, B+L H, D,) and cytosol (B, B+L, D) at 14 days.

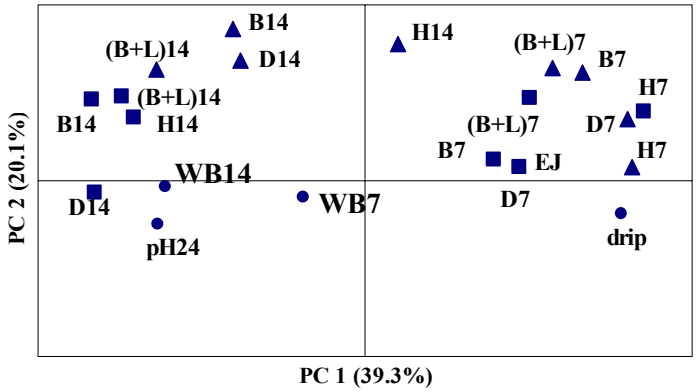

Figure 2. Principal component analysis of physico-chemical traits (●) and enzyme activities (■: in lysosome, ▲: in cytosol) at 7 and 14 days post-mortem for the whole set of samples.

Conclusions

The evolution of instrumental toughness and cathepsin activity in cytosolic and lysosomal extracts showed different pattern in the different genotypes studied. Homozygous or heterozygous animals for muscular hypertrophy presented higher enzymatic activity at early ageing periods and a higher tenderization rate than normal AV (+/+) or AM (+/+) bulls, for which the end of the tenderization process should be expected at longer ageing times. It seems that cathepsin D is the most implicated in later tenderization processes.

Acknowledgements

This study has been supported by the INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria) under project CAL03-074-C2. Dr. A C-M is a researcher from the Ramón y Cajal Program, Ministerio de Ciencia y Tecnología, Spain. We thank to Luis Guerrero for advice on statistical analyses.

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Table 2. Effect of genotype on WB shear force and enzyme activities and evolution of each variable during the ageing period.

	GENOTYPE				
	AV (*mh*/*mh*)	**AV (*mh*/+)**	**AV (+/+)**	**AM(+/+)**	**Sign.**
WB shear force (kg):					
WB – 3 days	4.95 A				
WB – 7 days	4.98 A	4.84 A	4.70	5.08	NS
WB – 14 days	4.24_{a} B	4.73_{ab} AB	4.54_{a}	5.24_{b}	*
WB – 21 days		3.91_{a} B	4.46_{ab}	4.92_{b}	NS
Sign.	*	NS	NS	NS	
Enzyme activity in lysosome extracts (units/mg protein):					
B – 3 days	0.83 A				
B – 7 days	0.88 A	0.86 A	0.88 A	0.76	NS
B – 14 days	0.34_{a} B	0.85_{b} A	0.69_{c} B	0.88_{b}	***
B – 21 days		0.41_{a} B	0.72_{b} AB	0.74_{b}	***
Sign.	***	***	NS	NS	
(B+L) – 3 days	0.83 A				
(B+L) – 7 days	1.21_{a} B	1.20_{a} A	0.75_{b}	1.05_{ab}	NS
(B+L) – 14 days	0.40_{a} C	1.25_{b} A	0.91_{b}	1.26_{b}	***
(B+L) – 21 days		0.44_{a} B	1.01_{b}	0.66_{a}	**
Sign.	***	***	NS	NS	
H – 3 days	0.32 A				
H – 7 days	0.45_{a} B	0.38_{b} A	0.22_{c}	0.22_{c} A	***
H – 14 days	0.14_{a} C	0.41_{b} A	0.34_{b}	0.38_{b} B	***
H – 21 days		0.16_{a} B	0.30_{b}	0.29_{b} AB	**
Sign.	***	**	NS	*	
D – 3 days	5.62 A				
D – 7 days	4.36 A	3.24 AB	3.09 A	3.53 A	NS
D – 14 days	1.60_{a} B	3.62_{b} A	5.22_{c} B	7.41_{d} B	***
D – 21 days		2.44_{a} B	2.89_{a} A	4.58_{b} A	**
Sign.	*	NS	*	***	
Enzyme activity in cytosol extracts(units/mg protein):					
B – 3 days	1.30 A				
B – 7 days	1.34_{a} A	1.38_{a} A	0.68_{b} A	0.78_{b} A	***
B – 14 days	0.60_{a} B	1.64_{c} A	0.84_{ab} AB	1.14_{b} B	***
B – 21 days		0.85_{a} B	1.01_{b} B	1.06_{b} B	*
Sign.	***	**	*	*	
(B+L) – 3 days	1.13 AB				
(B+L) – 7 days	1.49_{ac} A	1.56_{a} A	0.75_{b} A	1.08_{bc} A	**
(B+L) – 14 days	0.81_{a} B	2.07_{b} B	1.27_{a} B	1.88_{b} B	***
(B+L) – 21 days		0.95 C	1.34 B	1.26 AB	NS
Sign.	*	**	*	NS	
H – 3 days	0.32 A				
H – 7 days	0.51_{c} B	0.36_{b} A	0.25_{ab} A	0.21_{a} A	***
H – 14 days	0.15_{a} C	0.32_{b} AB	0.08_{a} B	0.12_{a} B	**
H – 21 days		0.19_{a} B	0.37_{b} C	0.35_{b} C	***
Sign.	***	NS	***	***	
D – 3 days	24.74 A				
D – 7 days	26.30_{a} A	21.85_{b} A	13.32_{c} A	14.63_{c} A	***
D – 14 days	8.52_{a} B	24.91_{b} A	17.61_{c} A	15.93_{c} A	***
D – 21 days		10.85_{a} B	30.11_{b} B	20.54_{c} B	***
Sign.	***	***	***	*	

Means in the same row followed by different subscripts are significantly different ($p<0.05$).
For a given genotype, values in the same column with different capital letters are significantly different ($p<0.05$).

SDS-PAGE ANALYSIS OF TCA SOLUBLE BOVINE MUSCLE EXTRACTS OVER THE POSTMORTEM AGEING PERIOD

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Background

Muscle proteins fall into three divisions according to their structure, function and/or degree of solubility (Ito *et al.,* 2003) and consists of; myofibrillar (salt-soluble), stomal or connective tissue (acid-soluble) and sarcoplasmic (water-soluble). In most publications dealing with meat proteins and/or polypeptides, the investigated fraction is usually determined by the extraction and separation methods applied (Claeys *et al.,* 2004). Much work concerning the proteolysis of ageing beef has focused on the myofibrillar fraction and the emergence of a 30 kDa fragment from Troponin T (Huff-Lonergan *et al.,* 1995 and Wheeler and Koohmaraie, 1999). However as fragments which are cleaved from myofibrillar proteins may themselves be soluble, analysis of the myofibrillar fraction only may not provide the researcher with a comprehensive overview of the proteolytic profile during postmortem ageing. Recently proteolytic fragments of troponin T were observed in a tricholoracetic acid (TCA) extract of beef (Nakai *et al*., 1995 and Stoeva *et al.,* 2000). Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) analysis can provide a clear profile of postmortem proteolytic pattern muscle extracts and maybe useful to view changes in a broad range of proteins/peptides over the ageing process. Altering the concentration of TCA used to obtain these extracts may be useful to optimise this visualisation technique.

Objectives

The purpose of the study was to monitor the changes in protein patterns in TCA extracts of bovine *M. longissimus dorsi* (loin, LD), over a fourteen-day ageing period, using SDS-PAGE and varying concentrations of TCA.

Materials and Methods

Sample collection Bovine *M. longissimus dorsi* (loin, LD) samples were collected at 1h, 3h, 2d, 7d and 14d post mortem, snap frozen in liquid nitrogen and stored at –80°C.

Protein Extraction Soluble protein extracts were prepared from two LDs at 1h, 3h, 2d, 7d and 14d using increasing concentrations of TCA, (0.5, 1.0, 5.0, 10.0 and 20,0%, w/v) following a modification of the method of Stoeva *et al.,* (2000).

Protein Concentration was determined using the Biuret method (Gornall *et al.,* 1949).
SDS-PAGE extracts were prepared by incubation in SDS-reducing buffer for 15 minutes at 50°C then stored at –20°C. Electrophoresis was performed according to the method of Laemmli (1970). Samples were applied to 12 % acrylamide 2mm dual vertical slab gels (BioRad) for a total of 3,500V/h then stained with either Coomassie Blue G-250 or Silver. Gels were then scanned using a colour image scanner (Epson Perfection 3200) for the visualisation of bands.

Repeatability study In order to determine the validity of the extraction method and its ability to return consistently similar degradation patterns, a repeatability study was carried out. To this end a single 2d muscle sample was independently extracted five times and run on an SDS-PAGE gel to determine if a quantitatively similar profile was obtained in each lane.

Results and Discussion

Results obtained show that increasing TCA concentrations have a negative effect on the extent of protein retrieval. The lower (0.5 and 1.0% TCA concentrations) gels have a higher degree of protein extractability,

compared to that of 5.0% (Figure 1). Higher TCA concentrations examined (10.0%) resulted in remarkably less protein extraction, with an absence of protein at 20.0% (results not shown).

The lower percentages of TCA (0.5 and 1.0%) provided extracts which were easily visualised on SDS-PAGE gels. As shown in Figure 1., clear banding patterns were observed from below 20kDa up to 205kDa. The most obvious changes over the ageing process were the increased intensities of bands appearing in the 20kDa to 36kDa region and the higher molecular weight 116kDa to 205kDa region. In terms of actual protein recovery, differences are visible between the two animals. Animal 2 has an absence of lower (< 29kDa) molecular weight proteins at lower TCA concentrations, with sharper more pronounced bands in animal 1 for the same concentration. Further characterisation of the individual animals will rule out if this is due to other factors such as, phenotypic differences.

A repeatability study was conducted on a single 2d sample. In this way the efficiency of the assay procedure to consistently isolate proteins in a reproducible manner was assessed. The results for the gel that was silver stained for greater sensitivity, show that the extraction procedure is indeed reproducible qualitatively, as the banding pattern of all five profiles are similar.

Conclusions

- SDS-PAGE separation techniques are useful in tracking changes in bovine TCA soluble protein extracts, over the post mortem ageing period.
- TCA concentration is a major factor in the extent of protein retrieval.
- Further work will be necessary in order to quantitatively categorise the specific changes observed.

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Acknowledgements

This project is funded by Enterprise Ireland.

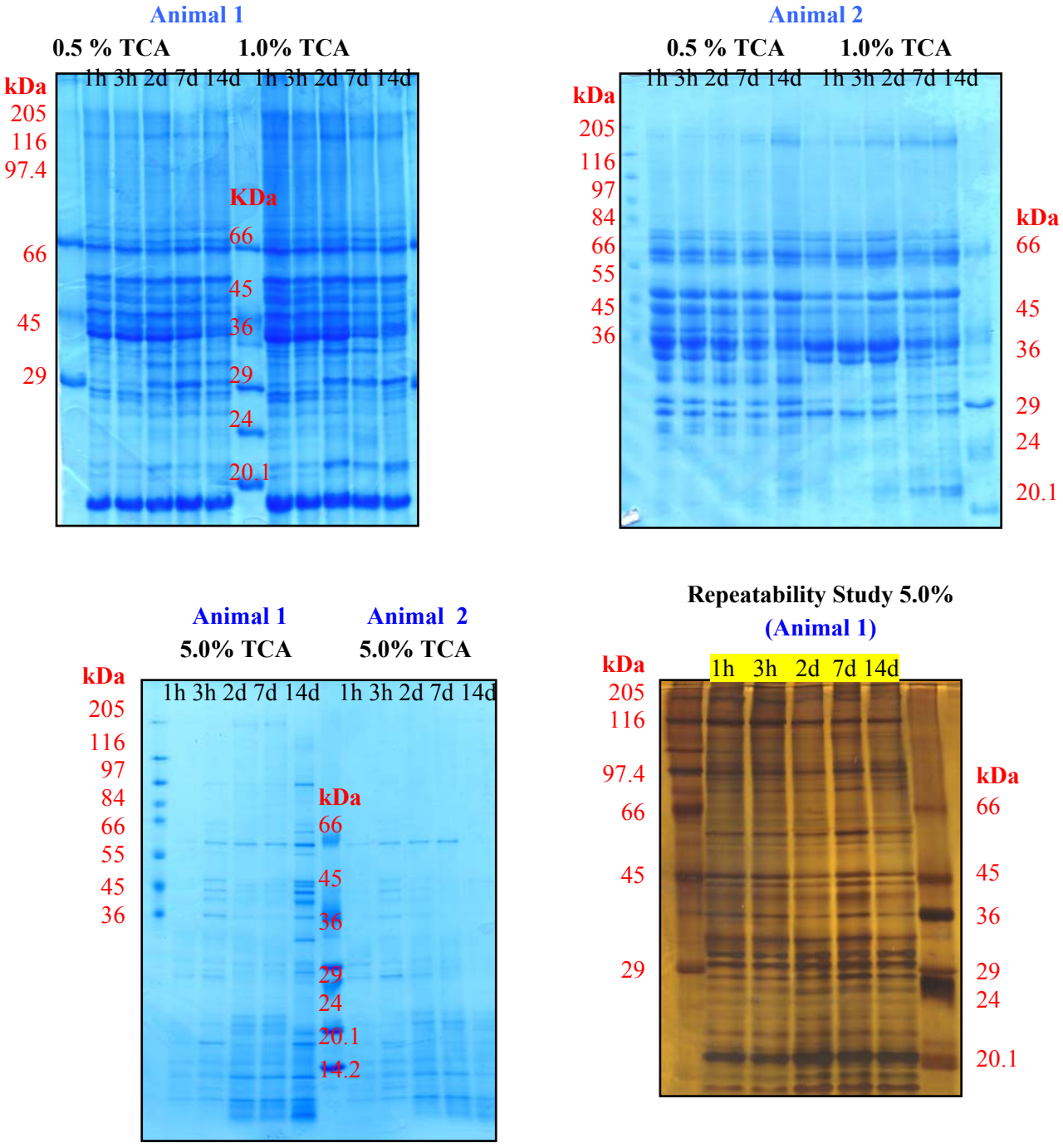

Figure 1. SDS-PAGE gels of two bovine LD samples (Animal 1 and 2) extracted into various concentrations of TCA (0.5, 1.0 and 5.0%, w/v) and stained with Coomassie Blue and a repeatability study showing a 2d sample (Animal 1) extracted into 5% TCA and Silver stained.

CHARACTERISTICS OF CARCASS COMPOSITION FOR HANWOO (COW, BULL, STEER) AND HOLSTAIN STEER

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Background

According the Korean animal products grading service, the frequency of carcass weight slaughtered from 1998 to 2002 decreased 2.9%, 14.7% and 12.2% for lighter than 200 ㎏, 200～250 ㎏ and 250～300 ㎏, respectively. On the other hand, that increased 8.1% and 14.8% for 300～350 ㎏ and 350～400 ㎏, respectively. Dairy cattle also showed the similar trend over the period. The tendency was understood as a consequence of improvement of feeding technique and long feeding period in response to consumer's demand of high quality beef. The rate of castration increased from 8.6% in 1998 to 38.2% in 2002. As a result, frequency of carcass heavier than 350kg increased from 16.3% in 1998 to 42.5% in 2002 which was the result of farmer's effort for producing high quality Hanwoo beef. According to the US National Beef Quality Audit in 2002, the most important task for beef producer included consistent product, yieldness, over-fattening. In the case of the US, carcass weight increased from 344kg in 1991, 339 kg in 339 to 357 kg. However, backfat thickness was 1.5cm in 1991, 1.2 ㎝ in 1995 and 1.24 ㎝ in 2000. In addition, average carcass weight for each quality grade was 370 kg for prime, 359 kg for choice and 355 kg for select grade.

Objectives

The current study was conducted to investigate characteristics of carcass quality and yieldness for Hanwoo cow, bull and steers within the range of market weight for each breed.

Materials and methods

The current analysis used industrial data collected from July 2003 to November 2003 across Korean best six slaughter houses. Seventy three carcasses of total 631 carcass were excluded for which a sum of deboned meat, bone and fat was higher than carcass weight, or which had higher than 15 kg of processing loss. Finally 558 carcasses were used for the analysis. Seven Hanwoo cows with carcass weight range of 350～400 ㎏, 77 Hanwoo bulls, 50 Hanwoo steers, 71 castrated daily cattle and thus total 212 cattle were used for analyzing carcass weight, cutability (10 primal cuts), body fat, bone weight. Carcass separation was performed according to the methods described by Ministry of Agriculture and Forest Act 1- 69. Subcutaneous fat was determined according to the beef regulation which requires remaining rate of 0 ㎜. Yieldness was calculated carcass weight against carcass weight without kidney and kidney fat. Back fat thickness and ribeye area were determined according to the method described by Animal products grading service.

Results and discussion

Table 1 shows characteristics of carcass traits. Average carcass weight of Hanwoo cow, bull and steer, and Holstein steer were 326.7 ㎏, 372.6, 378.6 and 396.7 ㎏, respectively. Holstein steer had the highest carcass weight, and that for bull and steer was similar. On the other hand, Hanwoo cow had a significantly lower carcass weight ($p<0.05$). Hanwoo cow had the most thinker back fat with 11.34 ㎜, followed by Hanwoo steer with 10.54 ㎜, Holstein steer with 7.69 ㎜, and Hanwoo bull with 6.16 ㎜ ($p<0.05$). Ribeye area was largest for Hanwoo bull with 85.7, followed by Hanwoo cow with 76.01 and Hanwoo steer with 74.20 ($p<0.05$). Yieldness was highest for Hanwoo bull with 67.8%, followed by Holstein steer with 64.0%, Hanwoo steer with 61.5%, and Hanwoo cow with 61.1% ($p<0.05$). Bone percentage was highest for Holstein steer with 15.0%, followed by Hanwoo bull 13.4%, Hanwoo steer with 12.0%, and Hanwoo cow with 11.8%. Difference in bone percentage between Hanwoo bull and Holstein steer was approximately 1.6%, while that between Hanwoo steer and Holstein steer was approximately 3.0% with higher percentage for Holstein steer. Body fat percentage was lowest for Hanwoo bull with 17.4%, followed by Holstein steer with 19.4%, Hanwoo steer with 24.6%, and Hanwoo cow with 25.0% ($p<0.05$).

With regard to carcass characteristics, Hamlin et al. (1995), Reiling et al. (1995) and Barber et al. (1981) reported that yieldness was linearly increased with carcass weight, but cutability against yieldness was decreased. Johnson et al. (1991) reported that high carcass quality with increased carcass weight resulted in reduction in cutability and increase in body fat percentage. In this regard, the current result of Holstein steer and Hanwoo steers supported the previous result. In addition, Abraham et al. (1980) and Talamantes et al. (1986) reported that body fat varied between breeds, sex, slaughter weight and between carcasses. Thus the percentage of body fat was a significant factor determining slaughter age for the best economic value of carcass.

Table 2 shows difference in carcass characteristics. Carcass weight did not differ with the selected range ($p>0.05$). However, retail cut percentage was highest for Hanwoo bull with 254.0 kg, followed by Holstein steer with 242.8 kg, Hanwoo steer with 229.6 kg, and Hanwoo cow with 221.2 kg($p<0.05$). Yieldness which reflects cutability was highest in Hanwoo bull with 67.8%, followed by Holstein steer with 64.5%, Hanwoo steer with 61.3%, and Hanwoo cow with 59.2% ($p<0.05$). Difference in cutability between Hanwoo and Holstein steers were 3.2% with higher percentage for Hanwoo steer. Bone percentage was highest for Holstein steer with 15.4%, while Hanwoo cow was lowest with 11.1% ($p<0.05$). Difference in Holstein steer and Hanwoo bull were 2.13%, and that between Holstein and Hanwoo steers were 3.45%. In body fat content, Hanwoo bull and Holstein steer had 17.6% and 18.5%, respectively. Hanwoo steer and Hanwoo cow were 24.8% and 27.1%, respectively ($p<0.05$).

Similar results with the current study were reported by Amer et al.(1994) and Jacobs et al.(1977) who reported in the study of comparing carcass characteristics between breeds and sex , steer had a higher back fat thickness and lower yieldness than those for bull at the same carcass weight. Hopkinson et al.(1985) buff had higher carcass weight, cutability, and dressing percentage than steer when these were slaughtered at the same live weight. On the other hand, body fat was higher for steer than bull. Miller et al.(1988) and Karima et al.(1986) studied the effect of sex on carcass traits for similar live weight and found that dressing percentage was higher for steer than bull and cow, while cutability was higher for bull than cow and steer.

Conclusions

Hanwoo bulls showed the highest yieldness with 67.8% between carcass weight 350~400 kg, followed by Holstein steer with 64.5%, Hanwoo steer with 61.3% and Hanwoo cow with 59.2% ($p<0.05$). In comparing yieldness between Hanwoo and Holstein steers, Hanwoo steer showed lower percentage by 3.2% then Holstein. In bone weight, Holstein showed the highest percentage with 15.4%, while Hanwoo cow was the lowest 11.1% ($p<0.05$). Difference in bone percentage between Holstein and Hanwoo bull were 2.13%, and that between Holstein and Hanwoo steers was 3.45%. Body fat was low in Hanwoo bull and Holstein with 17.6% and 18.5%, respectively, while that for Hanwoo steer and Hanwoo cow were 24.8% and 27.1%, respectively($p<0.05$).

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Table1. Comparison of carcass properties

Item	Hanow			Holstein	Overall
	cow	bull	steer	steer	mean
Numbers of animals	73	207	106	172	558
Carcass wt. (kg)	326.71^{c} ±4.89	372.61^{b} ±3.11	378.61^{b} ±3.88	396.73^{a} ±2.70	375.18 ±1.94
Retailcut wt .(kg)	199.48^{c} ±2.79	252.50^{a} ±1.99	232.35^{b} ±2.18	253.86^{a} ±1.60	242.16 ±1.30
inedible fat (kg)	82.40^{b} ±2.27	65.85^{c} ±1.33	94.14^{a} ±2.21	77.64^{b} ±1.12	77.02 ±0.90
Bone (kg)	38.49^{d} ±0.49	49.89^{b} ±0.34	45.33^{c} ±0.43	59.62^{a} ±0.35	50.53 ±0.36
By-products (kg)	120.89^{b} ±2.60	115.74^{b} ±1.48	139.47^{a} ±2.45	137.26^{a} ±1.28	127.55 ±0.99
% of bone	11.86^{c} ±0.13	13.46^{b} ±0.07	12.01^{c} ±0.08	15.07^{a} ±0.08	13.47 ±0.07
% of body fat	25.00^{a} ±0.44	17.47^{c} ±0.26	24.63^{a} ±0.42	19.47^{b} ±0.19	20.43 ±0.20
% of retail cut	61.19^{c} ±0.34	67.88^{a} ±0.20	61.52^{c} ±0.32	64.05^{b} ±0.15	64.61 ±0.16
Backfat thickness (㎜)	11.34^{a} ±0.47	6.03^{d} ±0.21	10.54^{b} ±0.34	7.28^{c} ±0.17	7.97 ±0.16
Loin area ( )	76.01^{c} ±1.05	85.70^{a} ±0.70	81.68^{b} ±0.77	74.20^{c} ±0.56	80.12 ±0.43

※ Mean ± standard error of mean.

Table 2. Comparison of carcass properties for animals having carcass weight ranges from 350～400 kg

Item	Hanwoo			Holstein	Overall mean
	cow	bull	steer	steer	
Numbers of animal	14	77	50	71	212
Carcass wt. (kg)	373.43 ±3.80	374.23 ±1.65	374.58 ±2.04	376.01 ±1.67	374.86 ±0.98
Retail cut wt. (kg)	221.22^{d} ±2.26	254.03^{a} ±1.60	229.66^{c} ±1.83	242.82^{b} ±1.27	242.36 ±1.13
inedible fat (kg)	101.31^{a} ±4.20	66.05^{c} ±1.40	93.25^{b} ±2.49	69.84^{c} ±1.04	76.06 ±1.25
Bone (kg)	41.64^{d} ±0.98	49.79^{b} ±0.40	44.90^{c} ±0.41	58.03^{a} ±0.42	50.86 ±0.45
By-products (kg)	142.95^{a} ±4.91	115.83^{c} ±1.39	138.15^{a} ±2.48	127.87^{b} ±1.03	126.92 ±1.11
% of bone	11.15^{d} ±0.24	13.31^{b} ±0.09	11.99^{c} ±0.10	15.44^{a} ±0.10	13.57 ±0.12
% of body fat	27.13^{a} ±1.07	17.65^{c} ±0.36	24.84^{b} ±0.60	18.56^{c} ±0.25	20.28 ±0.32
% of retail cut	59.28^{d} ±0.63	67.88^{a} ±0.31	61.35^{c} ±0.48	64.58^{b} ±0.20	64.67 ±0.27

A METHOD FOR DETERMINING THE MUSCLE FIBRE LENGTH

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Background

Muscle fibres are the result of the fusions of thousands of myoblasts to form a long tube. The length of a mammalian muscle fibre is variable, and can be the length of the muscle. They are not, however, usually as long as the muscle, but still the length may even be 340 mm in long muscles (Lawrie, review 1998, p. 39). Fibres are arranged end-to-end series with their tapering ends overlapping adjacent fibres. About 50% of their length is of constant diameter, and taper at both ends by 25% of their length the angle of the taper being about 1º. (McCormick, review 1994, pp. 30-31). There is no data about porcine muscle fibre lengths easily available.

Objectives

The purpose of this study was to develop a method to estimate the fibre length of a muscle in order to count the total number of fibres in a muscle.

Materials and methods

M. longissimus dorsi muscle from three pigs of about 110 kg live weight were excised on the day following slaughter caudally to 5th *Thoracic vertebra*. Three 1 cm thick slices were cut, from both ends at 1/5 of the total length and from the middle, of each excised muscles (Figure 1A). From each slice five 1x1x1 cm cubes were usedfor fibre length measurements and five smaller pieces, of about 300 mg, for fibre diameter measurements were cut as shown in Figure 1B.

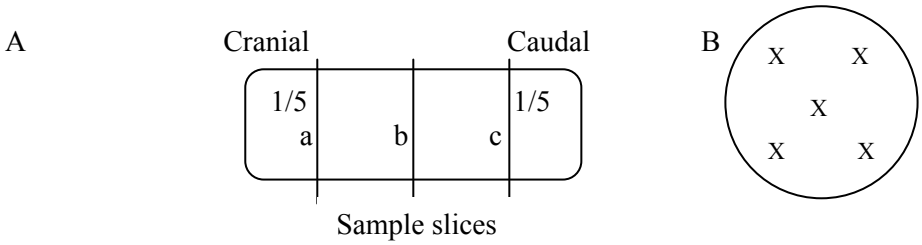

Figure 1. Sampling from *M. longissimus dorsi* muscle A: Slice locations; B: Fiber samples from a slice

Fibre length. Muscle fibres were separated by the Hooper method (1976; 1981). A sample cube was placed into a 50 ml beaker with 15 ml 4 N HNO_3. Beakers were covered with aluminium foil and kept overnight at room temperature. After the acid treatment the dimensions of the cubes were measured to determine the shrinkage (shortening) caused by the acid treatment. The cubes shrank longitudinally along the fibre axis 10%. Then each sample cube was inserted into a test tube with 5 ml Ringer-Locke solution. Test tubes were sealed and shaken vigorously by hand and by using test tube shaker, for one minute. Part of the suspension was poured on a slide and left to dry overnight.

The suspension was studied with a microscope (Olympus BH-2, Japan) at 40x magnification, with a commercial Zeiss AxioCam MRc digital camera (Zeiss GmbH, Germany) (Figure 1). Images were analysed with software package (Zeiss KS300 3.0, Germany). Individual fibre fractions were marked manually, on the images at their both ends, and the program then determined the length of the fibre. The results were stored cumulatively. Simultaneously, the number of fractions that visually seemed to be the very end of the tapered end of a fibre, was counted. From each slide sufficient fields were analysed so that the total number

of fibre fractions was at least 400. Totally over 20 000 fractions were measured (3 muscles x 3 slices x 5 cubes x more than 400 fractions).

The length of fibres was counted assuming that the tapered ends are equally distributed within the fractions (Equation 1).

$$L = \frac{\sum F}{(N/2)}$$

L= length of the fibres [mm]

F= total length of fractions [mm] (Eq. 1.)

N= number of the tapered ends.

Cross sectional area of slices. The slices were photographed, and the cross sectional area was determined using the image analysis program Zeiss KS300.

Number of fibres in a cross section. Due to an error in sample preparation, only two muscles were analysed for fibre numbers. The sample pieces were frozen in liquid nitrogen and kept then in -80 ºC. Cross sections (12 µm) were cut at -26 ºC in a cryostat (Reichert-Jung 2800 Frigocut E, Germany). The sections were not stained. From the sections, the counting area of the image and the number of fibres on the counting area were determined. The number of fibres per image was about 100. Two images per sample were analysed, totalling 10 images per slice (30 per muscle, 60 in grand total). The fibre number was counted by dividing the cross sectional area of the slice by the average cross sectional area of fibres.

Results and discussion

The fibres were well separated by the Hooper method. The fractions were clearly seen and marked for the automatic determination in the image. The tapered ends were also easy to identify, but the major obstacle was their small number. It was not possible to use a lower magnification or an automatic determination of the fractions directly from the image, because the first stage of the analysis had to be done manually. On one hand, the tapered ends of the fibres are very thin and difficult to see, but on the other hand, if this done always by the similar way, the fractions of certain size will always be counted similarly. An increase of the fields counted, however, would also much increase the work needed.

The aim of this study was develop a method for fibre length determination. Therefore, the conclusions below are only speculative, especially because the number of tapered ends was so small (varying between 4–14 within the total about 2000 fractions counted per slide). In such a small number, a change of one tapered end only has a marked effect on the results.

The fibres seem to be more than 100 mm long (Table 1). They seem to be longer in the middle of the muscle than at the end, which is in agreement with the statement of McCormick (review 1994). In this study we had actually only one end, the caudal (rear) end. The authors did not find any literature data about the length of porcine muscle fibre, and therefore no comparisons were made here. It seems, however, that the lengths obtained with this method are very long, and more analyses are needed for the validation of the method.

The fibre cross sectional areas were similar to that reported previously in Finland (Ruusunen and Puolanne, 2004) (Table 2). Because the fibres seem to be so long, theoretically there would be no need to have tapered ends at slice c, because it is closer (about 10 cm) to the caudal end of the muscle than the average length of the fibres. (On the contrary, the cranial end of the sample is not at the very end of the muscle, because a part of *M. longissimus dorsi* remained in the forepart of the carcass). Therefore, for reliable results, more slices taken at shorter spacings should be analysed, this would tremendously increase the amount of work needed.

The cross sectional area of fibres is larger in the middle of the muscle, which is a logical consequence of the observation (McCormick, review 1994) that the fibres are tapered at the end of the muscle. It must be noted, however, that the fibre axis in *M. longissimus dorsi* in not fully parallel with the muscle axis, and therefore the fibres have a slight pennate-type arrangement in the muscle. The number of fibres per muscle cross

section is about 1.1–1.3 million, on average 1.2 million. A very rough estimate of the total number of fibres in the whole muscle (length 60–70 cm, weight ca 4.0 kg) is about 4.2 million. Consequently, one fibre, 173 mm long and diameter Ø 40 µm, has a weight of about 1 mg.

Table 1. Muscle fibre lengths of porcine *M. longissimus dorsi* (n=3)

Slice (see Figure 1A on Page 1)	Muscle fibre length (mm)
a (cranial)	183 (156–298)
b (medial)	218 (186–346)
c (caudal)	137 (112–235)
Grand mean	173

Table 2. Muscle fibre cross sectional area and cross sectional fibre number in porcine *M. longissimus dorsi* (n=2)

Slice (see Figure 1A on Page 1)	Cross sectional area $x10^3$ µm^2	Number of fibres $x10^3$
a (cranial)	5.14	1147
b (medial)	5.17	1153
c (caudal)	4.67	1299
Grand mean	5.00	1198

Conclusions

A rough estimate of the muscle fibre length of porcine muscle length can be made by disintegrating muscular tissue and counting the tapering ends of muscle fibres in relation to the total length of the fibres counted. The method is, however, time consuming and hard to automatize. A fibre type staining could be included to increase the relevant data obtained from samples. The average cross sectional area of the fibres seems to vary within the muscle, being smaller at the ends of the muscle.

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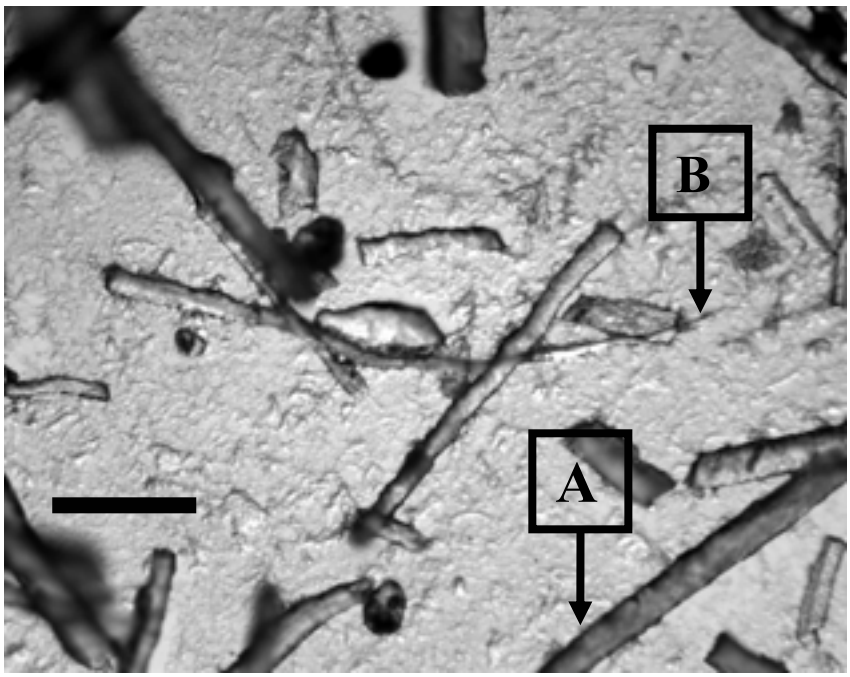

Figure 2. Fibre fractions. A: the thick middle part of a fibre; B: a tapering end. Bar: 200 μm. Microscopic magnification 100x.

GLYCOGEN, LACTIC ACID AND PH IN MEAT (META ANALYSIS)

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Background

Lactic acid has a pK_a-value of 3.86. This means that 90% is dissociated at pH 4.9, and 99% at pH 5.9, i.e. practically totally. Consequently, only ca. $3*10^{-5}$ M lactic acid solution would decrease the pH of distilled water to a value of 5.5. Analyses have shown that in meat the relationship between pH (a logarithmic measure) and the lactic acid content (a linear measure) is practically linear. On the assumption of a linear relationship, the buffering capacity of meat has been calculated from titration curves, to be 50–60 mmol H^+/(kg*pH) over the pH range of 7 to 5.5. Consequently, ca. 80 to 100 moles lactic acid per kilogram lean meat is needed to decrease the pH value from 7.2 of living muscle to 5.5.

In a simplified model glucose (glycogen) is broken down to lactic acid causing the pH to decrease. The protons formed are bound to buffering substances, such as proteins, organic and inorganic phosphates and dipeptides. The buffering capacity of all of these depend on pH. The buffering capacity of proteins is from about 20 mmol H^+/(kg*pH) (pH 7.0) to 35 mmol H^+/(kg*pH) (pH 5.5). The effect of the other substances ranges from ca. 30 mmol H^+/(kg*pH) (pH 6.8) to 15 mmol H^+/(kg*pH) (pH 5.5). At lower pH values, lactic acid becomes increasingly important, as its buffering capacity increases, while the capacity of other components decreases. Proteins, however, have another buffering capacity maximum at pH 4, due to the pK_a of the nonpeptidyl carboxyl group. Consequently, the buffering capacity is different at different pHs (Kivikari, review 1996).

In addition to the buffering capacity, there are difficulties obtaining unbiased determination of pH value of meat, as well as determining the glycogen, its derivates and lactic acid, especially in the small and inhomogenous samples generally used in meat research. The amount of water added during sample preparation and titration, added salt etc. have effects on the pH and also on the buffering capacity. This makes it understandable that the data given in the literature is somewhat variable.

Objectives

The purpose of this study was to analyse the relationships between glycogen and lactic acid contents and pH, based on the data presented in the literature (meta analysis).

Materials and methods

The data used in this meta analysis were collected from the literature relating to porcine *M. longissimus dorsi* (i) pH value, (ii) lactic acid content and (iii) glycogen content at (iv) different times post mortem. We used the value 7.2 (Kylä-Puhju, Ruusunen, Kivikari and Puolanne 2004) for the pH of zero lactate meat and buffering capacity of 52 mmol H^+/(kg*pH) (Kivikari 1996; Puolanne and Kivikari 2000). All data were converted into same units and calculated as follows (the underlinings refer to Table 1.):

The Measured pH value was the pH value that was given in the reference at the given time
The Calculated pH value was obtained as follows: pH calc. = 7.2 – lactic acid (mmol/kg)/52 mmol/(pH*kg)
Difference pH = pH measured – pH calculated
Measured Δ lactic acid LA (mmol/kg) = lactic acid time 2 – lactic acid time 1
Calculated Δ lactic acid LA (mmol/kg) = (glycogen time 1 – glycogen time 2)*2
Difference lactic acid LA (mmol/kg) = measured Δ lactic acid – calculated Δ lactic acid

Results and discussion

The differences between the measured and calculated lactic acid values did not show any consistent pattern, varying between -0.14 and +0.66 (Table 1.). The calculated pH values, however, tended to be lower than the measured.

The data in Table 1 are only a sample of the data available in the literature, and the following discussion is based on a more general analysis of the literature data. It was clearly seen in this study that the determination of pH values as well as lactic acid content and glycogen content are subject to large variation which may sometimes have lead to even biased results. This does not necessarily mean that the analyses have not been carefully done. The measurement of pH value is particularly difficult just after slaughter. Therefore it is recommended to utilise iodoacetate method to stop the glycolytic reactions which would also allow the measurement to be carried out at the room temperature. The sample sizes are sometimes small, from 10 mg (2 mg freeze dried) to several grams. Sometimes blood, fat and visible connective tissue had been carefully removed, but not always.

The intermediates of the glycolytic pathway which are not determined by the normal methods for detemining glycolytic potential may represent less than 10 mmol/kg as lactic acid (Kastenschmidt et al., 1968; Hamm and Fischer, 1980). These do not seem to be the major cause of the variability in the results. It was found that the value for buffering capacity is also very variable. Kivikari (1996) and Puolanne and Kivikari (2000) gave a buffering capacity of 52 mmol H^+/(pH*kg), but in her review Kivikari (1996) had found values from 42 to 64 mmol H^+/(pH*kg). In a previous study the value was 49 mmol H^+/(pH*kg) (Kylä-Puhju, Ruusunen and Puolanne, 2004), and in the current study the best fit was obtained with a value of 62 mmol H^+/(pH*kg). This was done by solving the value of the regression equation y = -0.1927x + 1.3843 at pH 5.5 (Figure 1). When the measured pH value x was 5.50, y was 0.32, meaning that the calculated pH value was 5.18 (5.50 – 0.32 = 5.18). From this it was possible to calculate that if the buffering capacity value was 62 mmol H^+/(pH*kg), the measured pH value and calculated pH value would be the same. The 62 mmol H^+/(pH*kg) gives ΔpH values between measured and calculated values that are on average zero independently of the pH value of the meat at the time of the determinations (Figure 2.). The buffering capacity values, however, should not be directly compared because they are from different sources. This still shows the uncertainty of the methodology involved.

Conclusions

It was concluded that much effort should be put on the analyses of pH values, glycogen contents and lactic acid contents. If they all are determined at the same times, it might be worthwhile to do the simple calculations in order to see, how well the figures fit together as glycolytic potential. It must, however, be pointed out, that during the post mortem reactions the calculations discussed above may be a over-simplification, and also other factors and components may have a marked role. In addition, it is recommended always to use the same control sample within the study to check the variation between the different sets of analyses. The buffering capacity of *M. longissimus dorsi* seems to depend on the method of determination, the values varying between 49-62 mmol H^+/(pH*kg).

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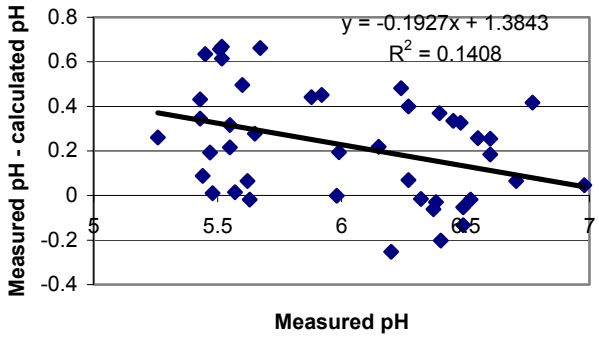

Figure 1. The difference of measured pH and calculated pH related to measured pH. Buffering capacity 52 mmol H^+/(pH*kg).

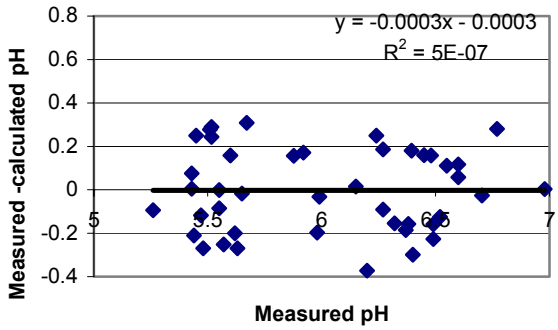

Figure 2. The difference of measured pH and calculated pH related to measured pH. Buffering capacity 62 mmol H^+/(pH*kg).

Table 1. Studies from the literature containing analytical data on pH, lactic acid content and glycogen content of porcine *M. longissimus dorsi* at different times post mortem. Calculations, see Materials and methods.

	pH				Lactic acid			
Reference	**Time**	**Meas.pH**	**Calc. pH**	**Diff.pH**	**Δ timeLA**	**Δ meas.LA**	**Δ calc.LA**	**Diff.LA**
Ahn et al. 1992	45 min	5.65	5.37	0.28				
	2 h	5.55	5.35	0.20	45–120 min	1	14	-13
	4 h	5.46	5.28	0.18	2–4 h	4	2	2
	24 h	5.45	4.80	0.65	4–24 h	25	6	19
							Total	8
D´Souza et al. 1999	40 min	6.48	6.15	0.33	5–40 min	11	24	-13
	24 h	5.55	5.23	0.32	40–-24 h	48	38	10
							Total	3
D`Souza et al. 1998a	45 min	6.4	6.60	-0.20	5–45 min	7	27	-20
	70 min	6.2	6.45	-0.25	45–70 min	8	27	-19
	24 h	5.62	5.55	0.07	70 min–24 h	47	39	8
							Total	-31
D´Souza et al. 1998b	40 min	6.6	6.35	0.25	5–40 min	2	11	-9
	24 h	5.48	5.47	0.01	40 min–24 h	46	50	-4
							Total	-13
Fernandez et al. 2002	40 min	6.52	6.54	-0.02	0–40 min	26	15	11
Hammelman et al. 2003	1 min	6.77	6.36	0.41				
	30 min	6.27	6.19	0.08	1–30 min	8	-5	13
	60 min	5.99	5.80	0.19	30–60 min	21	50	-29
	24 h	5.6	5.09	0.51	60 min–24 h	36	64	-28
							Total	-44
Henckel et al. 2002	24 h	5.60	5.18	0.42	1–15 min	10	6	4
					15–30 min	3	-6	9
					30–45 min	8	6	2
					45–60 min	4	6	-2
					1–3 h	29	30	-1
					3–6 h	12	10	1
					6–24 h	19	17	2
							Total	15
Klont et al. 1994	45 min	6.60	6.42	0.18	45 min–2 h	15	15	0
	18 h	5.57	5.56	0.01	2–4 h	9	17	-8
					4–18 h	20	24	-4
							Total	-12
Lambooij et al. 2004	0 h	6.49	6.54	-0.05				
	1 h	5.98	5.98	0.00	0–1 h	29	33	-4
	4 h	5.63	5.65	-0.02	1–4 h	17	13	4
	24 h	5.44	5.35	0.09	4–24 h	15	12	3
							Total	3
Schäfer et al. 2002	1 min	6.48	6.62	-0.14				
	15 min	6.38	6.43	-0.05	1–15 min	10	14	-4
	30 min	6.33	6.32	0.01	15–30 min	6	2	4
	1 h	6.16	5.95	0.21	30 min–1 h	19	16	3
	2 h	5.92	5.49	0.43	1 h–2 h	24	20	4
	24 h	5.51	4.85	0.66	2 h–24 h	33	30	3
							Total	10

Table 1 cont.

	pH				Lactic acid			
Reference	**Time**	**Meas.pH**	**Calc.pH**	**Diff.pH**	**Δ timeLA**	**Δ diff.LA**	**Δ calc.LA**	**Diff.LA**
Sayre et al. 1963								
Chester	0 h	6.45	6.12	0.33	0–24 h	54	96	-42
White	24 h	5.43	5.00	0.43				
Hampshire	0 h	6.38	6.41	-0.03	0–24 h	73	143	-70
	24 h	5.26	5.00	0.26				
Poland	0 h	6.27	5.87	0.40	0–24 h	45	68	-23
China	24 h	5.43	5.00	0.43				
Tarrant et al.	15 min	6.98	6.93	0.05				
1972	1 h	6.70	6.64	0.06	15 min–1 h	15	12	3
	2 h	6.55	6.29	0.26	1–2 h	18	21	-3
	3 h	6.39	6.02	0.37	2–3 h	14	12	2
	4h	6.24	5.76	0.48	3–4 h	14	28	-14
	5 h	5.88	5.44	0.44	4–5 h	17	26	-9
	6 h	5.67	5.01	0.66	5–6 h	22	14	8
	7 h	5.52	4.85	0.67	6–7 h	8	18	-10
	8 h	5.52	4.90	0.61	7–8 h	-3	6	-9
							Total	-32

ARE CATHEPSINS B AND D INVOLVED IN POST-MORTEM MYOFIBRILLAR DEGRADATION?

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Background

There is considerable evidence that the increase in tenderness that occurs during the post-mortem storage of muscle at 4°C is caused by limited proteolysis of the myofibril, although the detailed mechanism remains to be elucidated (Zeece et al. 1992, Jiang, 1998).
The principal proteinase systems in the muscle fibre are (a) the calpains, (b) the lysosomal cathepsins, and (c) the proteasome-ubiquitin system. Calpain seems to account for much of the post-mortem proteolysis in the myofibrils (Geesink and Koohmaraie, 1999), but the role of the cathepsins is unclear (Goll et al. 1983). The post-mortem activity of the proteasome has not been extensively investigated.
If cathepsins are to act post-mortem on the myofibril they must be released into the cytosol from the lysosome. Some studies, (e.g. Dutson, 1983) report post-mortem release of lysosomal enzymes, others (e.g. Lacourt et al. 1986) report no release. However, careful biochemical studies by Chambers et al. (1994) found limited release of lysosomal enzymes during the post-mortem storage of bovine muscle at 4°C and this finding was confirmed by Mobarak et al. (1999) using enzyme histochemistry and by Mobarak et al. (2000) using immuno electron microscopy.
Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) can detect the subtle proteolysis that occurs post-mortem in myofibrils. The release of easily releasable myofilaments (ERM) from myofibrils is another sensitive test of post-mortem myofibrillar degradation. ERM are a sub-population of myofilaments on the myofibril surface that are easily shed from the myofibril under 'relaxing' conditions, i.e. 0.1M KCl, 3mM Mg-ATP, 3mM EGTA, 1mM DTT, 2-mM Tris-Maleate, pH 7.1. Reville et al. (1994) showed that the yield of ERM in beef muscle rises steadily over the conventional 14 day post-mortem ageing period of 14 days at 4°C.

Objectives

Assuming that lysosomal cathepsins are released into the cytosol post-mortem the present study investigated whether catheptic activity against myofibrils *in-vitro* mimics myofibrillar changes known to occur during post-mortem ageing of beef. The effect of pre-treatment of bovine myofibrils with cathepsin B or cathepsin D on ERM release from these myofibrils was examined. The proteolysis of the myofibrils by cathepsin B and cathepsin D was monitored by SDS-PAGE.

Materials and methods

Myofibrils were prepared from bovine sternomandibularis muscle (18 month old Friesian heifers) by the method of Etlinger et al. (1976) and stored in Low Salt Buffer (LSB) – 0.1MKCl, 3mM $MGCl_2$, 3mM EGTA, 1mM DTT, 20mM Tris-Maleate, pH 7.0 containing 50% glycerol at –20°C until required.
Cathepsin B treatment of myofibrils at pH 5.5: Bovine spleen cathepsin B and its inhibitor leupeptin were purchased from Sigma. The enzyme was reconstituted in 2mM phosphate buffer, pH 7.0 at a concentration of 1mg/ml. Myofibrils were resuspended in 0.1M KCl, 1mM EDTA, 50mM Imidazole, pH 5.5 at a protein concentration of 3mg/ml and digestion was started by adding cathepsin B at a ratio of 1 part enzyme to 100 parts myofibril (w/w). Two controls were employed – myofibrils incubated for 60 mins in the KCl, EDTA, Imidazole buffer, and myofibrils plus enzyme plus leupeptin incubated for 60 mins at 37°C. The active treatment conditions were myofibrils plus enzyme for 5 minutes or for 60 mins at 37°C. Treatment was terminated by the addition of leupeptin at a concentration of 20µg/ml.
Cathepsin D treatment of myofibrils at pH 5.5: Bovine spleen cathepsin D and its inhibitor pepstatin were purchased from Sigma. Cathepsin D, was reconstituted in distilled water, but otherwise the treatment of myofibrils with cathepsin D was effected as described for cathepsin B, except that 0.1M NaCl substituted for

0.1M KCl and digestion of myofibrils with cathepsin D was terminated by addition of pepstatin to a concentration of 20mM.

Assay of Mg-ATP Stimulated Release of Protein from Cathepsin Treated Myofibrils: Control and enzyme-treated myofibrils were resuspended in LSB and assayed for Mg-ATP stimulated release of protein in the ERM assay as described by Reville et al (1994). Released protein is expressed as a percentage of the total myofibrillar protein. Release of protein in the presence of LSB alone (i.e. no ATP) was also measured.

Electrophoresis by SDS-PAGE: SDS-PAGE was carried out on control myofibrils and on myofibrils treated with cathepsin D or cathepsin B. Myofibrils were solubilised in 4M urea, 1M thiourea, 0.025M Tris-Acetate pH 6.8, 37mM DTT, 1.5% SDS, 0.025% Bromophenol blue. Samples were heated at 55°C for 15 mins. and subsequently stored at –20°C until SDS-PAGE was performed.

SDS-PAGE followed the method of Laemmli (1970), using a 5% stacking gel and a 7%-18% linear acrylamide gradient separating get. 30µg of protein was loaded per lane. The gels were stained with coomassie-blue.

Results and discussion

Figure 1 shows that pre-treatment of myofibrils with cathepsin B or D enhanced Mg-ATP stimulated release of protein from myofibrils, even after 5 minutes pre-digestion of the myofibrils. In the case of cathepsin D pre-treatment, Mg-ATP stimulated release of protein rose from a mean of 1.3% total myofibrillar protein in controls, through a mean of 1.89% after 5 mins., to a mean of 5.0% after 1 hour's pre-digestion of the myofibrils. The corresponding figures for cathepsin B were a rise from 1.05% for controls, through a mean of 1.8% after 5 mins, to a mean of 3.1% after 1 hour's pre-digestion. Neither cathepsin D nor cathepsin B treatment of myofibrils had any significant effect on release of protein in the absence of Mg-ATP.

Figure 2 shows the protein subunit composition of control myofibrils and myofibrils after digestion for 5 mins. and 60 mins. with cathepsin B or cathepsin D at pH 5.5 as assessed by SDS-PAGE. Five minutes treatment with cathepsin B had little effect on the myofibril. Treatment of myofibrils for 1 hour with cathepsin B had pronounced effects, of which the most notable are:- most of the high molecular weight material above myosin is removed; myosin and actin bands are notably diminished compared with controls; many new bands, presumably degradation products, are evident, e.g. a ladder of bands in the range 120 kDa to 150 kDa relative molecular mass.

The effects of treatment of myofibrils with cathepsin D are similar to the results described for cathepsin B, although there are differences in the pattern of degradative products produced (Figure 2). Five minutes treatment at pH 5.5 shows but a minor effect on protein subunit pattern, most notably on the very high molecular weight sub-units. Sixty minutes treatment with cathepsin D shows a marked effect – a big diminution in very high molecular weight material, a marked decrease in myosin band intensity, and some degradation of α-actinin (not seen after cathepsin B digestion).

Increased ERM yields from cathepsin D or cathepsin B 60 minute pre-treated myofibrils compare favourably with the natural increase in ERM yield in post-mortem bovine muscle from 1.4% at-death to 5.5% after 14 days storage at 4°C. However, proteolysis of the myofibril during conventional ageing of meat is very minor and there is no degradation of actin or myosin, whereas 60 mins. digestion of myofibrils with cathepsin B or cathepsin D produces marked degradation of myofibrillar proteins, including actin and myosin. Therefore the effects of primary interest in the present study are the effects of limited (5 mins.) digestion of myofibrils by cathepsins.

Five minutes digestion of myofibrils by cathepsin B or D had only minor effects on the protein subunit composition of the myofibrils. This amount of pre-digestion of the myofibrils allowed an increase of about 75% in the amount of protein released from myofibrils in the presence of Mg-ATP compared to controls in the case of cathepsin B and 46% in the case of cathepsin D. If both enzymes were acting together, and if the effects were additive, this would produce about a 120% increase in the yield of releasable protein. If cathepsins are released from the lysosome post-mortem, other cathepsins, in addition to B and D, could also contribute to the degradation of the myofibril, e.g. cathepsin L.

Conclusions

Concerted but limited action of cathepsins on myofibrils post-mortem could account for much of the increase in Mg-ATP stimulated release of protein from myofibrils that is seen during the conventional post-mortem storage of beef at 4°C.

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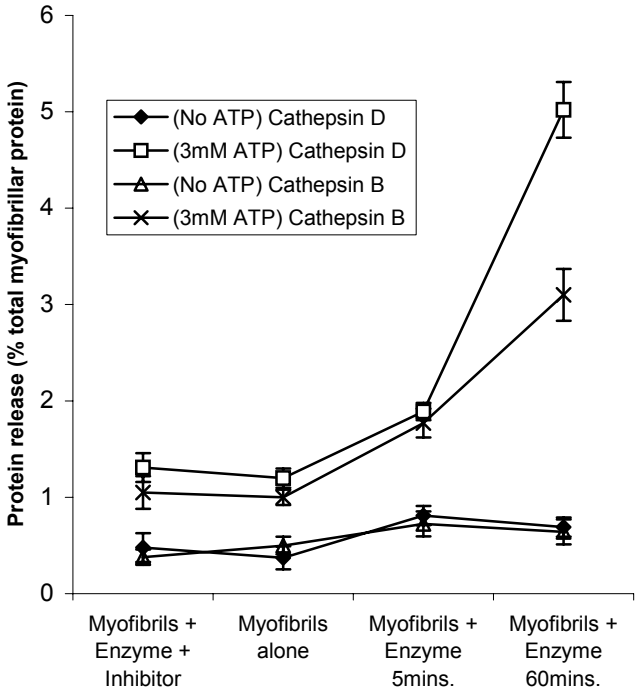

Figure 1 – Mg-ATP stimulated release of protein from cathepsin D or cathepsin B treated bovine myofibrils. Figures are means ± SD of releases from 5 animals.

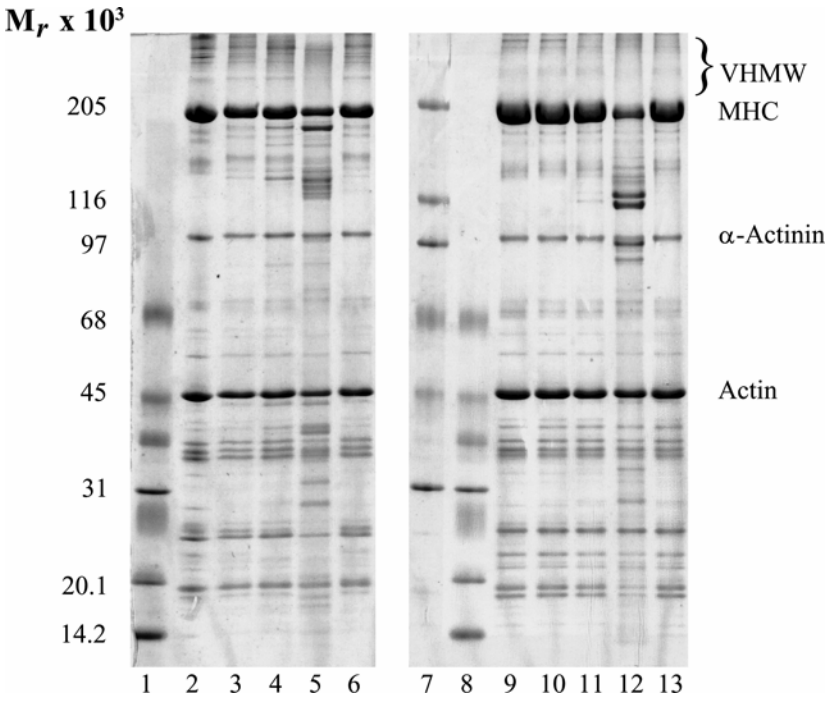

Figure 2 – Protein subunit composition of myofibrils as revealed by SDS-PAGE after treatment of the myofibrils with cathepsin B or cathepsin D at pH 5.5 at a cathepsin to myofibril ratio (w/w) of 1:100.
Lane 1: Low mol. wt. markers; Lane 2: Myofibrils from glycerol stock; Lane 3: Myofibrils held at pH 5.5, 1 hr., 37°C; Lane 4: Myofibrils + cathepsin B, pH 5.5, 5 mins. 37°C; Lane 5: Myofibrils + cathepsin B, pH 5.5, 60 mins, 37°C; Lane 6: Myofibrils + cathepsin B + Leupeptin, pH 5.5; Lane 7: High mol. wt. markers; Lane 8: Low mol. wt. markers; Lane 9: Myofibrils from glycerol stock; Lane 10: Myofibrils held at pH 5.5 for 1 hr. at 37°C; Lane 11: myofibrils + cathepsin D, 5 mins., pH 5.5, 37°C; Lane 12: Myofibrils + cathepsin D, 60 mins. pH 5.5, 37°C; Lane 13: Myofibrils + cathepsin D + pepstatin. VHMW = Very High Molecular Weight. MHC = Myosin Heavy Chain. M_r = Relative Molecular Mass.

MODELING CHANGES WITH AGEING IN THE MECHANICAL PROPERTIES OF BEEF CONNECTIVE TISSUE

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Background

Perimysium connective tissue is known to play an important role in the variations in meat toughness. It is a network of collagen fibers organized in ply and embedded in a ground substance made of various proteoglycans (Eggen, Malmstrom, Sorensen and Host 1997) which constitute the matrix of the tissue. During ageing, there is a weakening of the perimysium which separate into collagen fibers and fibrils. Whereas collagen remains unchanged at the molecular level, however, during ageing, there is a degradation of proteoglycans. This degradation as been put forward to explain the weakening of the perimysium connective tissue during storage. Nevertheless there are no data on the mechanical properties of this matrix and of these changes with treatments.

Objectives

The aim of this study was to quantify the mechanical changes occurring in the matrix of proteoglycans of perimysium connective tissue during storage.

Materials and methods

Muscles: *Semimembranosus* muscles were taken from a Charolais - Frisonne cow (3 ½ years old). The muscles were removed from the carcass at 24h *post-mortem*. They were divided into several parts which were vacuum packed and stored at 4°C for 2 or 14 days and then frozen.

Measurements :
All the samples were thawed in water at 10°C. Sheets of perimysium (approximately 10 mm long x 4 mm wide) were dissected and tested with a micro-tensile device developed in this laboratory, for the determination of breaking stress, breaking strain, Young's modulus, breaking energy and total energy. The width of the samples were determined using a microscope and the thickness determined with a Mitutuyo micrometer under a force of 0.2N applied on the whole surface of the sheets. During the tensile test the samples were immersed in meat drip. The procedure for handling samples is similar to that described by Lewis and Purslow (1989). After dissection, strips of perimysium were glued on aluminum foil frames with cyanoacrylate glue. The samples on the aluminum frames were then fixed on the micro-tensile device so that the direction of tensile strain corresponded to the direction of muscle fibers.
The extension rate was 130 µm/s. The software applied a slack toe correction to the force – displacement curves to remove the part of the displacement where collagen fibres are just unfolded. The Young's modulus was calculated as the slope in the straight region of the stress – strain curves.

Statistical analysis :
For all mechanical variables means were obtained from 80 to 120 measurements. Data were analysed using the general linear model procedure of SAS Software (SAS/Stat Cary, NC: SAS Institute Inc., 2000).

Mechanical model

The purpose of this model was to quantify the changes in Young's modulus and Poisson ratio of the matrix during ageing. The model proposed in this paper combines laminated membrane theory and reciprocal identification method to estimate the Young's modulus and Poisson ratio of the matrix. Evolutive anisotropy of the tissue due to the reorientation of collagen fibres with the perimysal strip elongation is taken into

account from measurements of the variation with strain of the angle between collagen fibres and the direction of strain. As a first approximation, the model does not account for the crimping effect along the collagen fibres.

Model description:

Four engineering constants are needed to describe the mechanical behaviour of a ply; the longitudinal Young's modulus E_L, the transversal Young's modulus E_T, the longitudinal Poisson ratio ν_{LT} and the longitudinal shear modulus G_{LT}. They are expressed in terms of engineering constants of the constituents and fiber volume fraction using a parallel system for E_L, ν_{LT} and a series system for E_T and G_{LT}. Then the on-axis stiffness matrix [Q] of each ply can be computed and the off-axis stiffness matrix [Q(θ)], expressed in terms of [Q] and a transformation matrix [T(θ)]. Next, laminated membrane theory is used to derive the mechanical behavior of the perimysal strip made of two plies lying at +θ and - θ to the muscle fiber direction (Christensen, 1991). As expected in the case of tensile test, the shear stress in a ply is balanced by the oppositely directed shear stress in the second ply, such that the net shear force over the tissue is cancelled out. The equations of in-plane behavior of the tissue are not linear as usual because of the dependency of fibers orientation on the strip elongation; so iterative methods must be used to compute the stress-strain curves. As the model does not account for the fibers crimping, the initial non-linear region of the tensile curve can not be described.

Reciprocal identification method:

It has been assumed according to Liu, Nishimura and Takahashi (1994) that the collagen of intramuscular connective tissue remains unchanged at the molecular level during *post-mortem* ageing of meat. The model is also based on the assumption that the matrix is mechanically isotropic, which is a simplification as proteoglycan matrix is not amorphous. The following data were used for the simulations: collagen fiber Young's modulus E_f= 1000 MPa, collagen fiber Poisson ratio ν_f = 0.5, fiber volume fraction V_f=8%. For given values of matrix Young's modulus E_m and Poisson ratio ν_m, a numerical stress-strain curve of the perimysal strip was computed and the gap between numerical and experimental curves was evaluated. Discrepancies were reduced by adjusting E_m and ν_m. The fitting were done on part of the stress-strain curves limited by strain = 0 and breaking strain.

Results and discussion

The data in Table 1 show the overall changes in mechanical properties of perimysium sheets due to storage. Significant reductions were observed in breaking stress and Young's modulus.

Duration of storage (Days)	Breaking Stress (MPa)	Breaking strain	Modulus (MPa)	Beaking energy (mJ)	Total energy (mJ)
2	0.76 *a*	0.77 *a*	1.34 *a*	2.9 *a*	5.2 *a*
14	0.51 *b*	0.70 *a*	1.05 *b*	2.6 *a*	4.1 *a*

Table 1: Mechanical properties of perimysium sheets
In each column the values followed by different letters are significantly different at a 5% level.

The results of the modeling are given in Table 2. It appeared (data not shown) that when both Poisson ratio and modulus of the matrix were adjusted freely by the software to give the best fit of the stress – strain curves then the final value of the Poisson ratio was systematically negative which means that during the tensile test there is an expansion of the matrix volume. It is not a classical behavior of materials although already mentioned by Vincent (1990). So, the Poisson ratio of the matrix was fixed in the range $0 < \nu_m < 0.5$.

Storage (days)	N	Modulus (MPa)
2	80	0.47 *a*
14	119	0.38 *b*

Table 2 : Matrix modulus obtained from modeling.
In each column the values followed by different letters are significantly different at a 5% level

There was a reduction of about 20% of the matrix modulus between 2 and 14 days of storage. The Poisson ratio being zero at 2 and 14 days.

Conclusions

The results obtained in this study agreed with those from Nishimura, Hattori and Takahashi (1995, 1996) in beef, which showed that there is a progressive weakening of perimysium connective tissue during storage explained by a degradation of proteoglycans. This study shows that after 14 days of storage there is a a 30% reduction in breaking stress and a 20% reduction in Young modulus measured in tension. A similar decrease in breaking stress has been found by Lewis, Purslow and Rice (1991). A mechanical model based on the composite theory has been developed in order to quantify the mechanical changes occurring in the proteoglycans matrix during storage. It appears that a decrease of the modulus of the matrix by 20 % associated with a null Poisson ratio can explain the changes observed at the level of the perimysium network between 2 and 14 days of ageing.
An improvement of the model is needed as it does not take into account the unfolding of collagen fibers and assumed that the matrix was isotropic.

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PROTEOMIC APPROACH TO CHARACTERIZE PALE AND DARK TURKEY BREAST MEAT SORTED BY IMAGE VIDEO

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Background

In the past decade, the turkey meat industry has developed new products based on cured and cooked meat. Meat processors need to adapt the raw material to further processing. The technological quality of the processed meat depends on its major component: the proteins. The state of the proteins will mostly determine the water holding capacity of fresh meat and colour, themselves correlated with pH value. A previous study has shown that early pH determines partly the colour of the fresh meat (Santé *et al*., 1996). Studying proteins requires the use of separating techniques such as 1-D electrophoresis. Utilisation of 2-D electrophoresis is developing since it allows separation of several hundreds of proteins. Further steps includes identification of spots of interest using mass spectrometry. Then, characterisation of meat could be performed through its protein mapping. Besides, Lametsch *et al*. (2003) showed that proteome was correlated to textural trait in pig meat.

Objectives

Predicting the process ability of meat is challenge for meat processors. Even if the turkey BUT9 represents 95% of slaughtered turkey in France and the environmental conditions (slaughter, chilling, etc..) are well defined, the variability still exist in terms of meat quality, i.e. WHC and colour. These traits are influenced by (denaturation of) sarcoplasmic proteins (Sosnicki *et al*., 1998; Wilson & Van Laack ,1999). The aim of the study is firstly to use video image colour on line to sort turkey breast meat according to their potential yield to prevent excess of purge and secondly characterize this meat according to its 1-D and 2-D electrophoretic protein patterns.

Materials and methods

Animals and samples

A total of one hundred and twenty turkey breast muscles were collected in a slaughter plant on the cutting line. The breast meat was obtained from 15-week old male turkeys (line BUT 9) after conventional chilling over a minimum of 8 hours. Firstly selected visually to obtain the largest range of colour, from lightest to darkest, the breast meat colour was measured using a tri-CCD DXC 990 P SONY camera and analysed according to the method developed by CEMAGREF (Marty-Mahé *et al*., 2002). The ultimate pH value (pHu) was determined 24 h post mortem using a portable pH meter equipped with a glass electrode directly inserted into the muscle. The results were expressed as L*, a*, b*, C* and h* coordinates for lightness, redness, yellowness, chroma ($C= (a^{*2}+b^{*2})^{1/2}$) and hue angle (h=arc tang (b*/a*)), respectively. Part of breast meat was cut into scallops, weighted Day1, wrapped and weighted again after 48 h.

The breast meat was subsequently vacuum packaged, immediately frozen and stored at –20°C for further analysis. After thawing, the cooking yield was measured on a muscle sample of standard size (15 x 5 x 2,5 cm; 337 ± 14 g, n=170) after injection of brine (136 g of nitrite salt x l^{-1}) at the rate of 15 %, vacuum packaging and cooking in a water bath until a 68°C internal temperature was reached.

Protein breast samples were frozen in liquid nitrogen and stored at –80°C until analysis.

Protein extraction/fractionation

The extraction buffer consisted of KCl 50mM, Tris 20mM, $MgCl_2$ 4mM, EDTA 2mM, pefabloc 5mM, DTT 2% (w/v) 1% pH 7. 150 mg of muscle were added to 1 ml of extraction buffer in an Eppendorf containing a glass bead. Homogenisation was performed using a Retsch MM2 agitator (Retsch, Haan, Germany) for 1 h at 4°C. Homogenates were centrifuged at 10,000g for 15 min at 10°C. Supernatant was collected, pellet was

washed 3 times using a washing buffer (KCl 75mM, $KH2PO_4$ 10mM, $MgCl_2$ 2 mM, EGTA 2mM, pH 7). Protein content was measured using a Bio-Rad Bradford protein assay kit.

1D eletrophoresis

SDS PAGE was performed on both pellet and supernatant using a Hoefer 250 cell (Amersham) on 11% polyacrylamide gels at 40 volts for 1h then 110 volts for 2 h. Gels were stained with blue coomassie. Gels images were acquired using a GS-800 densitometer and analysed using Sigmagel software (SPSS, Chicago, USA).

2D electrophoresis

2D electrophoresis was performed on 3 samples per group (C1 vs C4). Immobilised pH gradient (IPG) isoelectric focusing (IEF) was carried out in a Protean IEF cell (Biorad), using Bio-Rad ready strip , 17 cm, pH 3-10 non linear. 90µg of sarcoplasmic proteins were loaded onto the strips for analytical gels. Proteins loading on strips, IEF and SDS-PAGE were performed according to Morzel *et al*. (2004). Gels were silver stained following the protocol of Yan *et al*. (2000). Gels were produced in triplicate. Gels images were acquired using a GS-800 densitometer and analysed using the PDQuest software (Bio-Rad).

The meat quality data were subjected to analysis of variance using the general linear model procedure of SAS, and the means were separated using the Scheffe's test. 1D and 2 D electrophoresis data were analysed using student test.

Results and discussion

Meat quality

The muscle samples were distributed in 4 groups according to their L* value obtained by image analysis: 45-48 (C 1, n=18), 49-52 (C 2, n=66), 53-56 (C 3, n=58), and 57- 60 (C 4, n=30). Significant differences between C1, C2, C3 and C4 were found for the ultimate pH. Drip loss was higher in lighter meat (C4) but this difference remained not significant. Curing and cooking yield decreased as the lightness increased (Table 1). The Characterisation of turkey breast meat using image video was efficient to sort the meat according to its processing yield.

1D electrophoretic protein pattern

Sarcoplasmic proteins

The gels analysis showed that the density of some proteins increased in C4 group meanwhile others decreased (Table 2, Figure 1). Glycogen phosphorylase density tended to be lower for C4 group (4.7 vs 3.7), as well as creatine kinase (15.2 vs 14.3). In the C1 group, aldolase and enolase had lower density on gels. Glycogen phosphorylase and creatine kinase lower contents in C4 group could be explained by more protein denaturation or / and protein degradation. In unstructured ham, partly characterized by it very pale colour , it was reported that creatine kinase was proteolysed (Sayd *et al*., 2003). In fact, group C4 showed similar traits: pale colour, higher drip loss and lower curing and cooking yield.

Myofibrillar proteins

Small differences on the proteins pattern were found between the two groups C1 and C4 at 24 h. However, in C4 group, we observed the absence of a band at 35-40 kDa approximatly (Figure 2). Again for this group, a 30kDa band was present at a higher density. This band has been well described as a marker of proteolysis in muscle. For turkey, one day ageing was enough to get a 30kDa fragment, which has been well described as a product of troponin-T proteolysis (Ho *et al*., 1996; Negishi *et al*., 1996). 1D electrophoresis did not allow to show more differences as expected in such a group of meat quality.

2D electrophoretic protein pattern

Up to 600 spots were detected per gel. After removal of saturated or poorly reproducible zones, 334 spots were successfully matched across the whole set of the 2 goup (C1 and C4) images. The relative quantities of spots from each group were analysed. Differences were noted when the ratio of the relative quantity was above 2: 158 spots did not differ among the two groups, 26 spots had a higher quantity in the C1 group (darker meat) and 150 spots had a higher quantity in C4 group. 18 spots were only present in the C4 group gels. Among them, one spot is suspected to be a creatine kinase fragement by comparison to results of Sayd *et al*. (2003). With our data, we are not able to evaluate protein denaturation neither its level. However, Van

laack (2000) reported that other factors than protein denaturation are responsible for the low water-holding capacity of pale, soft, exudative chicken breast muscle. So, our results showed higher protein solubilisation, which could indicate either less denaturation or more proteolysis in the paler meat. In further work we will identify spots of interest (either proteins or fragments) by Maldi-tof spectrometry.

Conclusions

The characterisation of turkey breast meat using image video was efficient to sort the meat according to its processing yield : as the lightness increased, curing and cooking yield decreased. In 1 DE gels, proteins pattern differed in a small extent between the paler and darker meat (C4 group *vs* C1 group). Using 2DE gels allows the separation of 10 to 20 more protein bands. The meat with the lower processing yield differed by a higher level of protein solubilisation, indicating either less denaturation or more proteolysis in the paler meat.

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Table 1. Colour, pHu and cooking yield according to lightness (L* value) as measured by image analysis

Group (L* limits)	pH (24 h)	drip loss (%)	Curing and cooking yield
C1 (45-48)	6.01 ± 0.21 [a]	1.92 ± 1.25	93,7 [a]
C2 (49-52)	5.90 ± 0.13 [a]	1.93 ± 0.59	92,6 [a]
C3 (53-56)	5.94 ± 0.22 [a]	1.77 ± 0.57	90,5 [b]
C4 (57-60)	5.77 ± 0.16± [b]	2.48 ± 0.42	89,2 [b]

Table 2: density values of some sarcoplasmic proteins measured on 1 DE gels.

Protein band	C1	C4	anova
B1 phosphorylase	4.7± 1.9	3.7± 1	ns
B5 enolase	13.6± 1.2[a]	15.4± 1.1[ab]	0.04
B6 créatine phosphokinase	15.2± 1 [a]	14.3± 1[b]	0.038
B7 aldolase	13.5±1.5[a]	17.6± 1.6[c]	0.0012

Figure 1

1DE gel : sarcoplasmic proteins from groups C1 and C4

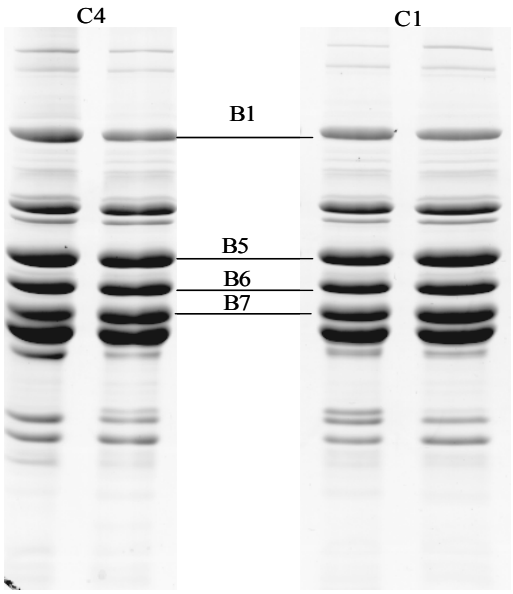


Figure 2

1 DE gel : myofibrillar proteins from groups C1, C2, C3 and C4

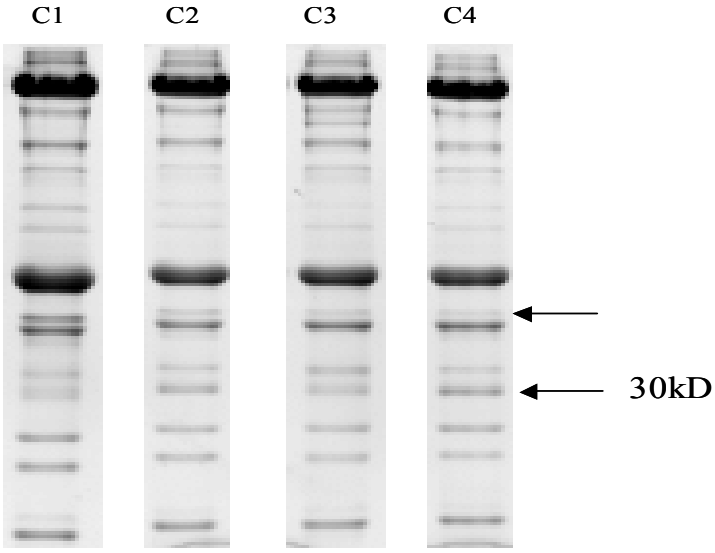
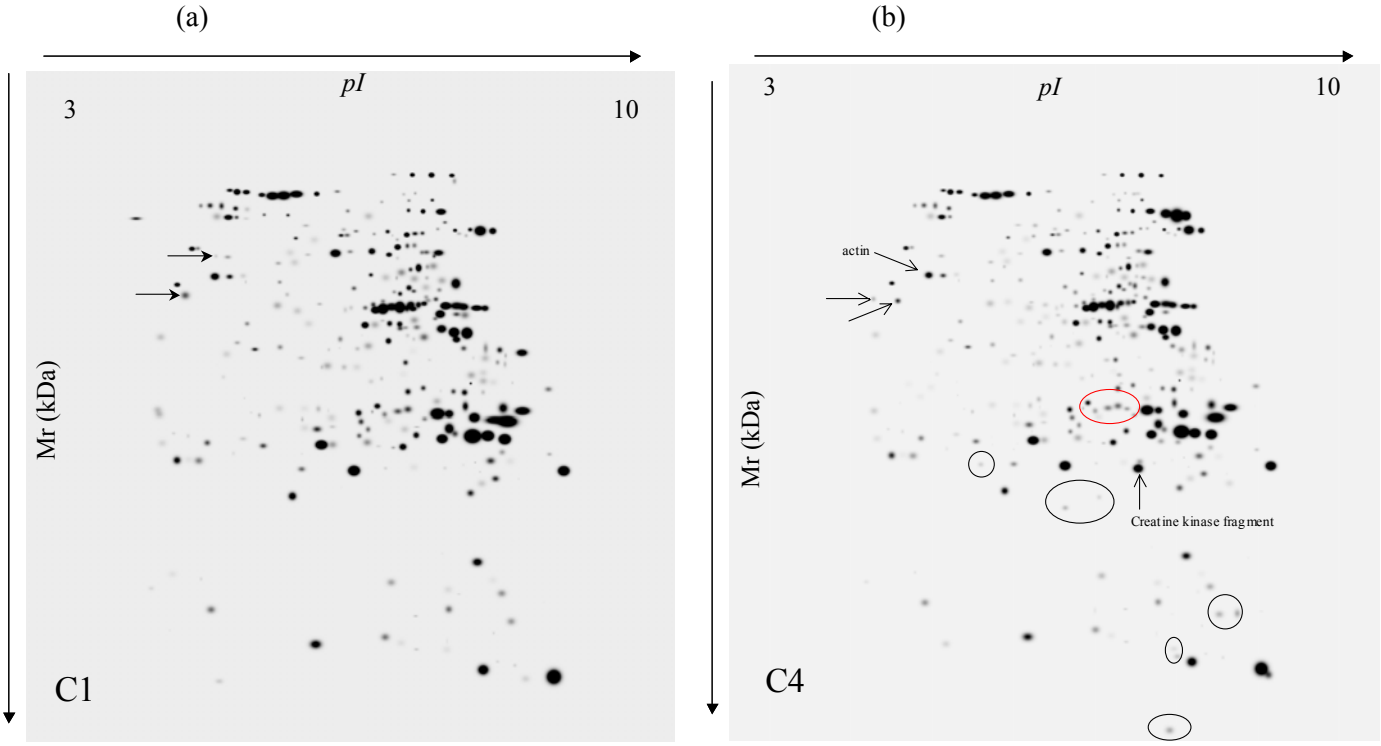


Figure 3 : Computer-generated master gels corresponding to all gels of *Pectoralis superficialis* muscle from C1 (a) and C4 groups (b)



BLOCKING AGENTS FOR ELISA QUANTIFICATION OF CATHEPSIN L IN BOVINE AND PORCINE MUSCLE CRUDE EXTRACTS

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Background

Consumers have considered from long time tenderness as the principal quality attribute of meat. Related to this, Jeremiah (1982) concluded that the most common cause of unacceptability in beef, pork and lamb meat purchases was toughness. This problem is strongly related with the inconsistency and unpredictable variability of meat texture, which avoids the standardization of meat quality and satisfaction of consumer demands, being one of the main problems that Meat Industry actually has to solve. The main origin of this variability is the large biological diversity of skeletal muscle [Pette and Staron, 1990]. Therefore it is not surprising to observe, in standardized processing conditions, a large animal variability in the rate and extent of postmortem meat texture development.

Endogenous muscle proteolytic enzymes are assumed to play a key role in this question, because they are responsible of myofibrilar disruption during ageing, a phenomenon directly related with the development of meat tenderisation [Sentandreu et al., 2002; Ouali, 1992]. So, the possibility to accurately quantify levels of some of these proteolytic enzymes has been proposed as a way to explain and predict meat texture variability. Cathepsin L is one of these target enzymes. Methods actually in use for quantification are based in the determination of its endopeptidase activity. However, quantification of cathepsin L activity in muscle crude extracts is imprecise due to absence of a specific substrate, being the interfering action of other peptidases, such as cathepsin B, always present. Immunochemical methods such as Enzyme Linked Immunosorbent Assay (ELISA) can be an interesting alternative for a more specific, sensitive and faster quantification of cathepsin L directly in crude extracts allowing a correct analysis of large number of samples per day. However, attention must be paid because ELISA can display important sources of error when not working in the appropriate conditions. Normally, the development of an ELISA test includes a first coating step consisting in the adsorption of antigens or antibodies to a plastic surface by non-specific binding (NSB). However, NSB of other undesired protein components during subsequent steps of the assay can give an overestimation of the signal not corresponding with the desired antigen-antibody reaction, which is detrimental for its sensitivity and specificity. Traditionally, undesirable NSB may be minimized by saturating the remaining binding sites of the plastic surface with different protein additives that must not interfere with the immunoassay. Not all blocking proteins that are commonly used for that purpose are adequate for each particular ELISA test, being necessary to determine the suitable one by empirical testing.

Objectives

In a previous work, a specific polyclonal antibody against highly purified bovine cathepsin L has been raised in rabbits [Sentandreu et al., 2004] with the aim to develop an immunoassay able to overcome the actual difficulties existing for cathepsin L quantification. The present work had as main objective the study of various blocking agents in their ability to prevent NSB of the reactants, other that capture IgG in the coating step, that will be utilized for the quantification of cathepsin L from both bovine and porcine muscle crude extracts by sandwich ELISA.

Materials and methods

Preparation of muscle crude extracts: 500 grams of either bovine or porcine muscle were used as the starting material to prepare a lysosomal-rich extract as initially described by Lardeaux (1983). The extract was then fractionated with ammonium sulphate, collecting the precipitated protein between 30-70 % saturation. The pellet was redissolved in a minimum volume of 20 mM Bis-Tris buffer, pH 6.0, containing 200 mM NaCl and 1 mM EDTA. The redissolved extract was aliquoted in volumes of 200 µl and immediately frozen and stored at –20 ºC until used.

Development of specific IgG against bovine cathepsin L: A polyclonal antibody against purified bovine cathepsin L was raised in rabbits as described in a previous work [Sentandreu et al., 2004]. The IgG fraction was obtained by chromatography on Q-Sepharose Fast Flow, being a part of this IgG fraction biotinylated.
ELISA protocol for the present study: Microlon microtiter plates, each one containing 96 flat-bottom wells of high binding capacity (600 ng/cm^2) were routinely used for the assays. Effectiveness of each blocking agent in preventing NSB was assayed over a range of eight different concentrations of crude extracts ranging from 0 to 4,600 and 0 to 8,600 µg/ml of bovine and porcine muscle crude extract concentrations, respectively. 1) In a first step columns of eight wells, corresponding to the different muscle crude extract concentrations, were incubated with either solutions of goat serum, skimmed milk, bovine serum albumin (BSA), fish gelatine (surcoating step, 5 % in PBS) or with PBS only (no surcoating step), for 1 h at 37 ºC. At the end of each incubation step wells were routinely washed five times with PBS containing 0.1 % Tween 20 (washing buffer); 2) After washing, wells were incubated with the eight different concentrations of bovine or porcine muscle crude extracts during 1 h at room temperature, using the washing buffer containing the corresponding blocking agent (1%) as diluent (assay buffers). Additionally, two columns per plate were not exposed neither to a surcoating step nor addition of any blocking agent to the assay buffer, thus serving as reference for determining total NSB on the plastic surface at each muscle crude extract concentration; 3) Wells were incubated with the biotinylated IgG fraction of the developed polyclonal antibody against cathepsin L in a 1/2000 dilution with each one of the assay buffers for 1 h at room temperature. After washing, wells were incubated with Extravidin®-horseradish peroxidase conjugate (Sigma, St. Louis, MO; 1/1000 dilution in assay buffers) for 1 hour at room temperature. Peroxidase activity was determined by incubation of wells with 3,3' 5,5'- tetramethyl-benzidine liquid substrate (TMB), 20 min for bovine samples and 10 min for the porcine ones. Reaction was stopped with a commercial stop reagent (Sigma), reading the O. D. values at 650 nm in an automated plate reader.

Results and discussion

The ELISA protocol developed in the present work aimed at the evaluation of different types of blocking agents in their capacity for avoiding unwanted NSB. So, the experimental protocol design presented here is basically the sandwich ELISA that will be used for cathepsin L quantification but avoiding the initial coating step of binding the capture antibody. In these conditions, the effectiveness of blocking agents will be proved by their ability to avoid any kind of binding to the plastic surface, which will be reflected in the absence of O. D. at 650 nm due to peroxidase activity.
The developed polyclonal antibody was raised against bovine cathepsin L, but previous studies have shown that this antibody cross-react with porcine cathepsin L so that quantification of this latter would be also feasible. In figure 1.A we can see results obtained for the ELISA carried out with different concentrations of bovine muscle extract, whereas figure 1.B shows the same ELISA but performed with a porcine muscle crude extract as antigen. In both assays the positive control, serving as reference for total NSB, was given by the curve developed in absence on any blocking protein.
In the case of bovine (figure 1.A), we can observe that skimmed milk was the most effective blocking agent, avoiding NSB for all of the assayed crude extract concentrations, obtaining fully comparable results with or without the surcoating step. Only for the highest concentration (4,600 µg/ml extract) we can observe a little increase in the O. D. value but negligible compared to the reference curve. This would indicate that using skimmed milk as blocking agent the surcoating step could be suppressed, always that it was contained in the reaction buffer. Similar results were obtained for porcine species except for the highest crude extract concentration (8,600 µg/ml extract), in which the blocking capacity of skimmed milk is lost (see figure 1.B). As reported in the literature, this can be due to electrostatic interactions between antigen, antibody and/or maybe other sample compounds because of the high concentration of the reactants. Like bovine, the surcoating step wouldn't be necessary, as previously pointed out [Vogt et al., 1987]. Other authors working with completely different samples also observed the effectiveness of skimmed milk in preventing NSB [Kaur et al., 2002; Zimmermann and Regenmortel, 1989].
Even if they yielded slightly higher O. D. values, both BSA and goat serum proved to be also effective blocking agents for bovine samples. However, and contrary to skimmed milk, results clearly indicated that the surcoating step is necessary to achieve an effective blocking effect (see figure 1.A, differences of BSA and goat serum with and without surcoating). This is in opposition to results obtained by Pruslin et al. (1991), where BSA exerted an optimal blocking effect with and without the previous blocking step. The similar behaviour between serum and BSA can be explained by the fact that albumin is one of the major

components of the serum [Kaur et al., 2002]. Opposite to bovine, neither BSA nor goat serum exerted an effective blocking of NSB for pork crude extracts. With the previous surcoating step, only a partial blocking effect was achieved, whereas in its absence similar O. D. values to the reference curve were obtained (figure 1.B), in accord to results obtained by Zimmermann and Regenmortel (1989).

The worst blocking results were obtained with fish skin gelatine. Even if it is commonly used and it has been recommended as a model blocking agent between gelatine preparations [Vogt et al., 1987], we realized that fish skin gelatine was only able to partially prevent NSB of bovine crude extract when the surcoating step is done (figure 1.A), whereas in the case of pork we did not observe any blocking effect, being the O. D. values obtained for all the concentrations in both cases (with and without the surcoating step) similar to that of the reference curve (figure 1.B).

Comparing the O. D. values obtained for all curves in the absence of muscle crude extract (zero values of the X-axis) we can observe that even if there are some differences, these are minimal. This indicated that in the absence of crude extract, biotinlylated anti-cathepsin L IgG and/or Extravidin®-peroxidase conjugate did not inespecifically bind to the plastic surface, being also true for the reference curve.

Conclusions

In the development of a sandwich ELISA test for bovine or porcine cathepsin L coming from muscle crude extracts, the choice of and adequate protein agent is essential to prevent non-specific binding (NSB) of components itegrating the immunoassay with the solid support. As previously suggested [Engvall and Perlmann, 1972; Mohammad and Esen, 1989], NSB of the anti-cathepsin L polyclonal antibody and/or Extravidin®-peroxidase conjugate can be minimized just with the inclusion of Tween 20 in the reaction buffer. However, and contrary to them, for reduction of background due to NSB of muscle crude extracts (antigen) to the matrix, it is necessary the use of a suitable blocking agent in addition to Tween 20. Binding of some components of the crude extract to the plastic surface is detrimental for sensitivity and reproducibility of the assay because we do not know if the obtained O. D. values were exclusively due to the detection of bound cathepsin L or to the union of the antibody to other absorbed components from the extract.

Skimmed milk revealed to be the most efficient of the assayed blockers, as also observed by other authors. In the case of pork, this would be the only suitable blocking agent but avoiding the highest assayed concentration. All the same attention must be paid, since some authors pointed out the possibility that skimmed milk may interfere the antigen-antibody reaction under certain conditions [Mohammad and Esen, 1989; Vogt et al., 1987]. For the ELISA quantification of cathepsin L from bovine species, goat serum and BSA, together with skimmed milk, could be used as effective blockers always that a surcoating steps be included in the assay in addition to be present in the assay buffer.

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Acknowledgements

This work was supported by a Marie Curie Individual Fellowship from European Commission (QLK1-CT-2002-51527) attributed to Sentandreu, M. A.

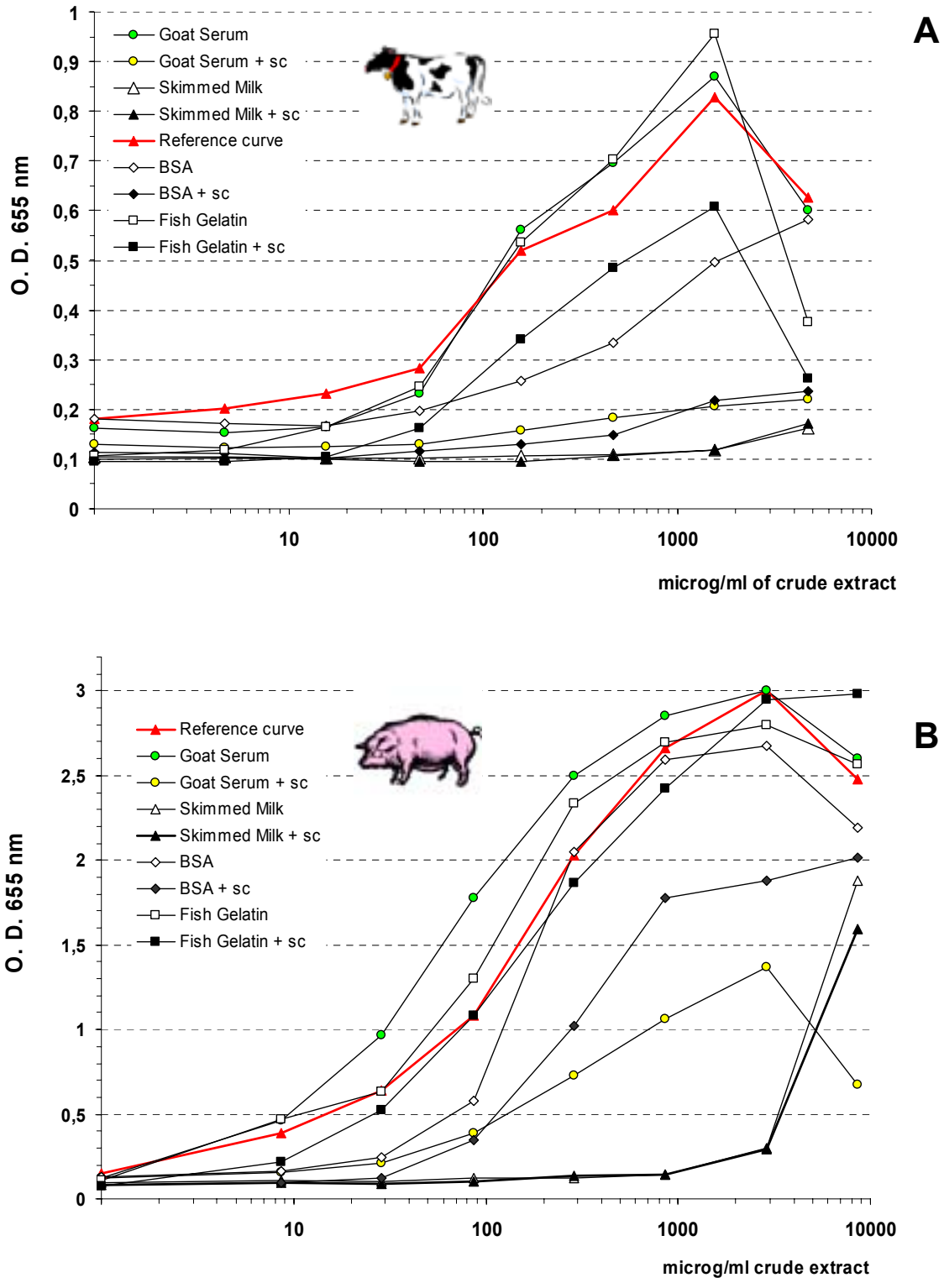

Figure 1: Effectiveness of blocking agents for preventing non-specific binding in indirect ELISA for cathepsin L using increasing concentrations of A) bovine or B) porcine muscle crude extracts. Legends showing "+ sc" indicate the curves in which a surcoating step has been carried out with the corresponding blocker. Those not showing "+ sc" belong to curves for which the surcoating step was not carried out, the corresponding blocking agent being only contained in the reaction buffer. Experimental points placed on the Y-axis correspond to 0 microg/ml of crude extract.

THE INDUCIBLE FORM OF HSP70 IN PORCINE SMALL INTESTINE, COLON AND SERUM

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Background & Objectives

Modern swine production includes practicalities that may be stressful to pigs. At cellular level, stress response includes synthesis of special stress proteins, heat shock proteins (HSP), which have several roles in helping to maintain the protein homeostasis. HSPs form a protein family, classified according to their molecular weights. One of the best-known is the 70 kDa family (HSP70), which contains both inducible (HSP72) and constitutive (HSP73) forms. Many environmental and pathological factors are known to activate HSP72 expression, also including psychological stress (Fukudo et al. 1997). Altough HSPs are intracellular proteins, small amounts of HSP72 can be found in serum, also in unstressed individuals (Pockley et al. 1998), but the source of serum HSP is still unclear. Among the organs that respond to stress, intestines belong to those that are most stress-sensitive (Söderholm & Perdue 2001). The main question asked in this study was whether HSP72 in the intestines varies with growth rate, and whether serum HSP72 correlates with known indicators of stress.

Material & Methods

40 experimental pigs (EP) used in this study were raised individually in 1.0 x 2.5 m pens and slaughtered two together at an experimental slaughterhouse. Additional samples were taken from 10 ordinary slaughterhouse pigs (SP). Five of the SP were physically injured (muscle and tail) and five showed no signs of injury. The animals were slaughtered in a commercial slaughterhouse and stunned with CO_2. The samples from small intestine, colon and blood were taken in the connection of slaughter. The inducible HSP70 was analysed immunologically, blood lactate concentration with a lactate analyser and serum cortisol by radioimmunoassay. Differences in the amounts between the two groups were calculated by paired t-test using GraphPad Prism software (GraphPad Software, USA). Differences were regarded as significant at $P=0.05$. Linear regression analysis was used to calculate the coefficients of correlation.

Result & Discussion

The carcass weight of the 40 EP was 81.0 ± 7.5 kg and that of SP significantly ($P<0.05$) lower, 74.7 ± 10.9 kg. HSP72 was detected in the small intestine, colon and serum. Amounts of HSP from all tissues studied were significantly higher in SP than EP ($P<0.001$), but also among SP the injured pigs had higher colon HSP72 than the apparently healthy pigs ($P<0.05$). There were positive correlations between the amounts of HSP72 in colon and small intestine ($r=0.54$; $P<0.001$; $n=50$), colon and serum ($r=0.66$; $P<0.001$; $n=50$), and small intestine and serum ($r=0.41$; $P<0.01$; $n=50$). A negative correlation was found between carcass weight and HSP72 content in colon ($r=-0.54$; $P<0.001$; $n=50$) or serum ($r=-0.45$; $P<0.01$; $n=50$). HSP72 in colon correlated with carcass weight also in both groups separately (SP, $r=-0.70$, $P<0.05$, $n=10$; EP, $r=-0.35$, $P<0.05$, $n=40$). Lactate and cortisol concentrations did not correlate with HSP72 in any tissue.

The negative correlation between HSP72 in colon and carcass weight suggests that pigs with high concentration of HSP72 were stressed and did not grow as well as less stressed animals with lower HSP72 concentration. This speculation is supported by a finding, which shows that chronic stress reduces the growth of the animal (Santos et al. 2000). We detected HSP72 also in serum and there was a positive correlation between HSP72 in serum and colon. A closer analysis of the data shows that the levels of serum HSP72 start to rise when colon HSP72 reaches approximately 3.9 μg/g tissue. If serum HSP72 comes from the colon, this may represent the point when colonal epithelium starts to loose its integrity.

Conclusions

The correlation between HSP72 in serum and colon suggests that at least part of the serum HSP72 comes from the colon, but a larger number of pigs is needed to show whether serum HSP72 is a useful stress indicator.

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Söderholm, J.D.; M.H. Perdue. 2001. Am. J. Physiol. 280: G7 - G13.

EFFECT OF LAIRAGE TIME ON INCIDENCE OF DIFFERENT QUALITY OF *M. SEMIMEMBRANOSUS* FROM HALVES OF MULTI-RACE HYBRIDS

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Background

Pigs are prepared for slaughter in the lairage, and the aim of resting is to bring back the animals to »normal« state, as they were at the breeding place, e.g. the resynthesis of glycogen in the muscles and removal of metabolyte products. The resting time should be proportional to the fatigue due to transport, elsewhere, longer time may have a contrary effect. While in lairage, unnecessary stress and disturbances should be avoided (Rede and Ljiljana Petrović, 1997; Lawrie, 1998). According to a number of authors, resting after transport of pigs affects positively the quality of meat. Fortin (1988) found decrease of PSE incidence by 27% in pigs slaughtered 3 hours after the transport at the slaughterhouse, compared with pigs slaughtered next to the arrival. Grandin (1989) found 1,3% of PSE meat incidence in pigs slaughtered 2,5 h after the arrival to the slaughterhouse, and in pigs slaughtered 15 min after the arrival the PSE meat incidence was significantly higher, up to 18%. The slaughter immediately or a short time after the arrival can increase the incidence of PSE meat (Eikelenboom et al., 1991). Malligan et al., 1998; van der Wal et al., 1997; Wariss et al. (1998) cite that the most favorable resting time is 2-3 hours. Bendal and Swatland (1988) and Wittmann et al. (1989) found that very long resting can induce the apearance of DFD meat.

Objective

The objective of this study was to investigate the quality of the halves of three-race pigs obtained in a mutual crossbreeding program at a farm in Serbia and Montenegro, and the influence of lairage time on meat quality. Further, to determine the incidence of different quality (RFN, RSE, PSE, DFD) on the basis of technological parameters pH_i, pH_u, WHC and L*, measured on *M. semimembranosus* as well as of our quality criteria (Tomović, 2002; Natalija Džinić et al., 2003, 2004).

Materials and methods

Two groups of pigs, multi-race hybrids, in all 33, were investigated. The pigs from the first group were slaughtered immediately after the transport (n=15) and the other group after resting of 48 hours in the lairage (n=18). The pigs were fed conventional feed during fattening, and heads of approximately same age and uniform mass were transported in autumn (october–november) to the slaughter-house, about 10 km from the farm. The pigs were stunned, debleeded and processed on the slaughterline applying standard technological procedure.

The quality of halves was determined on right halves at the end of the slaughterline, using the FOM-device (SFK-Technology, Denmark).

The temperature was determined in *M. semimembranosus* (MSM) of right halves 45 min post mortem (T_i) using the portable thermometer, HI 8757 (Hanna Instruments, Italy).

The pH_i was measured in right halves 45 min p.m., and pH_u 24 hours p.m. in the caudo-medial part (MSM) with pH-meter ULTRA X, UX-390 (Gronert, Germany) with INGOLD combined penetrating electrode.

Meat yield (%) was determined by partial dissection method (Walstra and Merkus, 1996) of cooled left halves.

Samples (200 – 300 g), taken at the caudo-cranial part (MSM) were used 24 hours p.m. for color and water holding capacity (WHC) determination. Colour was determined using the MOM Color 100 device (CIE L*a*b*) (Robertson, 1977). WHC was determined using the compression method and expressed as % of bound water (Grau and Hamm, 1957).

The incidence of different MSM quality depending on resting time in the lairage was determined on the basis of parameters and quality criteria for MSM quality (PSE: pH_i<5,8, pH_u<5,4, WHC<50%, L*>55; RSE: pH_i=5,8-6,0, pH_u=5,85-6,2; WHC=50-60, L*=50-55; RFN: pH_i>6,0, pH_u =5,4-5,85, WHC=60-70, L*=45-50; DFD: pH_u>6,2, WHC>70, L*<45).

Results and discussion

The average meat yield in halves of cross-breed pigs determined with FOM device was 52,60% and 52,15%, and the difference between two groups is not significant (P>0,05). The average meat yield determined by partial dissection is by 2% higher in both investigated groups of crossbreeds (54,21%, 54,48%) and the differences are not significant (P>0,05), compared with the average meat yield determined with FOM device.

Table 1. Quality of halves, of multi-race pigs rested for different time in lairage, determined with FOM device and by partial dissection

Lairage time	% lean meat (FOM)	% lean meat (partial dissection)
0 hour	52,60 ± 5,03	54,21 ± 4,78
48 hours	52,15 ± 3,87	54,48 ± 3,78

^{NS}P>0,05 *P≤0,05 ** P≤0,01

The results of investigation of technological parameters are presented in Table 2. It was found that the average pH_i of pigs slaughtered immediately after the arrival e.g. after 48 hours of rest in the lairage was 6,22 and 6,23, respectively, and these values respond to muscles of potentially normal quality (pH_i>6,0) (Tomović, 2002). Higher average pH_u (5,91) was found in MSM of multirace hybrids which were resting for 48 hours, and responds to muscles of RSE quality. The average pH_u (5,81) found in MSM of pigs slaughtered after arrival points to muscles of normal quality (pH_u=5,4-5,85). The average T_i of MSM from halves of pigs slaughtered without resting (42,2°C) was significantly higher compared to pigs rested for 48 hours (41,4°C). The average temperatures determined in MSM of multi-race hybrids are higher than the allowed temperature for meat with certificate (T_i=40°C) (Honikel, 1999, 2000). This finding indicates that the pigs were under great stress when slaughtered without resting, and that the stress was somewhat lower after 48 hours of resting. The stress induced fast glycolysis, which caused the increase of T_i, and this should reflect on the measured pH_i values, e.g. further decrease rate of pH_i values. Somewhat higher WHC (56,74%) was measured in MSM of pigs rested for 48 hours, however, not significantly higher than the WHC (56,25%) of MSM of pigs slaughtered without resting. On the basis of criterion for WHC, the investigated MSM were on the average of RSE quality (WHC=50-60%).
Regarding colour lightness L* of investigated MSM, it can be noticed that MSM of pigs slaughtered without resting are significantly lighter (L*=52,05) (P≤0,01) compared to lightness of pigs rested for 48 hours (L*=51,85). According to our criterion for colour lightness L*, the investigated MSM of pigs of both groups are of RSE quality on the average.

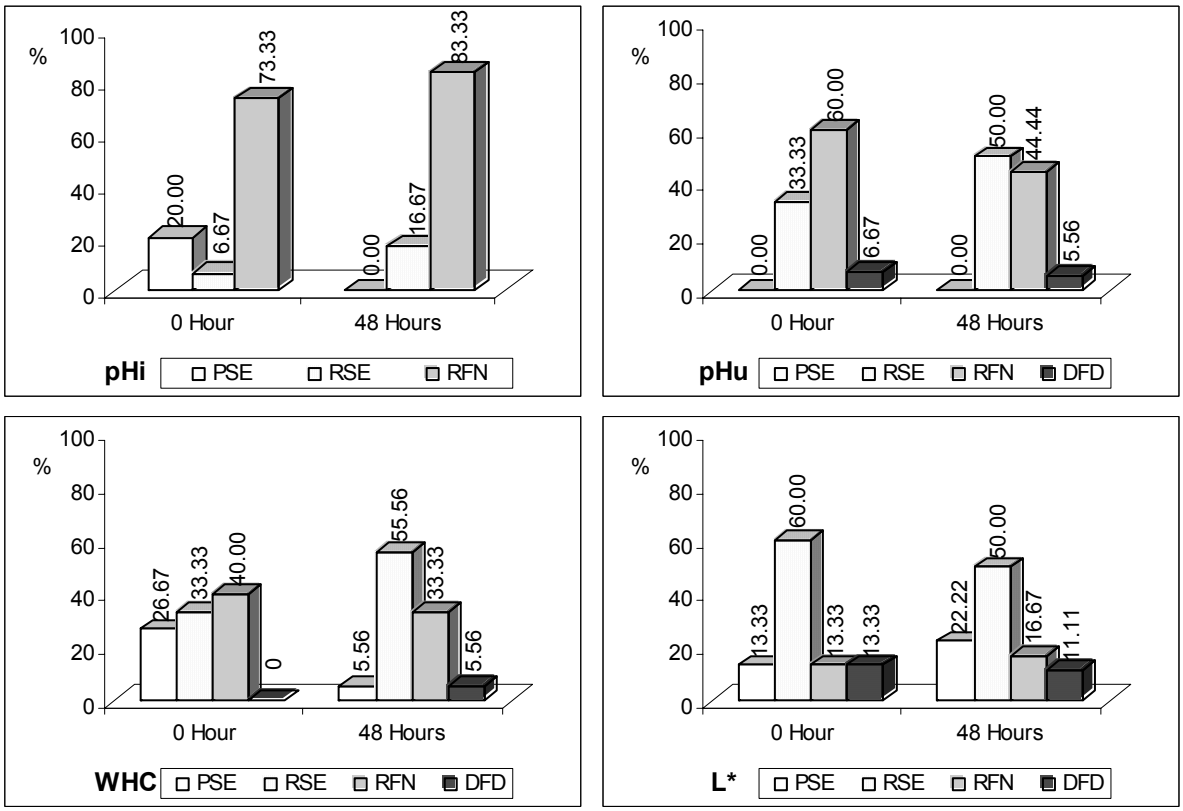
Table 2. Influence of lairage time of pigs on technological quality of *M. semimembranosus*

Lairage time	pH_i	pH_u	T_i	WHC	L*
0 hour	6,22 ± 0,30	5,81 ± 0,33	42,2 ± 0,74**	56,25 ± 7,91	52,05 ± 5,96**
48 hours	6,23 ± 0,23	5,91 ± 0,18	41,4 ± 0,58	56,74 ± 5,69	51,85 ± 4,94

^{NS}P>0,05 *P≤0,05 ** P≤0,01

The incidence of PSE, RSE, RFN and DFD quality of MSM of pigs rested for different time in the lairage is presented in Graph 1, on the basis of criteria for pH_i, pH_u, WHC (%) and L*.
According to the criterion for pH_i it was found that the potential incidence of MSM of RFN quality is higher (83,33%) in pigs which rested for 48 hours before slaughter, compared to pigs slaughtered without resting (73,33%). The obtained results show further that 60% of MSM of pigs slaughtered without resting and 44% of pigs slaughtered 48 hours after transport are of N quality, on the basis of criterion for pH_u. On the basis of criterion for colour lightness L*, it was found that the highest incidence of MSM (60%) is RSE quality in pigs slaughtered without resting and 13,33% of PSE quality, and in pigs slaughtered 48 hours after transport the incidence of PSE and RSE quality is 22,22% and 50%, respectively. Further, according to criterion for WHC, 40% e.g. 33,33% of MSM of pigs slaughtered without resting e.g. after 48 hours of lairage time are of RFN quality. A number of multi-race hybrids (three-race) was slaughtered after 24 hours of resting and the investigations showed that the incidence of MSM of RFN quality was 78,6% for the same season (autumn) (pH_i) (Natalija Džinić et al., 2004), 92,8% of RFN quality (pH_u), 7,1% of RFN quality (WHC) and 7,1% MSM of RFN quality (lightness – L*). It can be concluded on the basis of the presented results that MSM

quality of pigs was even lower (WHC and L*) when the resting time before the slaughter was 24 hours and regarding the results obtained in our investigations it is the most favorable to slaughter the pigs next to the arrival to the slaughter-house, e.g. that it is possible to decrease the incidence of lower quality meat prolonging the resting time to several hours (Malligan et al., 1998).



Graph 1. Incidence of different quality meat on the basis of criteria

Conclusion

The average meat yield in halves of cross-breed pigs after resting for different time in the lairage (0 and 48 hours) determined by FOM device was 52,60%, e.g. 52,15% (P>0,05). The average meat yield in halves determined by partial dissection was 54,21% and 54,48 (P>0,05). The investigated MSM of crossbreed pigs slaughtered immediately after the arrival were of RFN quality, on the basis of criterion for pH_u, and of pigs resting 48 hours in the lairage, according to the same criterion of RSE quality, on the average. The investigated MSM of both groups of crossbreeds was on the average of RSE quality, regarding the criteria for WHC and colour lightness – L*. The incidence of RFN quality of MSM, on the basis of criterion for pH_u is generally the highest 60% (without resting) and 44,44% (48 hours of resting). The incidence of RFN quality MSM is 40% e.g. 13,33% (without resting) e.g. 33,33% and 16,67% (48 hours of resting) according to criteria for WHC (%) and colour lightness – L*.

Acknowledgment

This work was supported by the Serbian Ministry of Science, Technologies and Development through the Project BNT.5.2.1.7101.B "Production of cooked ham"

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ZINC PROTOPORPHYRIN IX CONTRIBUTES TO THE BRIGHT RED COLOR IN PARMA HAM

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Background

Myoglobin in meat products to which nitrate and/or nitrite have been added is converted into stable red nitrosylmyoglobin coordinated to nitric oxide, and nitrosylmyoglobin changes into pink-reddish nitrosylhemochromogen after the meat has been heated. The north Italian traditional dry-cured ham "Prosciutto di Parma (Parma ham)" is made from only the leg of a fattened pig by salting with sea salt, drying, and maturing over a period of one year. Despite the fact that nitrite or nitrate has not been added, the color is an extremely stable bright red and is hardly changed by exposure of the ham to light. Morita et al. (1996) reported that the red heme pigment was easily extracted with 75% acetone and that it was a new myoglobin derivative unknown in meat and meat products. It has recently been reported that the amount of this lipophylic stable red pigment in Parma ham increases with aging (Parolari et al., 2003).

Objectives

The objective of this study was to identify the stable red pigment in Parma ham in order to obtain information for producing bright red meat products without nitrite and/or nitrate.

Materials and methods

Preparation of the red pigment from Parma ham: Minced Parma ham (5 g) was homogenized in 20 ml distilled water, and the homogenate was centrifuged (3,000 rpm, 5 min, 4 °C) and then filtered through a filter paper (No. 5C Toyo Roshi Co., Ltd., Tokyo, Japan). Three volumes of ice-cooled acetone were added to the filtrate, and the mixture was placed in ice for 15 minutes. The mixture was centrifuged at 3,000 rpm for 5 min at 4 °C. An equal amount of distilled water was added to the supernatant, and the mixture was applied to a disposable C18 column, Sep-Pak® Vac C18 Cartridge (12 cc/ 2g; Waters Co., MA U.S.A.) prewashed with 15 ml of methanol and 15 ml of distilled water. The column was washed with 25 ml 37.5% acetone, and then the red pigment preparation was eluted with 10 ml of 75% acetone. All of the operations were carried out under shading as much as possible.

Absorption and fluorescent analysis: The absorbance spectrum of the red pigment preparation was measured from 350 to 700 nm using a Model U-3210 spectrophotometer (Hitachi Ltd., Tokyo, Japan). The fluorescent and excitation spectra of the prepared red pigment were measured from 500 to 700 nm at 420 nm for excitation and from 300 to 500 nm at 590 nm for emission using a Model 650-60 fluorescence spectrophotometer (Hitachi Ltd., Tokyo, Japan), respectively.

Elemental analysis: Elemental analysis of the red pigment preparation was performed by scanning electron microscopy/energy dispersive X-ray microanalysis (SEM-EDX). The red pigment preparation from Parma ham was dried up using a centrifugal evaporator and was fixed on a metal stub using carbon tape. The sample was coated with carbon and was analyzed with a scanning electron microscope (S-800, Hitachi Ltd., Tokyo, Japan) equipped with an energy dispersive X-ray micro-analyzer (EMAX-2000, Horiba Ltd., Kyoto, Japan) with an accelerating voltage of 20 KeV and a spectral resolution of 10 KeV per channel.

Mass spectrometry analysis: Electrospray ionization high resolution mass spectrometry (ESI-HR-MS) analysis of the red pigment preparation from Parma ham was carried out using a JMS-SX120A (JEOL Ltd., Tokyo, Japan) equipped with an ESI ion source (JEOL MS-ESI 10, JEOL Ltd., Tokyo, Japan). The sample diluted in a chloroform/methanol/acetone (1:1:8 v/v) solvent mixture was infused into the ESI ion source at a flow rate of 1 µl/min. The needle voltage and capillary voltage were 2681 V and -1230 V, respectively. The chamber temperature was set to 105 □. A mixture of PEGs was used as an internal standard.

Results and discussion

The red pigment preparation from Parma ham was purplish-red, and the absorbance spectrum is shown in Fig. 1. A peak at 416 nm in the Soret band and peaks at 544 and 583 nm in the beta band were observed. Since this spectrum pattern was consistent with those previously reported (Morita et al., 1996; Parolari et al., 2003), the unidentified pigment in Parma ham could be prepared. Although metal-free porphyrins generally have four peaks in the beta band, the number of peaks in the beta band of metalloporphyrins decreases to two because of improvement in symmetry of the molecular structure. Therefore, the elemental analysis was carried by using a scanning electron microscope equipped with an energy dispersive X-ray micro-analyzer. As shown in Fig. 2, several peaks originating in zinc were observed, but no peak originating in iron was observed. This may indicate that the red pigment in Parma ham is not an iron-porphyrin complex but a zinc-porphyrin complex. A zinc-porphyrin complex emits fluorescence unlike many metalloporphyrins. Hence, the fluorescent and excitation spectra of the prepared red pigment were measured (Fig. 3). An excitation peak at 423 nm and emission peaks at 589 and 630 nm were observed. Since the fluorescent spectrum was similar to that of Zn protoporphyrin IX, we attempted to identify the red pigment prepared from Parma ham by ESI-HR-MS analysis. As shown in Fig. 4, the highest molecular ion peak was detected at m/z 624. Six main peaks were found when the peak region was expanded (Fig. 4, inset). This peak pattern agrees well with that of Zn protoporphyrin IX ($C_{34}H_{32}N_4O_4Zn$) (Fig. 4, inset), because Zn has five isotopes (m/z = 64, 66, 67, 68 and 70) and the isotopic ratio is characteristic. Iron has four isotopes (m/z =54, 56, 57 and 58) and its isotopic ratio is different from that of Zn. The exact mass of the principal molecular ion computed from the internal standard was 624.1711 and differed by only 0.4 milli- mass units from the monoisotopic mass (624.1715) of Zn protoporphyrin IX. On the other hand, peaks originating in Fe protoporphyrin IX (MW 616.49), Mg protoporphyrin IX (MW 584.95) or Cd protoporphyrin IX (MW 673.05) were not observed.

These results indicate that the unidentified red pigment in Parma ham is not an Fe-porphyrin complex but a Zn-porphyrin complex, i.e., Zn protoporphyrin IX. It was speculated that the Zn protoporphyrin IX in Parma ham was formed by substitution of Fe in heme to Zn. In a study on surface autofluorescent changes of Parma ham during processing, it was found that the amount of a fluorescent component that is likely to correspond to Zn protoporphyrin IX increased from raw to salted meat (3 months) and then hardly increased during the maturing process (Møller et al., 2003). Thus, Zn protoporphyrin IX might be formed during the early period of the manufacturing process, not during the maturing period. Further studies are needed to elucidate the mechanism of Fe-Zn substitution that occurs in Parma ham during the manufacturing process.

Conclusions

The red pigment extracted from Parma ham by water and then 75% acetone was crudely purified by solid phase extraction. The red pigment was purplish red and fluorescent metalloporphyrin. Element analysis using an SEM-EDX revealed that the red pigment preparation contained not iron but zinc. By mass analysis, the red pigment was identified to be Zn protoporphyrin IX. Zn protoporphyrin IX in Parma ham might be formed with heme and zinc contained in pork during the early period of the manufacturing process.

Acknowledgements

We thank Mr. Kenji Watanabe and Dr. Eri Fukushi of the GC-MS and NMR laboratory, Graduate school of Agriculture, Hokkaido University for performing the ESI-HR-MS analysis. We are also grateful to Mr. Toshiaki Ito of the electron microscope laboratory, Graduate school of Agriculture, Hokkaido University for performing the SEM-EDX analysis.

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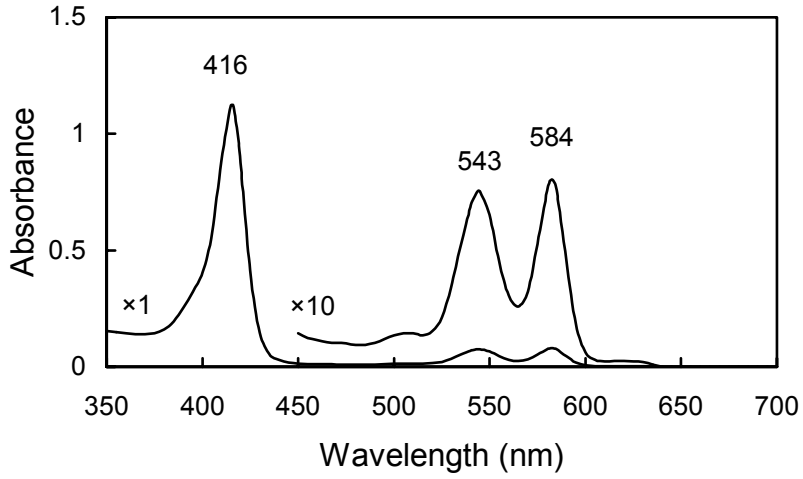

Fig. 1. Absorption spectra of the red pigment preparation from Parma ham. The maximum absorption wavelengths are shown for the preparation. The spectrum from 450 to 700 nm was enlarged 10-fold.

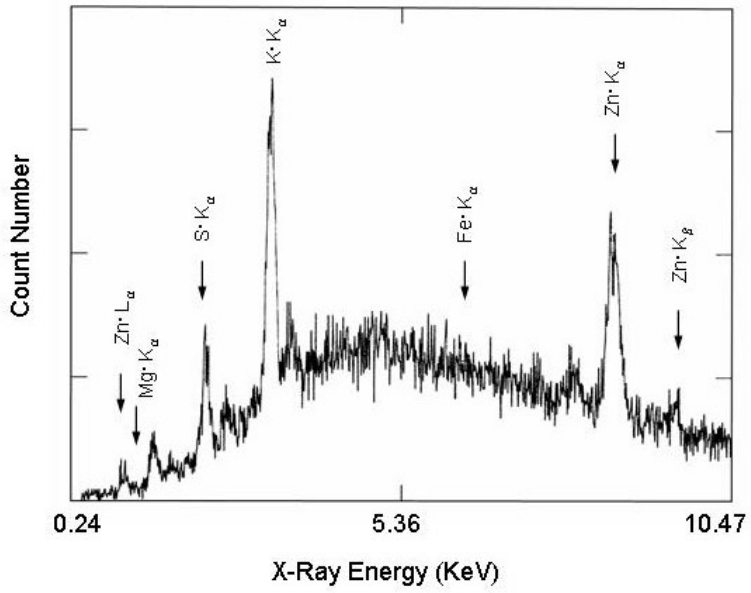

Fig. 2. SEM-EDX X-ray spectrum of the red pigment preparation from Parma ham.

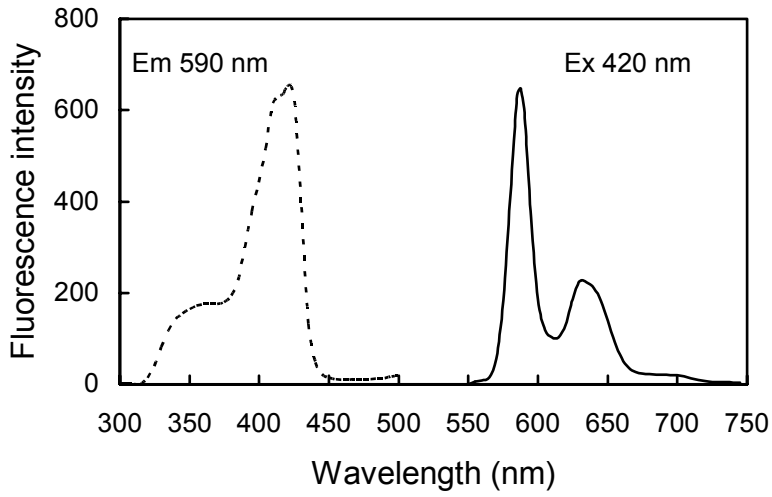

Fig. 3. Fluorescence and fluorescence excitation spectra of the red pigment preparation from Parma ham. Solid line, fluorescence spectrum at 420 nm for excitation; broken line, fluorescence excitation spectra at 590 nm for emission.

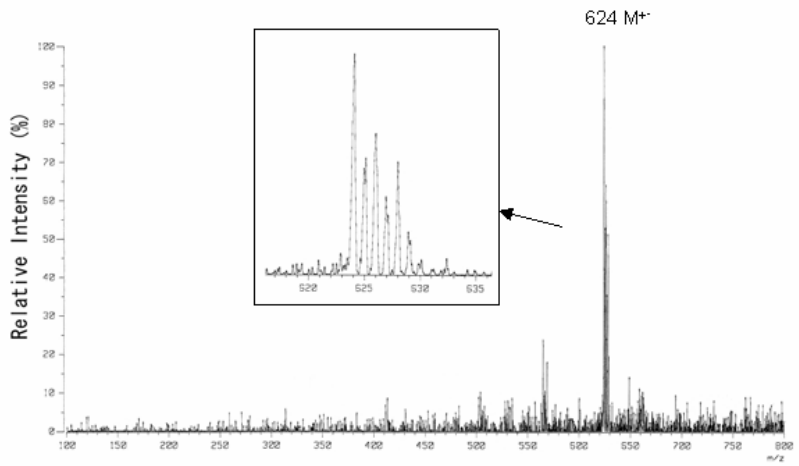

Fig. 4. ESI-HR mass spectrum of the red pigment preparation from Parma ham and magnification (inset).

MANIPULATION OF PRE-RIGOR GLYCOLYTIC BEHAVIOURS TO PRODUCE CONSISTENT BEEF TENDERNESS

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Background

One of the most important challenges met by the meat industry today is to provide a product of consistent eating quality in which tenderness is a strong attribute. Beef is generally variable in eating quality, which stems mainly from how the muscles are treated up to the time of rigor. (Koomaraie, 1996). Variance in temperature, pH and their relationship with time *post-mortem* is important in pre-rigor beef. Fluctuations within a carcass in the temperature/pH profile, post slaughter, is a root cause in the inconsistency beef tenderness. Hot boning is defined as the removal of muscles or muscle systems from the carcass prior to chilling (West, 1983) and is beneficial for both industrial and experimental reasons as each muscle can be treated individually according to its own glycolytic behaviours. Hot boning allows for the manipulation of muscles during the critical pre-rigor period, therefore, optimum conditions in terms of pH/temperature profiles can be determined for the production of consistently tender beef.

Objectives

The objective of this study was to alter rigor development via post mortem manipulation for the optimisation of consistently tender beef.

Materials and methods

Hereford cross Friesian heifers (n=8) were captive bolt stunned and exanguinated conventionally at the Meat Industrial Development Unit, National Food Centre, Dublin 15. From each animal both *longissimus dorsi* muscles were hot-boned within 90 minutes post slaughter. Each muscle was divided in 3 so that there was 6 samples per replicate. Each sample was randomly selected and submerged in water baths pre-set at the following temperatures; 0, 5, 10, 15, 20 and 25^{0}C. After eight hours the muscles were placed in a 2^{0}C chill and aged for 14 days post- slaughter. pH profiles were observed by inserting a glass electrode approximately 2 inches in to the muscle Sarcomere length, was determined by diffraction of a laser beam according to the method determined by Cross *et al.* (1980) 2 days *post-mortem*. Steaks were cut (2.5cm thick) at day 2, 7 and 14 *post-mortem* and stored at –20^{0}C for Warner Bratzler Shear Force (WBSF) analysis, which was carried out according to the method described by Shackelford *et al* (1991). Samples were taken at days 2, 7 and 14 to measure proteolytic activity.

Results and discussion

Pre-rigor incubation temperature had an effect on the rate of pH decline (Fig. 1), with higher temperatures giving a faster rate of pH decline. The 0^{0}C samples had the shortest sarcomere length followed by the 5^{0}C samples, whereas 10-25^{0}C samples did not cold shorten (Table 1). These results are reflected in the WBSF results (Fig. 2), where cold shortened muscle (0 and 5ºC) was found to be toughest. Fig. 3 shows the interaction of pH taken at 3 hours post-mortem (pH_3), WBSF at day 14 and temperature. The most tender and least variable beef was found at pH_3 between 5.9 and 6.2. However, this is not the case for cold shortened samples as cold shortened muscles were more variable in tenderness and pH. The absolute rate of tenderisation between day 2 and day 14 (ΔWBSF) was greatest for cold shortened muscles, particularly muscles incubated at 5ºC. Muscles with the highest ΔWBSF were also found to be most variable in tenderness at day 14. Therefore variability may be due to tenderisation of cold shortened muscles. Analysis of protein profiles showed that the appearance of the 30-kDa band was related to the rate of tenderisation, therefore proteolysis might be a root cause of variation in tenderness of cold shortened muscle.

The rate of pH fall is a good indicator of the rate of glycolysis (Bendall 1978) as pH fall is generally due to an increase in lactic acid, a product of anaerobic glycolysis. Therefore the incubation temperature influenced the rate of glycolysis. Cold shortening is a toughening of meat that occurs due to chilling meat below 10^0C before the onset of rigor, when pH reaches 6.2. This explains the results obtained following sarcomere length analysis. Each muscle aged over a 14-day period, irrespective of cold shortening, hence measuring the sarcomere length at day 2 *post-mortem* is a useful tool for predicting tenderness but not toughness as muscles with short sacomeres tenderise over time.

Conclusions

A pH_3 range of 5.9 to 6.2 produces consistently tender beef where the sarcomere lengths are long (above 1.5µm). Cold shortened muscle tenderises, the absolute rate of tenderisation between day 2 and day 14 was greatest at 5ºC where most variability in tenderisation exists. Proteolysis of cold shortened muscles may induce variability.

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Acknowledgements

The Irish National Development Plan under the Food Institutional Research Measure funds this project

Table 1. *Mean sarcomere lengths of M.longissimus dorsi at day 2 post-mortem (um)*

Temperature ^{0}C	0^0C	5^0C	10^0C	15^0C	20^0C	25^0C
Sarcomere length (nm)	1.2± 0.4	1.37± 1.15	1.6 ± 2.65	1.6 ± 4.42	1.7± 5.78	1.6 ± 7.4

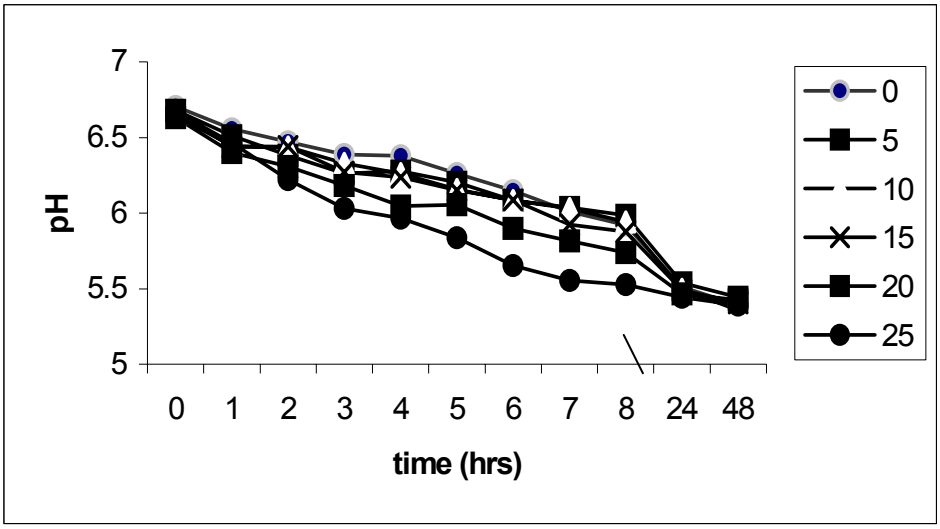

Fig 1; Mean pH decline for M. longissimus dorsi muscles incubated for 8 hours pre-rigor at temperatures from 0 to 25^{0}C.

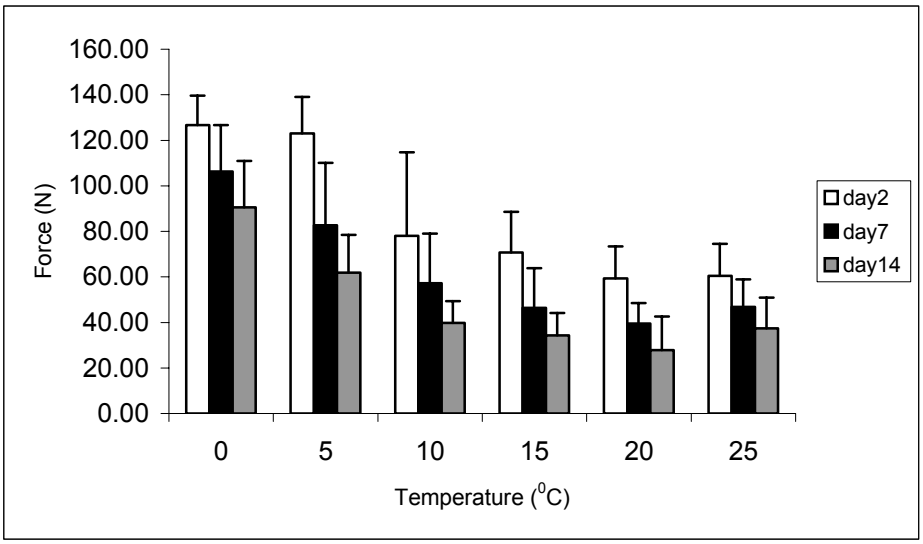

Fig 2; Mean WBSF (N) for M.Longissimus dorsi held for 8 hours at pre-rigor temperatures from 0 to 25°C.

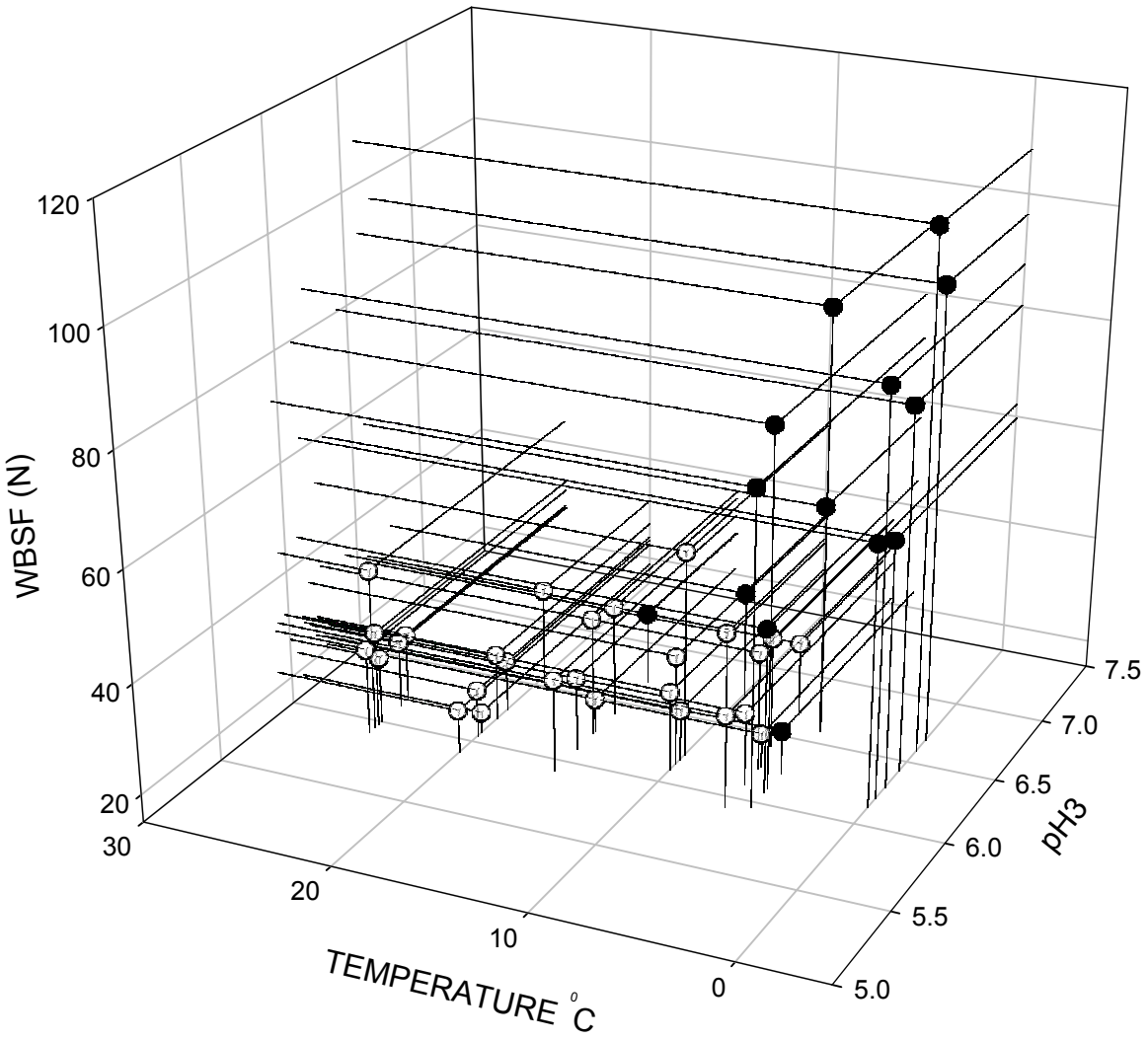

Fig. 3; Interaction between incubation temperature (8 hrs pre-rigor from 0 to 25ºC) and pH_3 and their effect on the tenderness of M.longissimus dorsi

THE SIGNIFICANCE OF SARCOMERE LENGTH AND PROTEOLYSIS ON THE TENDERNESS OF BOVINE *M. LONGISSIMUS DORSI*

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Background

Consumers surveys have revealed consistent eating quality, in which tenderness is a strong characteristic, is one of the most important attributes of beef. There are 3 phases *post-mortem* which have an influence on tenderness; the pre-rigor phase, rigor or the phase of maximum toughness and the tenderisation or post-rigor phase. The biochemical dynmaics of the pre-rigor phase influence the maximum contraction at rigor. Tenderisation may be defined as a decrease in toughness post-rigor (Hopkins and Thompson 2002) and mostly attributed to changes in the myofibrillar proteins. The extent of contraction at rigor may be monitored by measuring the length of the sarcomere and its influences on the rate of tenderisation (Herring et al 1967). The glycolytic behaviours pre-rigor are dependent on temperature and therefore can be altered through the chilling regime. Reducing the rate of the glcolytic pathway would enable the examination of the pre-rigor period and its effect on rigor.

Objective

Examination of the early post mortem period using different rates of glycolysis to monitor the effect of proteolysis and sarcomere length on the tenderness of beef.

Materials and methods

Hereford cross Friesian heifers were slaughtered at the research abattoir in The National Food Centre, Dublin. Hot-boned *longissimus dorsi* muscles (LD) (n=8) were split in half, one half was immersed in a water bath pre-set at 5ºC and the other half was immersed in 15ºC for the first 8 hours *post–mortem* before being transferred to a chill. Temperatures were selected on the basis of results presented by the authors in these proceedings. pH and temperature profiles were recorded. Samples were taken for sarcomere length at day 2, 7, 14 and 21 *post-mortem*. Steaks were cut on alternating days up to day 21and stored at –20º C for Warner Bratzler Shear Force (WBSF) analysis. Samples were taken at 1.5,4,8, 24, 48 hours *post-mortem* for analysis of myofibrillar protein profiles.

Results and Discussion

Temperature had an effect on the rate of pH decline; LD incubated at 15ºC had an accelerated rate of pH decline when compared to those incubated at 5ºC. WBSF carried out over a 21-day period illustrated that the muscles incubated at 15ºC were more tender then those incubated at 5ºC, suggesting the occurrence of cold shortening (Fig 1). The greatest degree of tenderisation in muscles tempered at both 5ºC and 15ºC occurred before day 3 *post-mortem*. Analysis of a gradient 3-15% SDS-PAGE (Fig 2) illustrated that the differences in the rate of myofibrillar proteolysis, between muscles held at 5 and 15ºC, are minor. Therefore it is unlikely that the difference in tenderness is due to degradation of the myofibrillar proteins (Fig 2). The emergence of the 30kDa appeared similar over a 21-day period for both 5 and 15ºC (not shown). Temperature was found to have an impact on sarcomere lengths. Cold shortening of muscles tempered at 5ºC caused a greater maximum contraction at rigor than those tempered at 15ºC. Cold shortened sarcomeres (5ºC) did not lengthen significantly over time (table 1).

Conclusions

The majority of tenderisation for both cold shortened and non cold shortened muscles occurred before day 3 post-mortem. Despite the fact that cold shortened muscle ages, it still remains tougher and than its non-shortened counterparts. A reduction in maximum toughness appears to be more favourable than an increase in tenderisation.

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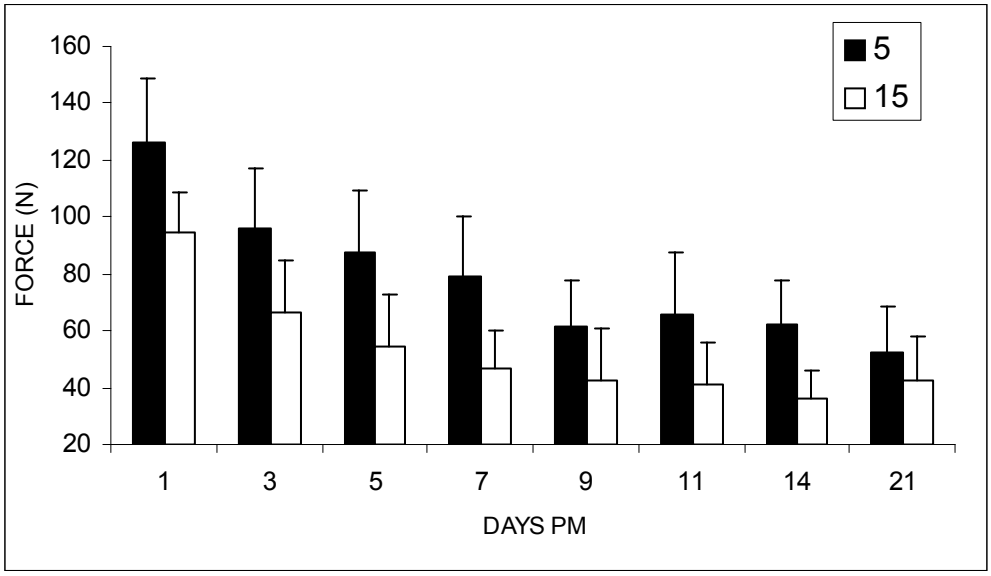

Figure 1: Warner Bratzler shear force measurements (n=8) for bovine *M. longissimus dorsi with* pre-rigor incubation temperature of 5 and 15ºC.

Table 1: Sarcomere length (µm) of bovine *M. longissimus*

	Day 2	**Day 7**	**Day 14**	**Day 21**
5ºC	1.51± 0.19	1.55±0.14	1.43±0.16	1.46±0.12
15ºC	1.64±0.17	1.76±0.12	1.73±0.11	1.69±0.12

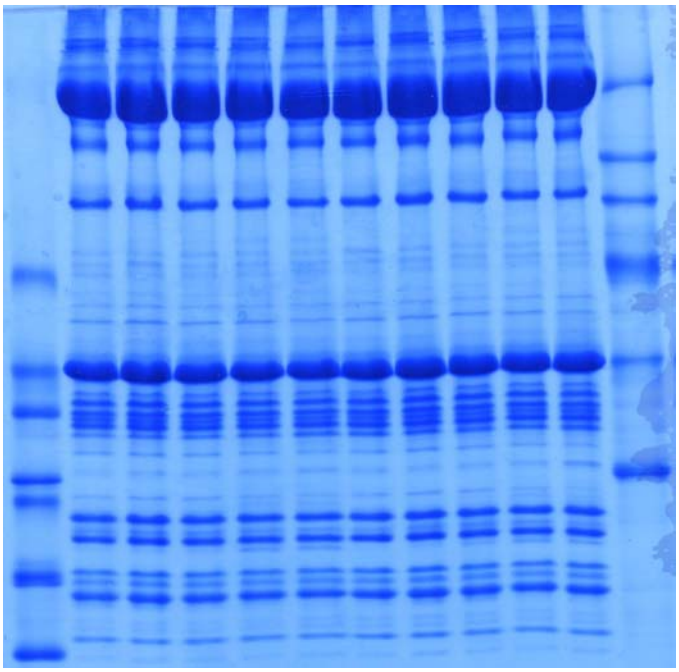

Figure 2 : Myofibrillar protein profiles. 3 –15 % gradient SDS-PAGE. Lane 1-12; low molecular weight marker; 5ºC time 0; 5ºC 4hrs; 5ºC 8hrs; 5ºC 24hrs; 5ºC 48 hrs; 15ºC time 0; 15ºC 4 hrs; 15 ºC 8 hrs; 15ºC 24 hrs; 15ºC 48 hrs; high molecular weight marker.

DIETARY CREATINE AFFECTS MEAT QUALITY OF PURE BREEDS OF DUROC AND LANDRACE DIFFERENTLY

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Background

Dietary creatine has been shown to increase total muscle creatine (Balsom, Söderlund, Sjödin & Ekblom, 1995), of which approximately two-thirds is creatine phosphate (Balsom, Söderlund & Ekblom, 1994; Casey, Constantin-Teodosiu, Howell, Hultman & Greenhaff, 1996). Increased availability of creatine phosphate is believed to contribute to the increased maximum and total work capacity (Casey & Greenhaff, 2000) and reduce the recovery period (Balsom et al., 1995) of humans supplemented with creatine. In relation to meat quality, it has been suggested that the increased creatine phosphate may delay the lactate formation in the muscle and consequently postpone the pH decline post mortem. On the basis of postponed pH decline, and possible increased water retention due to increased intracellular osmotic draw caused by the creatine phosphate, Berg and Alle (2001) proposed a hypothesis of increased WHC in meat from pigs supplemented with creatine. Alteration in the water mobility has been indicated in several studies by tendencies to greater myofiber hydration (Berg & Allee, 2001), reduced cooking loss (Maddock, Bidner, Carr, McKeith, Berg & Savell, 2002) and increased WHC (James et al., 2001).

Objectives

The objectives was to study interactions among breeds, creatine supplementation, and cooling profile in relation to WHC in pork.

Materials and methods

A total of 80 pigs, 40 Duroc and 40 Landrace pigs were allocated to 4 groups and supplemented with 0, 12.5, 25 or 50 g creatine monohydrate (CMH)/d for 5 days prior to slaughter. Pigs were fed 2 x 1375 g feed mixed with CMH for 5 days prior to slaughter. The body weight was registered in the morning 5 days before slaughter and before transport to the slaughter plant. The development in water mobility and distribution was measured in LD muscles of control and pigs supplemented with 25 g CMH/d by NMR T_2 relaxation (Bertram, Karlsson & Andersen, 2003) from 20 min to 210 min post mortem. The temperature was logged every min from 20 min until 24 h *post mortem*, and duplicate pH measurements were made in *longissimus dorsi* (LD) at 1, 15, 30, 45, 60, 120 and 1440 min (24 h) *post mortem*. After 1 h one half of the carcass was placed in a chilling room at 4°C (temperature profiles in Figure 1) and the other half in the freezer for 1 h at approximately –28°C, and then transferred to a separate chilling room at 4°C. Water holding capacity (WHC) was also determined as drip loss in LD in approximately 100 g samples using the plastic bag method (Honikel, 1998). The data was statistically analysed using the mixed procedure in SAS (SAS V8).

Results and discussion

Body weight gain of the pigs after 5 days of supplementation increased linearly with dietary inclusion of CMH and at a dose of 50 g/d the gain was increased by approximately 2 and 3 kg in Duroc and Landrace respectively (Table 1). When disregarding the breed, all groups of CMH supplemented pigs had a significantly increased weight gain compared to control animals. Weather this increased gain is as lean body mass (Balsom et al., 1995) or as increased water retention (Juhn, 1999) will be investigated further in this study.

In Duroc pigs pH was higher compared to Landrace at all time points, but in control animals and those supplemented with 12.5 g CMH/d this difference was only significant 24 h *post mortem* (Table 2). Contrary, in groups of pigs supplemented with 25 and 50 g CMH/d the Duroc breed had significantly higher pH at all time points, except after 15 and 30 min for pigs supplemented with 50 g CMH/d. Among Duroc animals pH was higher in pigs supplemented with 50 g CMH/d at 30, 45, 60 and 120 min *post mortem* compared to

control Duroc animals, whereas there was no systematic differences between supplementation groups within the Landrace. In general the difference in cooling profile (Figure 1) only affected pH of the meat after 2 and 24 h *post mortem*. As a whole, carcasses frozen for 1 h had a higher pH at 2 h *post mortem* ($P = 0.005$) and specifically Duroc supplemented with 50 f CMH/d and Landrace supplemented with 25 g CMH/d had increased pH upon freezing. After 24 h the effect of cooling profile was more pronounced; generally pH was higher in frozen samples ($P < 0.0001$) and specifically Duroc supplemented with 12.5 g CMH/d and all groups of Landrace had increased pH upon freezing.

Delaying the pH decline initially during the conversion of muscle to meat reduces protein denaturation and may consequently reduce the drip loss. In this context the drip loss is assumed to be reduced in meat from Duroc pigs compared to Landrace, and in Duroc pigs supplemented with 50 g CMH/d compared to control Duroc animals. Furthermore, different cooling profiles may affect the drip loss despite the pH of the frozen meat was only significantly higher 2-24 h *post mortem*.

In general terms this theory was confirmed, since drip loss, as determined by the bag drip method, was reduced up to 30% in Duroc but slightly increased in Landrace upon CMH supplementation (Table 1). The difference in cooling profile did not affect drip loss.

NMR T_2 relaxation data were analysed using distributed exponential fitting, which revealed the presence of three water populations in the muscles. The slowest T_2 population, T_{22}, expresses the amount of extra myofibrillar water and has been shown to be positively correlated to the drip loss (Bertram, Donstrup, Karlsson & Andersen, 2002). The difference in drip loss between breeds determined by the bag drip method was not directly reflected in the NMR T_2 population measured initially *post mortem* (Figure 2). This may be due to a difference in the contribution from intra muscular fat to the T_{22} population between the two breeds, since Duroc is known to have more intra muscular fat than Landrace. However the tendencies towards reduced drip loss in Duroc and increased drip loss in Landrace upon CMH supplementation was confirmed by the *post mortem* development in the NMR T_{22} population, which revealed a smaller and larger increase in the T_{22} population in muscles of Duroc and Landrace, respectively, upon CMH supplementation, although none of the NMR data was significantly different because of the variation in the material. A large increase in the T_{22} population *post mortem* is known to result in a high drip loss (Bertram et al., 2003).

Conclusions

In general, a breed by dietary CMH interaction was observed for *post mortem* pH and drip loss. Thus, in Duroc dietary CMH addition increased pH, and reduced driploss, while no significant effects of dietary CMH addition were observed in meat from Landrace pigs.

Table 1. LSMeans of weight gain (g) of the live animals and WHC of the meat expressed as drip loss (%) (n = 10). Weight gain expressed as difference before and after 5 days of creatine monohydrate (CMH) supplementation.

		Duroc CMH supplement (g/d)				Landrace CMH supplement (g/d)			
	SEM	0	12.5	25	50	0	12.5	25	50
Weight gain (G)	0.64-0.72	5.77^{a}	6.98^{ab}	7.32^{ab}	7.86^{b}	5.73^{a}	8.34^{b}	7.13^{ab}	8.70^{b}
Drip loss (%)	0.50-0.51	4.31^{ab}	3.04^{a1}	3.35^{a2}	2.98^{a3}	5.41^{bc}	6.35^{c}	6.22^{c}	6.24^{c}

^{a,b}Means lacking a common superscript differ ($P \leq 0.05$). $^{1\text{-}3}$Significance level in relation to control ^{1} $P = 0.07$, ^{2} $P = 0.17$, ^{3} $P = 0.06$.

Table 2. LSmeans of pH in *Longissimus Dorsi* of Duroc and Landrace supplemented with different creatine monohydrate (CMH) concentrations, measured at various time points *post mortem* (n = 10). Cool: cooling at 4°C after slaughter, Freeze: freezing for 1 h, from 1-2 h *post mortem*.

Time (min) *post mortem*	Cooling profile		Duroc CMH supplement (g/d)				Landrace CMH supplement (g/d)			
		SEM	0	12.5	25	50	0	12.5	25	50
1	-	0.045	6.55^{abc}	6.58^{ab}	6.58^{bc}	6.66^{c}	6.48^{ab}	6.51^{ab}	6.46^{a}	6.51^{ab}
15	-	0.046	6.47^{ab}	6.46^{ab}	6.54^{b}	6.54^{b}	6.44^{ab}	6.41^{a}	6.37^{a}	6.48^{ab}
30	-	0.043	6.40^{ab}	6.44^{bc}	6.50^{bc}	6.55^{c}	6.41^{ab}	6.40^{ab}	6.32^{a}	6.47^{bc}
45	-	0.044	6.42^{ab}	6.40^{ab}	6.49^{bc}	6.56^{c}	6.31^{a}	6.31^{a}	6.31^{a}	6.38^{ab}
60	-	0.051	6.35^{abc}	6.36^{abc}	6.38^{bc}	6.47^{c}	6.28^{ab}	6.28^{ab}	6.23^{a}	6.27^{ab}
120	Cool	0.063	6.19^{bc}	6.23^{bc}	6.24^{bc}	$6.32^{c,x}$	6.10^{ab}	6.11^{ab}	$6.01^{b,x}$	6.12^{ab}
120	Freeze	0.063	6.25^{a}	6.29^{ab}	6.25^{a}	$6.43^{b,y}$	6.17^{a}	6.14^{a}	$6.13^{a,y}$	6.12^{a}
1440	Cool	0.027	5.61^{b}	$5.64^{b,x}$	5.61^{b}	5.62^{b}	$5.49^{a,x}$	$5.48^{a,x}$	$5.49^{a,x}$	$5.47^{a,x}$
1440	Freeze	0.027	5.63^{b}	$5.68^{b,y}$	5.63^{b}	5.69^{b}	$5.53^{a,y}$	$5.55^{a,y}$	$5.57^{a,y}$	$5.52^{a,y}$

[a-c]Means within a row lacking a common superscript differ ($P \leq 0.05$). [x,y]Means within a column and within a specific time lacking a common superscript differ ($P \leq 0.05$).

Figure 1. Mean of temperature in longissimus dorsi (LD) of carcass sides cooled at 4°C throughout, and sides frozen for 1 h from 1-2 h *post mortem* (n = 80). Dotted lines indicate SEM.

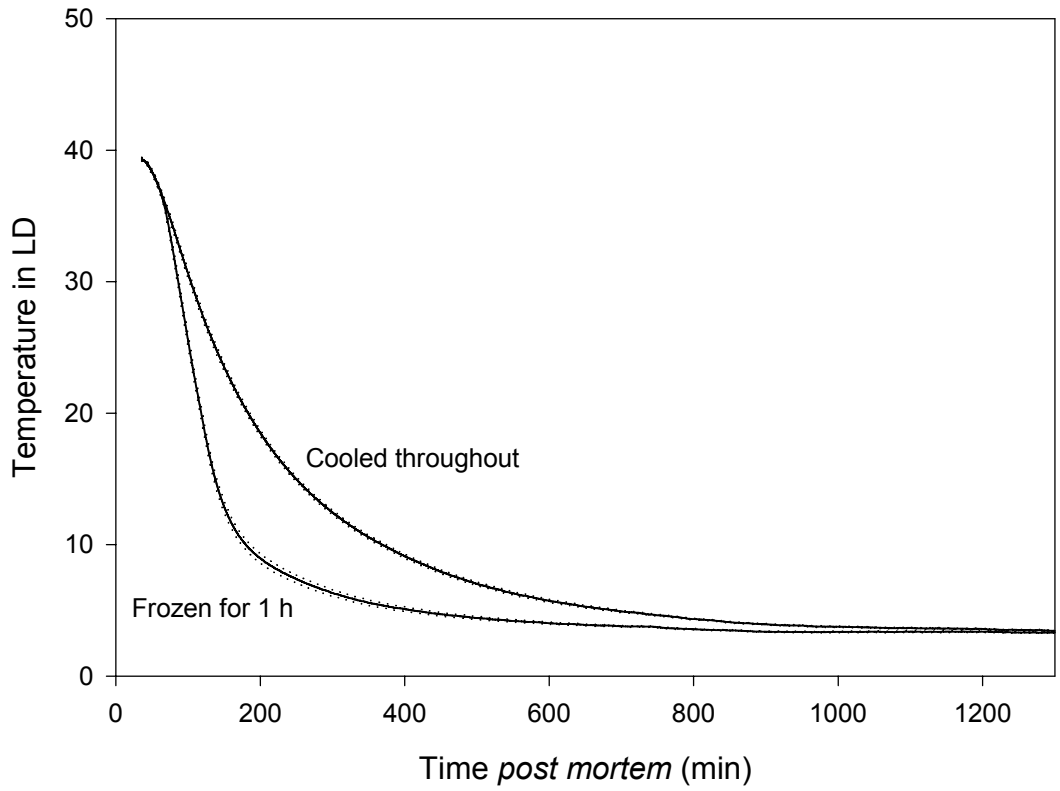
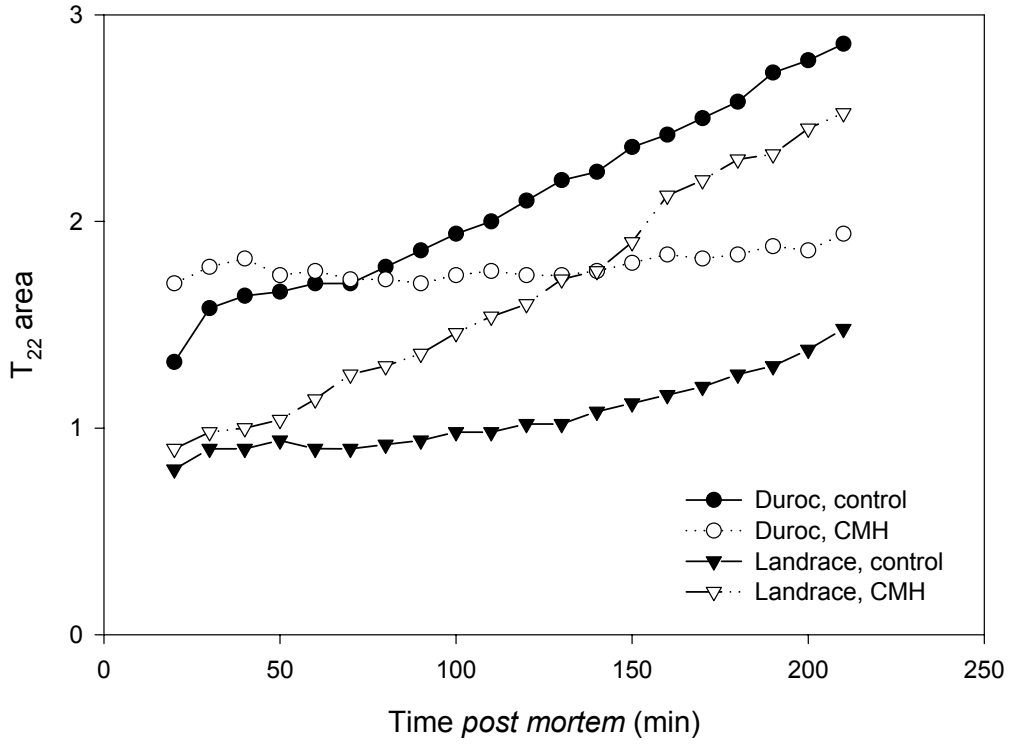


Figure 2. Development in NMR T_{22} population after slaughter in control pigs and pigs supplemented with 25 g CMH/d. LSMean values are shown, SEM = 0.38 (n = 10).



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Session 2
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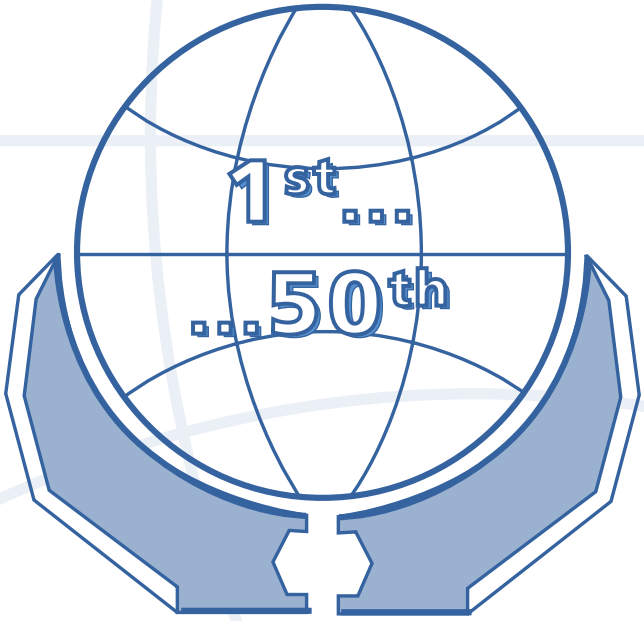

INTRAMUSCULAR CONNECTIVE TISSUE AND ITS ROLE IN MEAT QUALITY

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http://www.uoguelph.ca/OAC/foodsci/directory/faculty/purslow.html

Abstract

The amount, spatial distribution and composition of the connective tissue within muscle vary with muscle position and the carcase and with animal age. This has long been recognised to influence the tenderness of cooked meat. This paper builds upon some historical perspectives with a review of some recent clarifications of the biological function of intramuscular connective tissue IMCT and of its contribution to meat texture, which is clearly multifactorial. The perimysial component of (IMCT) varies most in amount between muscles, and is also the IMCT structure most involved in defining the mechanical integrity of cooked meat. The distribution of perimysium defines muscle fascicle size (muscle "grain" size), which is also still regarded as an indicator of tenderness. Postmortem conditioning of meat has consistently been shown to reduce the strength of intramuscular connective tissue in the raw state, but equally consistently, this has been shown not to affect the toughness of cooked meat. Cooking increases IMCT strength in the range 20-50°C and decreases its contribution at higher temperatures and longer cooking times. Crosslinking of collagen in older animals is generally considered to result in tougher meat, although definitive links between mature crosslink content and cooked meat toughness have been difficult to prove. In the last quarter-century IMCT has been increasingly viewed as a "background" contributor to meat texture, which is difficult to change. However, the large variation in perimysial content of muscles in one animal represents an incredible range of expression. This appears to be firmly fixed to the functional properties of different muscles. In particular, it is hypothesised that definition of muscle fascicle size and shape by the bounding perimysium is related to the need for sub-sections of the whole muscle to slip past each other in the normal contractile function of the tissue. However, the amounts and composition of IMCT can be manipulated by animal nutrition and exercise, and factors affecting the turnover of IMCT may especially be a future target for manipulation of meat texture.

(**Note!** Figures are on Pages 15–19**)**

Introduction

The morphology, composition and amount of intramuscular connective tissue (IMCT[1]) vary tremendously between muscles, species, breeds and with animal age. We intrinsically acknowledge the large influence of IMCT on meat toughness in age-old preferences in choosing cuts of meat to purchase. The large price differential at retail between fillet steak (loin steak) and shin beef reflect our expectations of eating quality, based principally on their different IMCT content, and the preference for meat from younger, less mature, animals reflects the fact that the IMCT contribution to cooked meat toughness is higher in older animals, due to its increased mechanical and thermal stability. Although only a small component (in terms of mass fraction), the disproportionately large influence of IMCT on meat toughness ha resulted in studies trying to relate its amount, or various aspects of its composition and distribution in muscle tissue to variations in meat texture for more than three-quarters of a century (Lehmann, 1907; Hammond, 1932; Carpenter, Kauffman, Bray, Briskey, Weckel, 1963; Marsh, 1977; Light and Bailey 1989; Brooks and Savell, 2004). Modern production practices for pork, lamb and especially beef clearly aim to minimise the variations in texture due to animal maturity, so that young animals of a narrow age range at slaughter of the same breed show variation in the texture of a meat from a given muscle that is only minimally related to characteristics such as collagen content and solubility. In recent years, work to reduce unwanted meat toughness has largely been focussed on postmortem proteolysis of the cytoskeletal and myofibrillar proteins within muscle fibres, as this process can be influenced by post-mortem handling to a great extent. (This focus has led to the view that, whilst the IMCT contribution to meat texture is certainly important, it is also rather immutable and forms a "background toughness" that we can do little about in practical terms (Ouali, Demeyer, Raichon, 1992; Sentandreu, Coulis, Ouali, 2002). However, the amount, composition and distribution of IMCT is possibly the
most variable phenotypic difference between muscles within an animal, and represents a tremendous variation in protein expression and turnover. Given the large influence of IMCT on meat texture, even small manipulations of its expression and turnover may have considerable potential for reducing unwanted variations in meat tenderness.

Structure and composition of intramuscular connective tissue

The basic structure and composition of IMCT has been comprehensively reviewed by several previous authors (Borg & Caulfield, 1980; Mayne & Sanderson, 1985; Sanes, 1986; Bailey & Light, 1989; Purslow & Duance, 1990; McCormick, 1994; Greaser, 1997; Purslow, 2002; Kjær, 2004) and only a brief description will be given here as background. IMCT is principally comprised of fibres of the proteins collagen and elastin, surrounded by a proteoglycan (PG) matrix. The total collagen content in beef muscles can vary from 1-15% of dry weight; elastin is a smaller component varying from 0.6% to 3.7% (Bendall, 1967). Collagen types I, III, IV, V, VI, XII and XIV have been identified in IMCT (Listrat et al., 1999,2000). Types I and III are the major fibre-forming types in endomysium, perimysium and epimysium, and type IV collagen is the principal non-fibre-forming component of the basement membrane linking the fibrous (reticular) layer of the endomysium to the muscle cell membrane (sarcolemma). In mammalian species type V is a minor component of IMCT, although it is much more abundant in the major IMCT structure of fish muscle, the myocommata (Sato et al., 1998). As reviewed in detail elsewhere (Purslow, 2002), the relative concentration of different types of collagen varies through embryonic development in bovine (Listrat, Picard, Geay, 1999; Listrat et al., 2000) and chicken (Lawson & Purslow, 2001) muscle. There have been a number of PG components identified in IMCT, principally decorin (Eggen, Malmstrom, Kolset, 1994; Nakano, Li, Sunwoo, Sim, 1977). Heparan suphate-containing PGs are associated with the basement membrane and higher concentrations of chrondroitin sulphate and dermatan sulphate are found in the perimysium (Carrino and Caplian, 1984; Andersen, Klier, Tanguay, 1984; Nishimura, Hattori, Takahashi, 1996a). Vellman and

[1] The abbreviation IMCT has been used in the past, e.g. by Mohr and Bendall (1969) and others subsequently. In more modern biological parlance connective tissues are referred to as extracellular matrix (ECM). Although "ECM within muscle" has been used in some papers, the historical and shorter "IMCT" will be used here. Needless to say, these terms are synonymous.

coworkers (Vellman, Liu, Eggen, Nestor, 1999) reported the high levels of chondroitin sulphate-containing PGs in early developmental stages of turkey pectoralis muscles to be replaced by heparan sulphate-containing PG's later in embryonic development.

There are three main structural components of IMCT. (1) The *endomysium* is the thin connective tissue layer separating individual muscle fibres. The vast majority of its thickness is made up of a near-random feltwork of fine, wavy collagen fibres, as shown in Fig. 1. This collagen feltwork can easily reorientate with changing muscle length (Purslow & Trotter, 1994; Trotter, Richmond, Purslow, 1995). (2) The *perimysium* is the connective tissue layer that separates each muscle into muscle fibre bundles, or fascicles. There are large (primary) fascicles and smaller (secondary) fascicles, and therefore primary and secondary perimysial layers separating them. Collagen fibres in the perimysium are arranged in a crossed-ply arrangement of two sets of wavy collagen fibres (Rowe, 1981), with the fibres in each ply parallel to each other but at an angle to the muscle fibre axis (typically $\pm$54° in resting muscle, but this angle changes with changing muscle length; Purslow, 1989). Again, reorientation of this collagen network allows the perimysium to easily follow elongation or shortening of the muscle fascicles. (3). The *epimysium* is the connective tissue sheath delineating and separating individual muscles. In many muscles (e.g. bovine sternomandibularis; Fig. 2) collagen fibres in the epimysium take on the same crossed-2-ply arrangement in the perimysium. In pennate muscles, however (e.g. gastrocnemius), or in muscles where the epimysium clearly participates in transferring load to adjacent structures (e.g. bovine semitendinosus), the collagen fibres are more close-packed and longitudinally arranged, like a tendon.

The role of perimysial IMCT in meat texture

Whilst cooked meat toughness and the total collagen content of a muscle often rank similarly, precise correlations between textural measures such as Warner-Bratzler shear force (WBSF) and collagen content are poor. Dransfield (1977) showed some of the best correlations between Volodkevitch bite force on cooked meat and collagen content (measured by hydroxyproline concentration), but even so the correlation explained less than half the total variation in texture. Previous structural and mechanical studies of cooked beef (Carroll, Rorer, Jones, Cavanaugh, 1978; Purslow, 1985) have clearly demonstrated that separation of the perimysium from the endomysium of fibres on the surface of the fascicle is relatively easy in cooked meat, but that the individual perimysial layers are strong and so dominate the fracture behaviour of meat and therefore is the major contributor to toughness. The high strength of the perimysium relative to the weaker endomysial-perimysial interface in cooked beef was quantified by Lewis and Purslow (1990). Fig. 3 shows a steak of bovine semitendinosus muscle cooked to 80°C being pulled apart. In the early stages of separation (Fig. 3 top), fascicles separate from each other, by endomysial-perimysial separation, leaving intact perimysium clearly visible in the gaps between them. At later stages of rupture (Fig. 3 bottom), the perimysial layers have to be broken down to separate the whole sample into pieces. This process clearly mimics the breakdown of cooked meat in the mouth during mastication, which also involves separation of intact fascicles in the first few chew cycles (Lillford, 1991, 2000).

The collagen content in the perimysium varies much more between muscles than endomysial content (Light, Champion, Voyle, Bailey, 1985; Purslow 1999; Nakamura, Iwamoto, Ono, Shiba, Nishimura, Tabata, 2003). In a study of six bovine muscles, Light et al. (1985) show a range of 1.4% to 7.0% in perimysial mass as percentage of muscle dry weight but only a range of 0.1% to 0.5% in endomysial mass between six beef muscles. A more extensive study of fourteen beef muscles reported by Purslow (1999) showed perimysial collagen as % of muscle dry weight ranging from 0.45% to 4.76%, whereas endomysial collagen % varied from 0.47% to 1.20%. Thus endomysial content varied by a factor of 2.5, whereas perimysial content varied by more than an order of magnitude. Although techniques of separating endomysial IMCT from perimysial IMCT are far from perfect, these studies indicate that it is the amount of perimysium that varies most between functionally different muscles. Brooks and Savell (2004) report that perimysial thickness in bovine semitendinosus muscle is on average 2.4 times thicker than in psoas major from the same animals. There are indications from the work of Swatland and coworkers. (Swatland, Gullett, Hore, Buttenham, 1985), Carpenter et al. (1963) and Cooper and coworkers (Cooper, Breidenstein, Cassens, Evans, Bray, 1968) that thicker perimysium is associated with reduced tenderness, although in some of these studies animal age is a

confounding variable. Unpublished results from the study on 14 beef muscles reported by Purslow (1999) showed the maximum thickness of primary perimysium to be 2.53 times greater than the thinnest, with variations in secondary perimysial thickness being slightly smaller (1.9). However, correlations between shear force values of muscles cooked to 80°C and perimysial thickness were low. Light et al. (1985) were able to show similarities in ranking between toughness and perimysial content of their six muscles, but correlations between WBSF and perimysial content or perimysial thickness (Brooks and Savell, 2004) remain low. Light et al (1985) demonstrated that variations in ratio of type I:III collagen, the diameter of collagen fibrils and the amount of divalent (immature) crosslinks are also seen in the perimysium of the six muscles they studied, suggesting that there are multifactorial contributions to the IMCT component of meat texture.

Muscle "grain" and meat texture

The division of muscles into fascicles by the perimysium can easily be discerned by eye, or by the ball of the thumb moving over a cut surface, as the "grain" of a muscle. The USDA grading scheme for beef has for many decades contained a reference to grain size; fine grain is a requirement for the top grades. (Large grain size is primarily mentioned in the current scheme as an indication of meat from older animals.) Grain size ("graininess") is included in the sensory parameters of note in a review of the history of sensory evaluation of meat by Szczesniak (1986). Much of the work cited in the literature on muscle grain and meat texture is 40-70 years old, and so does not appear in modern databases. For the sake of completeness, it is reviewed in some detail here. In these early works the term "texture" was specifically used to describe the visual perception of graininess of muscle cut across the fibre direction, i.e. how smooth or divided the cut surface appeared, and the size/nature of the divisions. It is assumed in the majority of the literature that grain size is synonymous with muscle fibre bundle (fascicle) diameter. Figure 4 compares fascicular architecture in cross-sections of three muscles from the same (bovine) animal: pectoralis profundus, sternocephalicus and rhomboideus cervicus. Large differences in primary fascicle size, the thickness of the perimysium, and the degree of adipose tissue associated with the perimysium can be seen in theses sections.

Hammond (1932) reviews the experimental work of his group from 1913 to 1922, together with contemporary work of others in this area in his large monograph on sheep meat quality. He notes a general belief at that time that cooked meat from fine-grained muscle is more palatable than from coarse-grained muscle, which is often described as "stringy". Thigh muscles with the highest rates of post-natal growth were felt to have the largest grain (e.g. semimembranosus, vastus externus, biceps femoris).
Hammond (1932) examined the effect of sex, age and breed differences on grain size. His comparison between 23 different leg and thigh muscles (Table CXXV, p 512) shows consistent patterns of variations in grain size between muscles between individual animals. Due to the ranking system used, the absolute variation in grain size from the biggest to the smallest cannot be quantified. However, from the examples of histological sections presented, fascicle size can easily be seen to vary by a factor of two or more. Hammond also compared the grain size of 7 muscles in rams at birth, 5 months and "adult" age, and 8 muscles in wethers at 5, 11 and 22 months. He concludes that muscle fibre bundle size increases with animal age. Rams, on average, have coarser grain sizes in the same muscles at comparable ages than wethers, and these in turn are larger than in ewes. Hammond also investigated differences in grain size between sheep breeds by ranking each of 7 muscles from 6 animals from 5 breeds. On average across the 7 muscles, grain size ranked with breed as follows:

Suffolk > Merino > Shetland > Soay > Hampshire.

Although the individual animals were all at different ages (Shetland 5yrs; Soay 2yrs; Merino 5yrs; Suffolk 4 yrs; Hampshire 11 months), the general inference drawn by Hammond is that muscle grain size is related to animal size.

In quantitative terms, this work by Hammond (1932) contains only a small amount of non-parametric data on the effect of breed on grain size. There is no correlation attempted between grain size and tenderness, but he infers that coarse grain size leads to less palatable, "stringy" meat. Hammond's conclusions (that coarse

texture is found in male animals and large-framed animals, and that this frame size effect also holds between breeds and species) are reiterated by Lawrie in his standard text on meat science (Lawrie, 1985).

Brady (1937) reports results from experiment using 6 yearling Hereford x Shorthorn steers and 7 mature Holstein cows. Measurements of the number of fibres per fascicle and fibre diameter were made on histological sections of 4 muscles (Triceps brachii, Longissimus, Adductor and Semitendinosus) in the raw and cooked state. Warner-Bratzler shear force measurements and taste panel scores for texture and tenderness were also taken. To confuse the issue, non-intuitive definitions of texture were used in the taste panel; large muscle fibre bundle size (coarse graining) was described and rated as the most desirable ("finer") texture. Brady concludes that these correlations are significant and that the data warrant the interpretation that the larger the bundle (which Brady confusingly defines as the finer the texture), the more tender the meat. Note that this conclusion is apparently in contrast to Hammond, who asserted that small grain size is associated with tenderness.

There is therefore an important difference in the use of the term "fine" to describe texture or grain size between Brady (1937) and Hammond (1932). Hammond uses "fine" to describe small grain (small bundle or fascicle size). Brady uses the term "fine" to indicate most desirable texture; in this case the "finest " texture is actually the largest grain size, i.e. the largest bundle diameter. So, although it is true that both Hammond and Brady relate "fine texture" with tenderness, they mean completely opposite things by the word "fine".
Ramsbottom, Standine and Koonz (1945) studied variations in tenderness between the muscles within the carcasses of 3 heifers of unspecified breed. They define texture as being determined by the size of the muscle fibre bundles (fascicles), but also the amount or thickness of the perimysial connective tissue separating them. They associate these rankings in grain size and definition with the same order of ranking in muscle toughness, i.e. the larger the grain size, the tougher the meat.

Cooper et al. (1968) found that the muscle bundle size increased greatly with maturity in bovine longissimus muscle. In their study, bundle size was significantly associated with visual scores of texture ($r = 0.37$, $P<0.01$), positively related to shear force ($r = 0.39$, $P<0.01$) and negatively correlated to taste panel tenderness ($r = -0.41$, $P<0.01$). They also found a significant negative correlation between the overall opinion of acceptability and bundle size ($r = -0.30$, $P<0.05$). .

Carpenter et al. (1963) studied muscle fibre bundle size and the coarseness (thickness) of perimysial connective tissue strands in 78 loins from pigs aged 4-42 months. Bundle size and CT coarseness were rated subjectively on a 5-point scale, and related to tenderometer and taste panel scores of tenderness. They give correlations of -0.39 ($P<0.01$) between muscle bundle size and tenderness, and -0.38 ($P<0.01$) between connective tissue thickness and tenderness. The trend of these relationships agrees with that found by Cooper et al. (1968) and inferred by Ramsbottom et al. (1945) and Hammond (1932), and so disagrees with Brady (1937).

Lepetit and Culioli (1994) review much of the French work on connective tissue morphology in meat. From work by Dumont (1985), they point out that if the force required to shear raw muscle is divided by the overall collagen content of the muscle, then 30-40% of the variance in shear force value remains, which they feel may be related to IMCT morphology. They cite Dumont (1983) as showing a high correlation between raw muscle shear force and the linear density of the perimysial network (i.e. the number of tracts encountered per unit length of transect). However, as discussed below, shear force measurements on raw meat are poor predictors of cooked meat texture, and so these results should be viewed with caution.

Taken together, these studies point to some consistent pattern that tenderness is partly correlated with small diameters of muscle fascicles, but as is so common in meat science, this single variable is of extremely limited value in predicting the toughness of cooked meat, due to the highly multifactorial nature of texture.

Variations in the Amount of Intramuscular Connective Tissue Relate to Muscle Function

From the forgoing two sections it is clear that IMCT varies in amount and, especially at the perimysial level, in spatial distribution between muscles. It is generally accepted that this must reflect differences in

physiological (mainly biomechanical) function between muscles, but until recently it has not been clear what the functional role of IMCT is. However, in the last decade there has been a great advance of our understanding of role of IMCT in coordinating force transmission in muscle. Evidence for load sharing via the endomysium between muscle fibres within a fascicle and via the perimysium between fascicles within a muscle is comprehensively reviewed by Trotter et al. (1985), Huijing (1999a,b) and Monti, Roy, Hodgson & Edgerton. (1999). Endomysial connective tissue facilitates load sharing within a fascicle by linking adjacent muscle cells by shear (Trotter & Purslow, 1992; Purslow & Trotter, 1994; Trotter et al, 1995). It has been shown that the easily-deformable tensile properties of the endomysial network allow it to follow necessary shape changes in contracting muscle fibres without restraining this process, but the shear properties though the thickness of the endomysium are suitable for efficient load transfer over a wide range of physiological muscle fibre lengths (Purslow, 2002). These endomysial linkages can be thought of as coordinating the deformations within a fascicle, especially in the usual physiological circumstances where not all motor units are contracting.

In terms of the very variable spatial distribution of perimysium, and via the perimysium, (Purslow, 1999) proposed that division of a muscle into fascicles by the perimysium reflects the need to accommodate shape changes as the muscle contracts, and that this is achieved by allowing fascicles to slide past each other. Ultrasonic imaging data of Fukunaga and colleagues on changes in muscle geometry during human locomotory function (e.g. Fukunaga, Ichinose, Ito, Kawakami, Fukashiro, 1997) clearly demonstrates that that these shape changes do occur in living muscle. Purslow (2002) calculated the shear strains that occur between fascicles within various muscles over their physiological range of contraction based on the data from a series of papers by Fukunaga and colleagues and showed them to be substantial, and variable between muscles. Functionally different muscles have very different requirements to accommodate the shear strains that necessarily occur as the muscle contracts and changes shape, and this begins to explain why the amounts and distribution of perimysial connective tissue varies so much between functionally different muscles. Variations in IMCT between functionally different muscles represents a great variation in expression from a given genome, and whilst genomics and proteomics approaches to describing such variation in expression may assist our understanding of this in relation to meat quality (Eggen & Hocquette, 2003), in terms of meat science a prime question is what possibilities exist to manipulate variability in IMCT expression so as to improve meat texture? Although there is clearly some possibilities, as evidenced by differences between breeds and in some hypertropic genetic conditions, these considerations of the physiological function of IMCT imply that the variations in expression of perimysial IMCT between muscles is tightly related to muscle activity, and so may not easily be manipulated without compromising muscle function in the live animal.

Postmortem effects on IMCT

Although little in the way of new concepts has come up in work on both the effects of cooking temperature and time, and on effects of postmortem storage (conditioning or ageing) of meat on IMCT since previous reviews (Purslow, 1999, 2002), it is perhaps worthwhile to highlight some salient points where there still seems to be contrary interpretations of previously established views in the last 5 years.

Cooking influences on IMCT contributions to toughness.

The toughness of meat increases with temperature of cooking. A sharp increase in toughness occurs between 40ºC and 50ºC, followed either a dip between 50º and 60ºC or a plateau in toughness depending on measurement technique, and then a second phase of increasing toughness above 65ºC. Several studies have intuitively correlated these changes with the thermal denaturation of myosin in the range 42-65ºC and collagen above 65ºC (Davey & Gilbert, 1974; Davey & Winger, 1979; Martens, Stabussvik, Martens, 1982). Although this interpretation has been proved incorrect, it still is used by some to infer that collagen and shrinkage above 65ºC is primarily related to toughness development at higher temperatures (e.g. McCormick, 1999, 2001).

The evidence for IMCT actually contributing to the first phase of toughening, at 40-60°C, goes back to Bouton, Harris & Ratcliff (1981), who inferred from their Warner Bratzler PF-IY measurements that the connective tissue contribution to toughness is high at low cooking temperatures and decreases above 60ºC. Lewis and Purslow (1989) showed by direct measurements on perimysium isolated from cooked beef meat that perimysial connective tissue strength increases in meat cooked up to 50ºC and decreases above this temperature. Mutungi, Purslow & Warkup (1996) showed that the strength of porcine muscle fibres continually increases up to 90ºC. Christensen, Purslow & Larsen (2000) confirmed these two previous findings by examining the strength of both perimysium and individual muscle fibres from the same muscle samples cooked to various temperatures. These studies all add confirmation to the interpretation of Bouton et al. (1981); cooking increases the IMCT contribution to toughness in the range 20ºC-50ºC, with myofibrillar contributions being more prominent above 60ºC. Because isolated IMCT forcefully shrinks above 65ºC when rapidly heated (Kopp and Bonnet, 1987), there is still a feeling that high-temperature shrinkage of collagen could cause the shrinkage of meat seen at 65º-80ºC, and that this shrinkage cause volume reduction in the muscle fibres, so increasing their toughness (Lepetit, Grajales, Favier, 2000). Powell and coworkers (Powell, Hunt, Dikeman; 2000; Powell, Dikeman, Hunt, 2000) have provided some interesting new findings in this area by relating the pronase-extractable fraction of collagen to its thermal denaturation above 55ºC. They report strong correlations between this fraction and peak shear force in experiments; salt-soluble collagen is not nearly as well correlated to toughness. Their explanation is that this insoluble but enyzmically extractable fraction is more related to changes in the structural integrity of the fibrous IMCT network on cooking. However, it has been pointed out previously (Purslow, 1999) that the ratio of transverse to longitudinal shrinkage in meat on cooking, and especially how these vary with sarcomere length, is not simply explainable on the basis of collagen network shrinkage. It is possible that other events, such as cytoskeletal protein denaturation, cause the shrinkage to toughen myofibrillar components at higher temperatures. Obuz, Dikeman, Grobbel, Stephens and Loughin (2004) conclude that variations in cooking losses between different sample sizes and different cooking methods alter the WBSF values seen and also complicate the relative contributions of IMCT softening and myofibrillar hardening.

Post-mortem proteolysis has no effect on the integrity of IMCT after cooking

During post mortem storage (conditioning) of meat there is significant proteolysis of both collagenous and PG components of IMCT, which increases the amount of collagen that can be solubilised from meat (Stanton and Light, 1987) and significantly reduced the strength of perimysium in raw meat after conditioning (Lewis, Purslow, Rice, 1991), as shown in Fig 5.

Over the last ten years there has been a large amount of work to reaffirm that conditioning increases the amount of collagen that is easily extracted from muscle, decreases their structural integrity as viewed by SEM, degrades PG components of IMCT and reduces the strength of IMCT networks, as demonstrated for example by shear measurements on raw muscle or on uncooked IMCT structures embedded in acrylamide gels or by Warner-Bratzler measures on raw meat (Nishimura, Hattori, Takahashi, 1994; Nishimura, Hattori, Takahashi, 1995;Nishimura, Hattori, Takahashi, 1996a,b; Nishimura, Liu, Hattori, Takahashi, 1998; Liu, Nishimura, Takahashi, 1994, 1995; Nakano, et al., 1997; Eggen, Ekholdt, Host, Kolset, 1998; Fang, Nishimura, Takahashi, 1999; Palka, 2003). Similarly, Torrescano and coworkers (Torrescano, Sánchez-Escalante, Giménez, Roncalés, Beltrán, 2003) recently found strong correlations between the total collagen content and insoluble collagen content of 14 beef muscles and raw Warner-Bratzler peak shear force values.

We must remember, however that the overwhelming majority of meat is cooked before it is eaten; textural measurements on raw meat miss out the interactions of proteolysis with effects of cooking, and it is this which leads to measurements on raw muscle being a poor predictor of cooked meat texture. Previous work by Bouton and Harris (1972) showed that measures of the connective tissue component of toughness were unaffected by extensive conditioning when followed by cooking. Lewis et al. (1991) demonstrated this clearly by measuring the strength of perimysium in conditioned meat both before and after cooking.; while there is a reduction in strength of IMCT with conditioning in raw meat, these effects are negated after cooking to temperatures of 60ºC and above, where both aged and unaged perimysial IMCT has the same strength. It is work reiterating this result, shown in Fig. 5, to caution against the recent trend to interpret properties of IMCT in raw meat and degradations during conditioning as if these will inevitably translate into

cooked meat toughness. They do not, as has been known from the work of Harris and colleagues for more than 30 years. Palka (2003) recently was compelled to revisit previous conclusions that the texture of raw meat is poorly related to cooked meat toughness.

Animal maturity, crosslinking and turnover of IMCT

Animal maturity is associated with increased thermal and mechanical stability of IMCT (Bailey and Light, 1989). Meat tenderness generally decreases with animal age, and collagen-rich muscles show this effect more than those with a low IMCT content (Shorthose & Harris, 1990). Increasing collagen fibre diameter and especially the development of mature crosslinks from immature divalent forms accompany this. Although an in-depth investigation of the relationships between a large number of types of collagen crosslinks and cooked meat toughness showed no conclusive correlations in pigs of similar maturity (Avery, Sims, Warkup, Bailey, 1996), or in beef animals in the age range 400-800 days (Avery, Sims, Warkup, Bailey, 1998), it is generally accepted that the development of mature crosslinks with age in IMCT cause it to have increased contributions to cooked meat toughness (Bailey& Light, 1989; McCormick, 1994, 1999). The mechanisms of crosslinking on maturation and ageing of collagen are extensively reviewed by Bailey and coworkers (Bailey, Paul, Knott, 1998). The formation of the immature, divalent crosslinks is controlled by post-translational modifications and can be assumed to be positively directed to provide the mechanical stability required for collagen to fulfil its functional roles.

Collagen in muscle is relatively slow to turnover. Rucklidge, Milne, McGaw, Milne & Robins (1992) report a half-life of 45 days, and although Laurent (1987) inferred a faster turnover, there are good reasons to suppose that the long residence time reported by Robins is more realistic. Relatively slow turnover of IMCT components gives time for slow modifications, including the conversion of divalent to trivalent crosslinks between collagen molecules, slowly increasing the thermal and mechanical stability of IMCT with age. This consequence of slow turnover may not be a functional adaptation, but simply an ageing process. Thus, if the turnover of collagen in muscle could be increased, there is a possibility that less mature, less thermally stable IMCT could result in more tender meat.

Woessner (1968) long ago pointed out that for growth to occur there must be degradation of collagenous networks; during animal growth the hypertrophy of a fixed number of muscle fibres s responsible for muscle weight gain and to accommodated bigger muscle fibres and fascicles, endomysial and perimysial networks must necessarily be remodelled, otherwise they will limit growth.

Turnover of connective tissue is principally under the control of matrix metalloproteinases (MMPs) and their specific inhibitors, TIMPs. Fifteen out of sixteen known MMPs and 3 out of 4 TIMPs have been found in either bovine skeletal muscle, or IMCT, fibroblasts and myoblasts isolated from it (Balacerzak, Querengesser, Dixon, Baracos, 2001). Both muscle cells and fibroblasts within muscle are known to secrete MMPs and TIMPs. MMPs are secreted in inactive forms and activated in a cascade involving plasmin, tissue kallikrein and other factors (Parsons, Watson, Brown, Collins, Steele, 1997). Injury of (cardiac) muscle stimulates MMP expression by fibroblasts in response to the cytokines IL-β and TNF-α (Siwik, Chang, Colucci, 2000). Both TNF-α and other growth factors such as bFGF stimulate increased MMP activity in skeletal myoblasts (Allen, Teitelbaum, Kurachi, 2002). Mechanical stimulus (e.g. exercise) has long been known to be a factor controlling muscle growth and turnover (Goldspink, 1999, 2003). Turnover of IMCT is especially evident in repair of injury or hypertrophic response to exercise (Koskinen et al., 2001) and down regulated during immobilization (Ahtikoski, 2003). It is not known whether turnover of IMCT structures is solely mediated by the fibroblasts that sparsely populate the IMCT, or whether MMPs are expressed by muscle cells in response to mechanical signals. Interactions between muscle cells and extracellular matrix structures involved in muscle development and in cell signalling have been reviewed recently (Purslow, 2002). Muscle strain or injury leads to a rapid production (fibrosis) of endomysial and perimysial connective tissue (Stauber, Knack, Miller, Grimmet, 1996). The remodelling of muscle tissue during rapid the hypertrophy involved in compensatory growth following feed restriction necessarily involves remodelling of the IMCT structures which link and coordinate the various level of structure within the tissue. It has previously been recognised that animals slaughtered after a period of rapid growth will have less contribution to toughness from IMCT (Aberle, Reeves, Judge, Hunsley, Perry, 1981; Miller, Tatum,

Cross, Bowling, Clayton, 1983). Allingham, Harper & Hunter (1998) showed that rapid compensatory growth after weight loss can reduce the strength of IMCT in bovine semitendinosus. Sylvestre, Balcerzak, Feidt, Baracos and Bellut (2002) have demonstrated that dietary manipulation of growth rate in lambs does alter the amounts, activation and activity of MMPs.

Conclusion

The expression of connective tissue within muscle is amazingly variable, depending on developmental stage, muscle position/function, animal breed, nutrition, exercise and injury. Much of the work on IMCT in relation to meat texture in the last century has bee devoted to documenting and understanding the variations between muscle and breed and brought on by animal age. Investigations of post-mortem treatment of meat to reduce the IMCT contribution to meat toughness has met with extremely limited success outside the sphere of long, slow cooking to dissolve extracellular matrix components in tough muscles, and has led to the view that the IMCT contribution to texture is a "background" feature that little can be done about. In the post-genomic era it is possible to return attention on how expression of IMCT components may be manipulated, so as to improve tenderness. The amounts and distribution of connective tissue in muscles seem to vary for very good reasons of muscle development, growth, and especially function. This may limit the scope for manipulation of its expression. Manipulation of the state of IMCT maturity may, however, be a possible means of reducing unwanted variability in meat tenderness.

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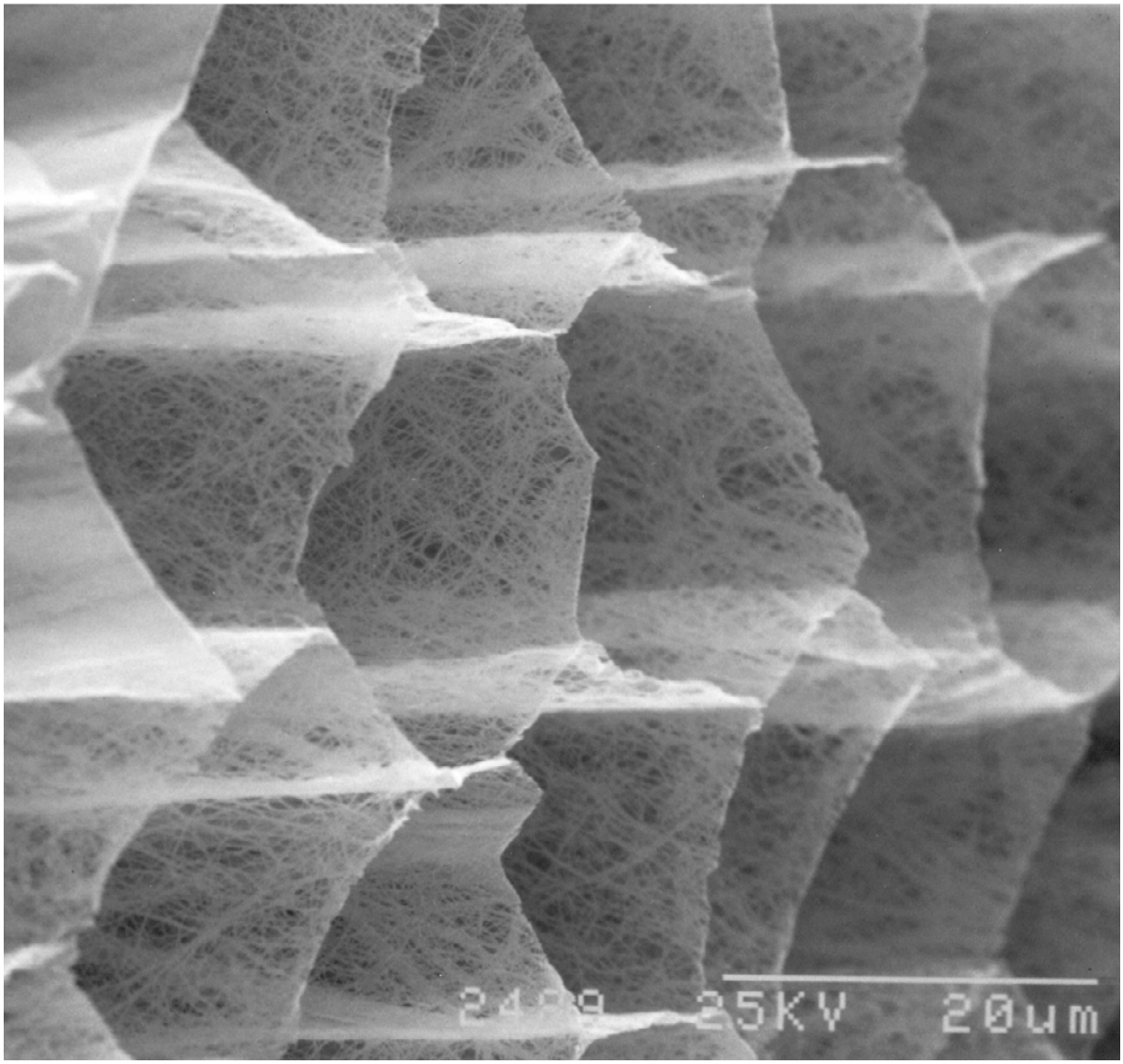

Fig. 1. Scanning electron micrograph of endomysial network in bovine sternomandibularis muscle after extraction of myofibrillar components by NaOH. The feltwork of fine wavy collagen fibres in the endomysial structures separating individual muscle fibres is clearly seen. From Purslow and Trotter (1994).

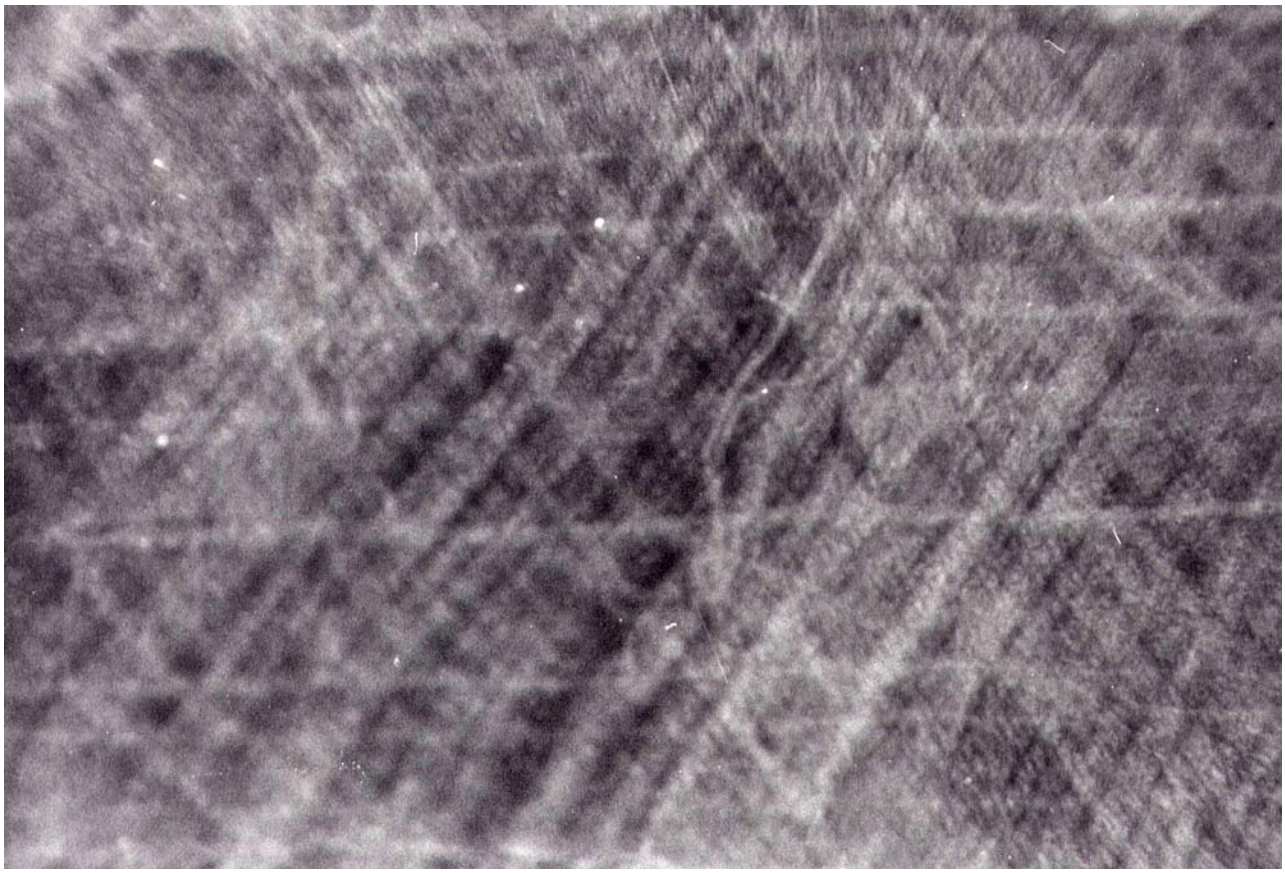

Fig.2. Light micrograph showing the arrangement of collagen fibres in the epimysium of bovine sternomandibularis muscle. Horizontal lines show the edges of individual muscle fibres in the surface of the muscle. The collagen fibres of the overlying epimysium lie in two plies of parallel fibres, at approx. $\pm$ 54° to the muscle fibre direction.

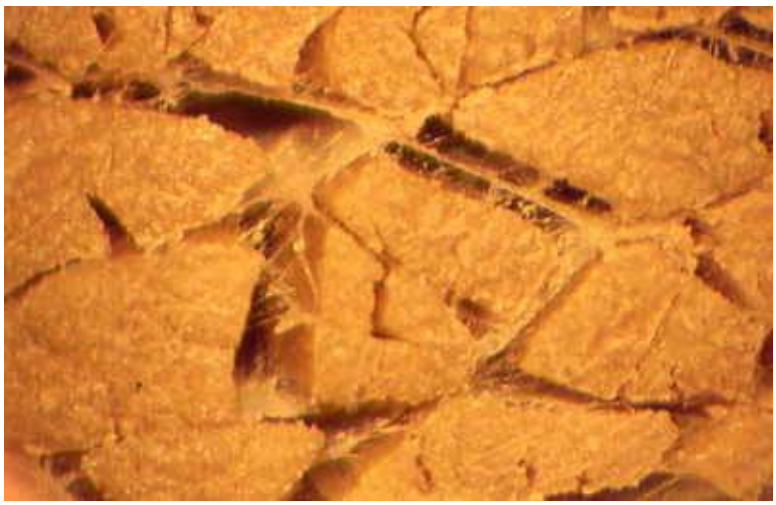

Fig.3. Bovine semitendinosus muscle cooked to 80°C and pulled perpendicular to the muscle fibre direction. For full details of preparation see Purslow (1985). Top: initial stages of separation, showing intact fascicles separating from each other, leaving perimysial sheets in the gaps between them. Bottom: subsequent pulling apart of the sample causes complete rupture after the separated perimysial sheets are broken.

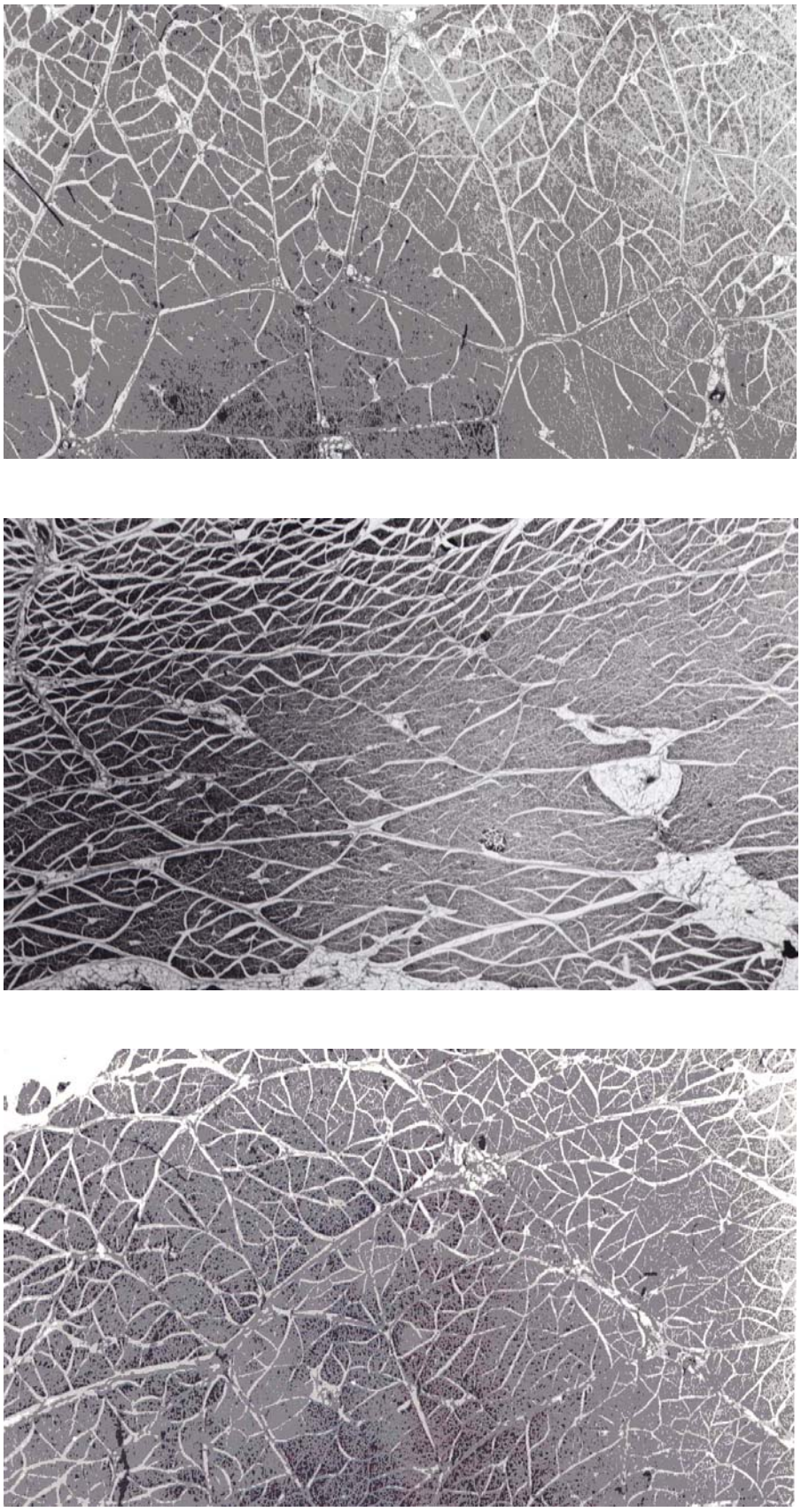

Fig 4. Comparison of the fascicular architecture in cross-sections of three muscles from the same (bovine) animal: pectoralis profundus (top), strenocephalicus (mid) rhomboideus cervicus (bottom). Clear differences in fascicle size, shape, and perimysial thickness can be seen between them.

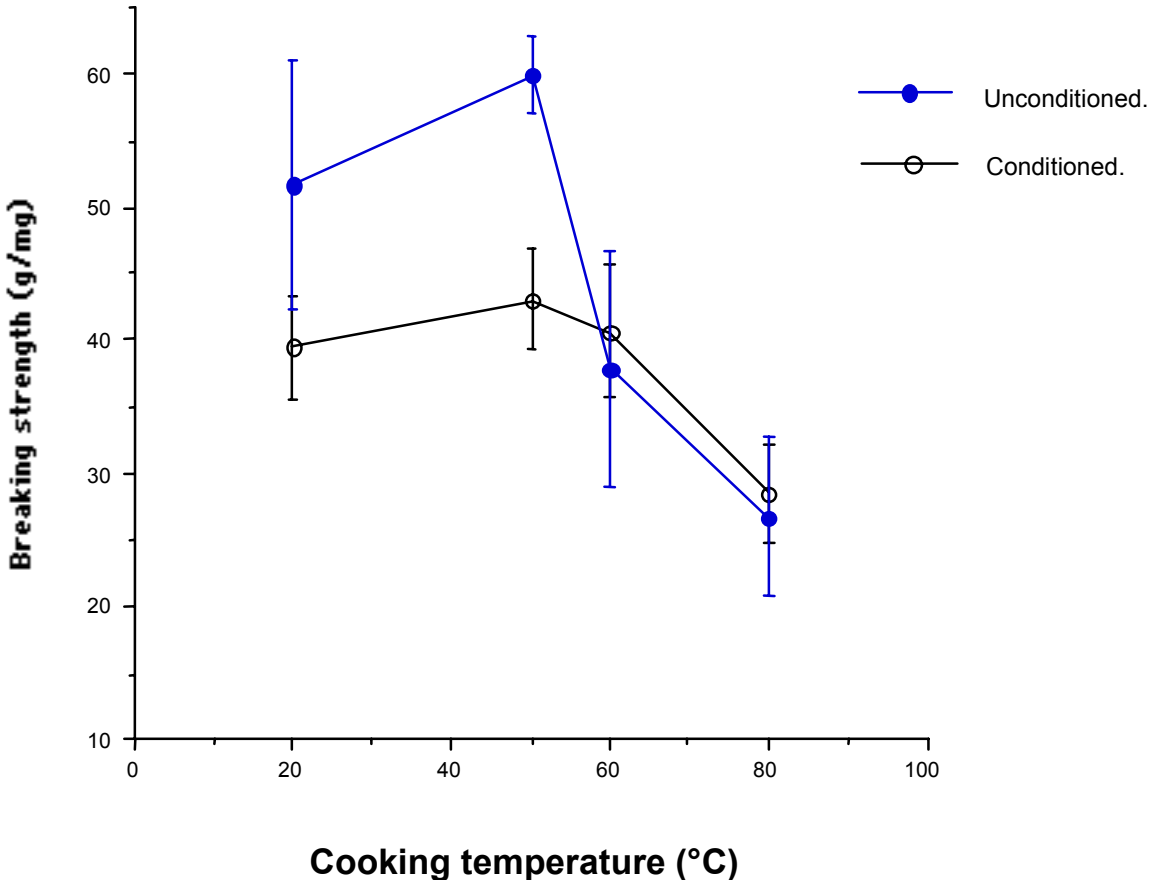

Fig. 5. Strength of perimysium isolated from meat after cooking to the temperatures shown; comparison of perimysium from one day post mortem muscle (unconditioned) or muscle stored for 14 days (conditioned). Vertical bars represent mean ± SE. From Purslow, 1999; redrawn from Lewis et al., 1991.

THE CHILLING OF CARCASSES

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Abstract

Biochemical processes and structural changes that occur in muscle during the first 24 hours postmortem play a great role in the ultimate quality and palatability of meat and are influenced by the chilling processes that carcasses are subjected to after the slaughter process. For beef and lamb, employing chilling parameters that minimize cold shortening is of the greatest importance and can be best addressed by ensuring that muscle temperatures are not below 10°C before pH reaches 6.2. For pork, because of the impact of high muscle temperatures and low pH on the development of pale, soft, and exudative (PSE) pork, a more rapid chilling process is needed to reduce PSE with the recommended internal muscle temperature 10°C at 12 h and 2 to 4°C at 24 h. Spray chilling, a system whereby chilled water is applied to carcasses during the early part of postmortem cooling, is used to control carcass shrinkage and to improve chilling rates through evaporative cooling. Delayed chilling can be used to reduce or prevent the negative effects of cold shortening; however, production constraints in high-volume facilities and food safety concerns make this method less useful in commercial settings. Electrical stimulation and alternative carcass suspension programs offer processors the opportunity to negate most or all of the effects of cold shortening while still using traditional chilling systems. Rapid or blast chilling can be an effective method to reduce the incidence of PSE in pork but extreme chilling systems may cause quality problems because of the differential between the cold temperatures on the outside of the carcass compared to the warm muscle temperatures within the carcass (i.e., muscles that are darker in color externally and lighter in color internally).

Keywords: Chilling; Cold shortening; Color; Meat tenderness; Shrinkage

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Introduction

Most postmortem chilling processes of livestock carcasses are primarily employed to ensure food safety, maximize shelf-life, and reduce shrinkage with less emphasis on maintaining tenderness and color factors of the finished product. Whether chilling conditions are being met for regulatory requirements, as part of a critical control point of a HACCP system, or as best practices, other factors may be more important than those affecting direct consumer satisfaction of the product.

Heat dissipation initially was accomplished through natural means such that animals would have been slaughtered in cooler seasons and product would have been stored in caves as a way to prolong keeping quality. Throughout the centuries, more sophisticated means were developed to reduce the heat from freshly slaughtered animals. Today, advanced refrigeration systems are used throughout most parts of the world to accomplish the task of chilling carcasses during the critical time period after slaughter and through the development of rigor mortis. The efficiency of heat removal became so great that a half century ago, it was discovered that the process of chilling could negatively affect the eating quality of beef and lamb (Lawrie, 1998). Conversely, with increases in pork carcass weights and mass over the years, the challenge oftentimes for this species is to get the product chilled more rapidly to reduce problems associated with temperature/pH relationships. These two examples reflect the importance of developing and implementing chilling systems that are neither too severe nor too mild for the particular species involved.

This is a review of past and current research on the effects of chilling systems on meat quality. Special attention is given to the impact of chilling systems on tenderness, color, and shrinkage.

The first 24 h postmortem

There are many biochemical and structural events that take place in the first 24 h period after the animal is slaughtered and the muscle is converted to meat. This period greatly impacts meat tenderness and muscle color and is species-specific in how the initial cooling process results in positive or negative consequences in meat quality.

Rigor mortis

After exsanguination, glycolysis proceeds without oxygen and produces lactic acid as a result of anaerobic glycolysis. This creates a lactic acid build-up and therefore a decrease in pH. In a normal setting, muscles begin the process of rigor mortis where permanent cross-bridges, called actomyosin, are formed between the actin and myosin filaments. Rigor begins in normal meat at pH values of 5.7 to 5.8 (Hannula and Puolanne, 2004). During the first phase of rigor, the delay phase, the muscle is still extensible because there is still ATP available to bind with Mg2+, which helps disconnect the actin/myosin cross-bridges and in turn allows the muscles to relax. Creatine phosphate is depleted during this phase, which inhibits the phosphorylation of ADP into ATP. This causes a sharp decrease in ATP production, which is the signal of the start of the onset phase of rigor. Because there is little ATP available to break down the actin and myosin bonds, muscles cannot relax and therefore become inextensible (Aberle et al*.,* 2001).

pH decline

Normally, the pH in the muscle decreases from 7.0 upon slaughter to approximately 5.3 to 5.8 (Smulders, Toldra, Flores, & Prieto, 1992). In extreme cases, this decline can take only 1 h. The typical decline for pork is in the range of 6 to 12 h, and beef usually completes its pH decline in 18 to 40 h (Smulders et al., 1992). Howard and Lawrie (1956) suggested that the rate of pH decline has an inverse relationship to tenderness. As the pH drops, it nears the iso-electric point. At this point, all of the negatively and positively charged amino acid side chains equal, which causes the maximal attraction between the two. This attraction holds the filaments closely together and does not allow any water to get in, greatly reducing the water-holding capacity (Smulders et al., 1992).

Because pork experiences a quicker pH decline than other species, it is more likely to experience elevated temperatures during the onset phase of rigor, which can be detrimental to the myoglobin pigment. This

causes the myoglobin structure to be "open" and scatter light, creating a pale colored product (Lawrie, 1998). When pork is not able to hold water, it loses color and firmness. This condition is described as pale, soft, and exudative (PSE). Quickly lowering the temperature postmortem will decrease the velocity of these chemical and biochemical reactions, therefore decreasing the rate of pH decline (Lawrie, 1998). Blast chilling will help with the reduction of this type of reaction (Meisinger, 1999). Offer (1991) reported that the effect of chilling on PSE conditions is strongly dependent on the rate of pH decline. Meisinger (1999) suggested chilling pork to muscle temperatures of less than 10°C in 12 h, and 2 to 4°C in 24 h to reduce the occurrence of PSE.

Tenderness

Koohmaraie et al. (1987) suggested that at slaughter all animals with the same pre-slaughter treatments have the same tenderness level, and that differences in tenderness are created in the first 24 h post mortem. It has been shown that there is a decrease in tenderness of muscles before the aging process begins (Koohmaraie, 1996). Koohmaraie (1995) showed that there is a large amount of variation in tenderness (shear force) after one day of postmortem storage, and that maximum toughness has been observed in the range of 9 to 24 h (Koohmaraie, 1996). After 24 h, an increase in tenderness is observed as a result of enzymatic degradation of muscle tissue. Temperature of storage can affect this enzymatic degradation, as well as other factors including: pH, muscle fiber type, amount and degree of cross-linking of connective tissue, and animal species (Smulders et al., 1992). Enzymatic degradation is caused by proteolytic enzymes such as calpains and lysomal proteases (Smulders et al., 1992). The aging process typically takes 1 to 2 d in chicken, 3 to 6 in pork, and 10 to 20 d in beef (Smulders et al., 1992).

The cause of toughening during the first 24 h has been debated, and there are numerous studies that suggest various reasons for this increase. Goll, Geesink, Taylor, and Thompson (1995) proposed that toughening was caused by the change in strength of the binding state of the actin/myosin interaction and the possibility of this change causing severe shortening in the first 24 h postmortem. Another theory is myofibril fragmentation index (MFI), which has been shown to be useful in characterizing tenderness groups (Culler, Parrish, Smith, & Cross, 1978; Parrish, Vandell, & Culler, 1979). Culler et al. (1978) found that MFI accounted for more than 50% of the variation in loin steaks, and that the MFI was a more important effecter of tenderness than collagen solubility or sarcomere length. In addition, many studies have shown that sarcomere length does not affect tenderness (Culler et al., 1978; Smith, Kastner, Hunt, Kropf, & Allen, 1979; Seideman & Koohmaraie, 1987; and Shackelford, Koohmaraie, & Savell, 1994). However, the majority of the research suggests that sarcomere shortening is the causative factor of the decrease in tenderness of muscles from the time of slaughter to 24 h postmortem. Bouton, Harris, Shorthose, and Baxter (1973) found significantly shorter sarcomere lengths in the *M. semimembranosus, M. gluteus medius*, *M. biceps femoris*, and *M. longissimus dorsi* muscles, as well as significantly tougher *M. adductor* and *M. vastus lateralis* muscles conditioned at 0 to 1ºC compared to those conditioned at 15 to 16ºC. They also found strong relationships between Warner-Bratzler shear force values and sarcomere lengths when sarcomeres were shorter than 2.0 µm. Overall, Bouton et al. (1973) found that shear force values decreased exponentially as sarcomere lengths increased. When comparing "less tender" and "more tender" steaks, Davis, Smith, Carpenter, Dutson, and Cross (1979) found that "more tender" steaks had longer sarcomeres (based on mean values) than "less tender" steaks did. Davey, Kuttel, and Gilbert (1967) found that myofibrillar contraction state affected the level of tenderness that could be achieved by aging, and specifically at 40% contraction, there was almost no effect from aging. Marsh and Leet (1966) found that decreases up to 20% of the initial excised muscle do not have a significant effect on tenderness, but toughness increases rapidly with further shortening, peaking at 40%. However, after 40%, the meat becomes progressively more tender. In addition, it was found that meat with 55 to 60% shortening had Warner-Bratzler shear force values similar to those that shortened less than 20%. Marsh and Leet (1966) concluded that the degree of rigor onset at time of cold application is inversely related to the degree of cold shortening.

Cold shortening

Cold shortening has been studied since the 1960's (Locker, 1985). Locker and Hagyard (1963) defined cold shortening as a rapid decline in muscle temperature to less than 14 to 19ºC before the onset phase of rigor mortis. When carcasses are cooled quickly, they have the potential to be affected by cold-induced shortening and/or toughening. Temperature and pH relationships at the moment of onset of rigor can be considered the decisive factor of degree of cold shortening (Hannula & Puolanne, 2004). When the muscle temperature is

reduced to 0 to 15ºC before the onset phase of rigor, the sarcoplasmic reticulin cannot function properly and is unable to bind calcium, which leaves an abundance of calcium in the sarcoplasm. Because there is still ATP left in the muscle, the muscle contracts at a maximum level, causing the filaments to slide over one another basically eliminating the I-band of the sarcomere. At internal temperatures of 1 to 2ºC, the sarcoplasmic reticulin is the least functional (Aberle et al., 2001).

The relationship of cold shortening and sarcomere length to toughness was first demonstrated clearly by Herring, Cassens, and Briskey (1965) who showed the direct relationship of sarcomere length to fiber diameter and toughness. Their theory is that the more contracted the sarcomere, the larger the fiber diameter becomes due to sliding of the filaments over one another. After cooking, meat with larger fibers is tougher.

Muscle types vary in their potential to cold shorten, with red being more susceptible than white (Bendall, 1973). Because white muscle fibers tend to have higher amounts of glycogen, they experience a more severe drop in pH earlier in the rigor process. This is especially relevant with pork. Because pork is comprised primarily of white muscle fibers, they are not as affected by cold shortening to the extent as beef and lamb. Pork takes approximately 6 h to complete the rigor mortis process, and only 15 min to 1 h to begin the onset phase of rigor. The combination of time, temperature, and pH differs between and within muscle, and also between species (Hannula & Puolanne, 2004). This indicates that all muscles are not affected by cold shortening to the same degree (Hannula & Puolanne, 2004).

Additional sarcomere shortening conditions are thaw and heat rigor. These conditions are caused when carcasses are exposed to extreme cold or hot temperatures pre-rigor. Thaw rigor is a form of rigor mortis that develops when muscle that was frozen prerigor is thawed (Aberle et al., 2001). Aberle et al. (2001) stated that when this muscle is thawed, contraction is produced by the sudden release of Ca^{2+} into the sarcoplasm resulting in a physical shortening of 60 to 80 percent of the original muscle length and a release of large quantities of meat juices and severe toughening. Heat rigor occurs when muscles are maintained at elevated temperatures up to 50°C resulting in a rapid depletion of ATP, which creates severe shortening and the early onset of rigor (Aberle et al., 2001). The severity of these two extreme conditions show the importance of designing chilling conditions that do not negatively impact meat quality.

Prevention of cold shortening

Although there is no way to prevent rigor and the shortening of sarcomeres completely, there are ways to reduce the extent and toughening effects of this process before, throughout, and after slaughter. Fat thickness can play a significant role in the reduction of cold shortening during the chilling processes of beef (Dolezal, Smith, Savell, & Carpenter, 1982) and lamb (Smith, Dutson, Hostetler, & Carpenter, 1976). Increased thicknesses of subcutaneous fat were found to improve tenderness by allowing the carcass to chill more slowly and to increase enzyme activity (Smith et al., 1976). The authors postulated that increased fatness either decreased chilling rate because of a greater amount of insulation or because of increased total mass.

Dolezal et al. (1982) found that carcasses possessing 2.54 mm of external fat received the lowest ($P < 0.05$) sensory panel ratings for myofibrillar tenderness, and had the highest ($P < 0.05$) shear force values. In addition, as fat thickness increased up to 7.61 mm, palatability also increased. Smith and Carpenter (1973) reported that a fat covering of 2.5 mm for lamb carcasses would prevent excessive postmortem shrinkage during chilling and transit. A subcutaneous fat depth of 0.62 cm at the 12th rib is suggested as the minimum level to prevent cold shortening in cattle.

Carcass weight and composition have both been deemed important factors of chilling rate (Kastner, 1981). In a study by Hippe et al. (1991), sides from thinner cows had a higher percentage of cooler shrink than sides from steers after 24 h. This is substantiated by research from Johnson, Hunt, Allen, Kastner, Danler, and Shrock (1988) that lean tissue retained less water than adipose after 20 h postmortem. More moisture loss occurs from lean than from fat tissue (Johnson et al., 1988). The major variables associated with reducing shrinkage are decreased surface area and/or increased subcutaneous fat covering (Smith & Carpenter, 1973). Increased fatness may decrease shrinkage by serving as a barrier against moisture loss (preventing evaporation from the lean), or it may act to minimize the total moisture content in the carcass (Smith & Carpenter, 1973).

Smith et al. (1976) determined that lamb carcasses with increased quantities of fat chilled more slowly, had less sarcomere shortening with less perceptible connective tissue, and were more tender. Increased levels of subcutaneous or intramuscular fat can increase lamb tenderness by altering the postmortem chilling rate, especially with little external fat (Smith et al., 1976).

Electrical stimulation helps prevent cold shortening by using up energy (ATP) before the onset stage of rigor. An electrical current is passed through the carcass during the slaughter process causing the muscles to violently contract and accelerating anaerobic glycolysis, which increases the rate of pH decline and reduces the overall time of rigor mortis. This in turn reduces the muscle's susceptibility to cold-induced shortening. Electrical stimulation of beef produces significant positive effects on lean maturity, overall maturity, and Warner-Bratzler shear force tenderness (Savell, Smith, & Carpenter, 1978; Calkins, Savell, Smith, & Murphey, 1980). Lean color also was found to be positively impacted, and heat ring (the distorted appearance of the outside edge of muscle, caused by rapid chilling and little external fat) was reduced. Also, Warner-Bratzler shear force values were significantly decreased for 21 d aged, electrically stimulated carcasses vs. control carcasses, and more interestingly, this was also true for 7 d electrically stimulated carcasses vs. 21 d controls (Savell et al., 1978). Shorthose, Powell, and Harris (1986) found this to be the case with lamb as well. Electrical stimulation had minimal (Johnson, Savell, Weatherspoon, & Smith, 1982) and even adverse (Swasdee, Terrell, Dutson, Crenwelge, & Smith, 1983) effects on quality, palatability, and weight loss in pork.

During chilling, the extent of shortening in a carcass depends on the physical restriction imposed by the attachment of the muscle to the skeleton (Hostetler, Landmann, Link, & Fitzhugh, 1970). Another method used to reduce sarcomere shortening is to alter the method of carcass suspension. Hostetler, Link, Landmann, and Fitzhugh (1972) looked at several methods including: vertical hanging (conventional hanging using suspension by the Achilles tendon), horizontal (side placed on table, with limbs tied perpendicular to the vertebra), neck-tide (side suspended from the cervical vertebra, with pelvic limb tide to thoracic limb), hip-free (side suspended from the obturator foramen and pelvic limbs free), and hip-tied (side suspended from the obturator foramen with the thoracic limb tide to the pelvic limb). The hip-free method was determined to be the most beneficial in improving sarcomere lengths and the ultimate tenderness of the muscles, especially in the loin and round. Hostetler, Carpenter, Smith, and Dutson (1975) found that carcasses suspended by the obturator foramen had muscles with 17% longer sarcomeres than those from carcasses suspended normally, and that this created less overlap of thick and thin filaments.

Times that the carcasses are placed into the cooler and temperatures at which they are held could be the most plausible way to affect cold shortening after slaughter. Bendall (1972) reported that muscles with temperatures less than 10ºC are susceptible to cold shortening until a muscle pH of less than 6.2 is reached, and at 16ºC cold shortening is less severe. Hannula and Puolanne (2004) found that the effects of aging should be more effective if the carcasses are maintained above 7 to 10ºC until the onset of rigor (pH 5.7). Olsson, Herzman, and Tornberg (1994) have set 7ºC internal muscle temperature as the upper limit for cold shortening, and Bendall (1973) recommends against cooling beef carcasses below 12ºC internally in less than 15 h, or before completion of rigor mortis. There are many different opinions on the exact temperature necessary to eliminate or reduce cold shortening. Conventional chilling allows for control of these factors that can promote or inhibit cold shortening. At the same time, there are additional techniques that can be applied during the chilling process that can alter the level of cold induced shortening.

Delay Chilling

Delay chilling has been defined by Smulders et al. (1992) as the process of keeping intact carcasses out of the chill room for some period of time. This is not to be confused with high temperature conditioning, which is subjecting primals or cuts to elevated temperatures after boning (Smulders et al., 1992). Marsh, Lochner, Takahashi, and Kragness (1980-81) indicated that beef sides delay chilled at 37°C for 3 h postmortem were more tender than conventionally chilled sides. Delayed chilling has been reported by some to have a positive influence on postmortem tenderness. In a study by Fields, Carpenter, and Smith (1976), steaks from steer and cow sides held at ambient temperatures of 14 to 19°C for 20 h were more tender than those from

normally chilled sides. Also noted was the fact that elevated temperature conditioned steaks stored for 7 d received higher panel ratings for tenderness than control steaks. In this study, pre-rigor storage at elevated temperatures improved the appearance of steaks from steer and cow carcasses (Fields et al., 1976). The authors reported that 115 out of 120 steaks from sides held at 14 to 19°C received higher ratings than control steaks for muscle color, consumer acceptability, and discoloration during 5 d of retail storage. Martin, Murray, Jeremiah, and Dutson (1983) also reported decreased toughness when carcasses were held at elevated ambient temperatures in the range to 10 to 42°C, but were unable to clearly define the mechanism responsible. Delay chilling in pork was studied by Møller and Vestergaard (1987), and *M. longissimus dorsi* with a high initial pH above 6.1 was determined to be tough, while *M. longissimus dorsi* with a low initial pH below 6.1 was not affected.

On the other hand, Will and Henrickson (1976) determined that shear force and penetration values between chill (1.1°C for 48 h) and delay chill (held 3, 5, or 7 h postmortem at 16°C) treatments were small (1.65 kg and 0.18 cm, respectively), and led them to the conclusion that there were no significant tenderness differences observed between the two regimes. Phoya and Will (1986) reported that a taste panel was able to detect differences between delay chilled steaks (16°C for 4 h, then some muscle excision and 1°C storage for another 44 h) and conventionally chilled steaks (1°C for 48 h before fabrication). Warner-Bratzler shear force values and preference and hedonic scale scores showed that delay chilling for 4 h with hot-boning did not increase beef tenderness when carcasses were suspended through the obturator foramen (Phoya & Will, 1986). In a similar study performed by Jeremiah, Martin, and Murray (1985), researchers determined that delay chilling (12.5°C for 2 h) along with electrical stimulation did not produce consistent changes in physical, cooking, or palatability traits in the *M. semimembranosus*, *M. longissimus dorsi*, or *M. triceps brachii* muscles of beef.

As noted by Smulders et al. (1992), the potential benefits of high temperature conditioning, which include prevention of cold shortening and increased proteolysis, must be weighed against the possibility of heat shortening. Also, Smulders et al. (1992) stated that the effect of increased tenderness of delay chilled muscle can disappear during storage, and could result from an increased rate of aging or accelerated start to aging because of increased temperatures. There are multiple mechanisms such as heat shortening or cold shortening prevention that can dictate the effect of delay chilling on tenderness.

4. Spray Chilling

Shrinkage represents a serious economic concern for the beef packing industry today (Hamby, Savell, Acuff, Vanderzant, & Cross, 1987). Conditions that must be kept in order to minimize shrinkage include maintaining low temperature conditions, minimal air circulation, and a high relative humidity (Smith & Carpenter, 1973). The principal purpose of spray chilling is to reduce carcass weight loss during chilling, especially during the first 24 hours postmortem (Allen, Hunt, Luchiari Filho, Danler, & Goll, 1987). Spray chilling systems are currently in use in North America, Europe, and elsewhere in the world for beef, lamb, poultry (Brown, Chourouzidis, & Gigiel, 1993), and pork (Brown & James, 1992). The process of spray chilling involves the intermittent spraying of cold water onto carcasses during the first three to eight hours post-slaughter (Hippe, Field, Ray, & Russell, 1991) to replace moisture lost by evaporation (Gigiel, Butler, & Hudson, 1989). With spray chilling, the surface remains wet allowing for maximum mass transfer and evaporative cooling without increasing carcass weight loss (Gigiel et al., 1989).

Postmortem shrinkage is the result of evaporation and drip loss of carcass wash water and loss of moisture by the carcass components (Smith & Carpenter, 1973). Evaporative weight losses of up to 2% of the hot carcass weight have been reported during the initial 24 h of conventional air chilling of beef, pork, and lamb (Greer & Jones, 1997). In a study conducted on 1,000 lamb carcasses, during the first 36 hours, 92% of the 72 h weight loss had already occurred (Smith & Carpenter, 1973). This study determined that shrinkage continues to increase with increased time in the cooler, but at a decreased rate (Smith & Carpenter, 1973). The majority of the initial carcass weight loss that was experienced in this study was due to evaporative losses of water added during washing. The approximately 39% of the weight loss remaining occurred in the next 60 h due to evaporative losses of moisture from carcass components (Smith & Carpenter, 1973). Lamb

can be considered fairly susceptible to cold shortening because of exposed musculature and high surface-to-volume ratio that leads to excessive evaporative weight loss (Brown et al., 1993).
In commercial practice, there is considerable variation in the duration of spray chilling, and the amount of water deposited on carcasses in a specified period of time is usually not known (Greer & Jones, 1997). It appears that spraying carcasses in the initial 3 to 12 h of carcass chilling is relatively common within the commercial setting (Greer & Jones, 1997).

Shrinkage
Shrouded beef carcasses chilled overnight in conventional systems typically shrink from 0.75 to 2% (Kastner, 1981). There have been numerous studies conducted on spray chilling systems that significantly reduce this inherent level of shrink. Heitter (1975) described the Chlor-Chil system used by Swift and Company as an innovative method to reduce carcass shrink while reducing the amount of spoilage bacteria normally present on carcasses in the cooler. This system involved the intermittent spraying of a mild solution of chlorine and water to achieve reductions of carcass shrink that varied from 0.5 to 1.25% (Heitter, 1975). Heitter (1975) went on to say that this shrink reduction that was realized in the chilling operation was carried through to the trimmed cuts. In one of multiple studies that compared spray chilled sides to conventionally chilled sides, Allen et al. (1987) found that after 24 h, spray-chilled beef sides shrank 1.14% less than conventionally chilled sides. In a similar study conducted by Jones, Murray, & Robertson (1988), beef sides spray chilled for 12 h and unshrouded beef sides spray chilled for 8 h had significantly lower carcass weight shrinkages to 6 d than control sides. Jones and Robertson (1988) found that shrinkage was reduced by 0.48 to 0.89 as the spray-chilling period was increased from 4 to 12 h. Hippe, Field, Ray, and Russell (1991) determined that the percentage of cooler shrink for beef sides at 2, 4, 6, and 24 h was lower for spray chilled versus conventionally chilled at all times postmortem.

Lee, Hawrysh, Jeremiah, and Hardin (1990) found that when comparing spray chilled and conventionally chilled beef sides that were both shrouded and unshrouded, unshrouded, spray chilled sides displayed the lowest percent carcass shrinkage after 24 h. They found that spray chilling unshrouded sides would result in a 1.3% savings in side weight. In a similar study by Strydom and Buys (1995), spray chilled beef sides resulted in a 1.1% savings in side weight versus conventionally chilled sides, regardless of duration of spray chilling. On a contrary note, Greer and Jones (1997) discovered a linear relationship between spray chill duration and carcass weight loss, and determined that carcass shrinkage was only reduced by 0.08 g/100 g for every hour of spray chilling.

Shrinkage of conventionally chilled pork can average from 1.85 to 100 g (Jones, Jeremiah, & Robertson, 1993). Conventional pig chilling systems work to reduce pork temperature to 4°C (Brown & James, 1992). James, Murray, and Robertson (1988) determined that a spray-chilling regime could have a significant effect on pork carcass shrinkage while increasing cooling rate of the muscles in the loin and ham. This study revealed that an average 79 kg pork carcass could receive an approximately 1.61 kg advantage in cooler shrink if spray chilling were utilized.

Quality characteristics
An additional benefit of spray chilling discovered by several researchers is an improvement in carcass quality. Allen et al. (1987) observed skeletal maturity to be significantly younger in beef sides that were spray chilled due to visual differences in skeletal ossification. This group determined that it would be advantageous to use spray chilling to improve quality grade when chilling carcasses with maturity that lies near the line drawn between younger and older classed grades. Jones and Robertson (1988) found that beef muscle color was not influenced by the spray chilling process, but fat from spray chilled sides where the spray period was 8 h or more had significantly higher Y^* values than control sides. Greer and Jones (1997) expressed similar results with no real effect of spray chilling on beef lean color, but fat L^* values that were consistently higher on spray chilled carcasses at most locations after 4, 8, 12, and 16 h of spray chill. They recovered generally lower a^* and b^* values after 12 and 16 h of spray chill, and fat developed a washed out, grey appearance after 16 h of spray chilling. It also has been reported that shear values, and therefore tenderness, are not affected by spray chilling (Hippe et al., 1991; Greer & Jones, 1997; Jones & Robertson, 1988; & Lee et al., 1990).

Purge loss

There is some evidence that the initial advantage noted in spray chilled beef sides is lost as purge during the first 15 days of vacuum storage (Allen et al., 1987). Greer and Jones (1997) demonstrated that spray chilling did not affect weight losses from the *M. longissimus thoracis* muscle in comparison to air chilled controls, after spray durations of 4, 8, 12, or 16 h, or storage temperatures of 1, 4, 8, and 12°C for up to 44 days of vacuum storage. Hippe et al. (1991) also reported that purge losses from vacuum packaged top rounds stored 5 or 10 weeks were not significantly different between spray chilled and conventionally chilled sides. Jones and Robertson (1988) determined that drip or purge losses over 6 days in vacuum packaged ribs and inside rounds were similar between spray chilled and conventionally chilled sides. Brown et al. (1993) also reported no increased drip-loss or cooking-loss from samples taken from spray chilled lamb sides. Strydom and Buys (1995) reported that 17 h of spray chilling had no effect on purge loss of vacuum packaged wholesale (7d) or retail display (3d) beef cuts. With this experiment, none of the moisture conserved during chilling was lost during vacuum packaging, retail display, or cooking. However, Allen et al. (1987) reported small (0.26%) increases in purge from inside rounds of spray chilled carcasses.

Shelf-life/microbial quality

There is some concern that the economic advantage of decreased shrinkage may be affected by a slight decrease in microbiological quality (Hippe et al., 1991). However, there is evidence that spray chilling does not affect bacterial counts (Hamby et al., 1987; Strydom & Buys, 1995; Brown et al., 1993), or may slightly reduce bacterial counts (Greer and Dilts, 1988). Greer and Jones (1997) showed that spray chilling had no effect on bacterial growth on the *M. longissimus thoracis* muscle (44 d) or retail cuts as compared to commonly chilled sides even after they were subjected to extremes in spray duration of up to 16 h and vacuum storage temperatures of up to 12°C. Dickson (1990) reported that spray chilling did not affect survival or growth of *Listeria monocytogenes* for up to 42 d of vacuum storage of beef. In a study performed by Jeremiah and Jones (1989), retail case life of chops from spray-chilled pork sides was typically shorter than those from conventionally chilled sides.

Rate of temperature decline

There are many variables influencing chilling rate including: size, shape, and fatness of the carcass, temperature, relative humidity, and flow pattern of the air (Smulders et al., 1992). It has been hypothesized by James (1996) that higher rates of heat transfer, and the heat extracted to evaporate the added water used during spray chilling, should substantially reduce carcass chilling time. Jones and Robertson (1988) indicate that spray chilling significantly affected rate of cooling in the *M. semimembranosus* (SM) and *M. longissimus dorsi* (LD) of beef. This group saw 1 to 2°C increases in the temperature declines of these muscles when sides were both shrouded and unshrouded with spray chilling duration of 8 and 12 h. This effect was most pronounced in the SM because of its position in the carcass and the fact that muscles in the round are closest to the source of water used in the spray chilling process. This effect is also enhanced by a relatively thin fat cover (Jones & Robertson, 1988).

In another study performed by Lee et al. (1990), after 8 h, LD and SM muscles from spray chilled sides had lower muscle temperatures than those that were conventionally chilled with both shrouded and unshrouded carcasses. Brown et al. (1993) also noticed small differences in chilling rate between lamb legs and loins of multiple spray chilled carcasses and those that were conventionally or double spray chilled. On a contrary note, Hippe et al. (1991) reported no differences in postmortem temperatures of *M. semitendinosus*, *M. longissimus*, or *M. serratus ventralis* muscles between sides that were spray chilled and those chilled normally.

Rapid/Blast Chilling

Systems to rapidly reduce carcass temperatures have been investigated for a variety of reasons and with a variety of results. Terms used to describe this category of chilling include but are not limited to “rapid,” “ultra-rapid,” “blast,” “very fast,” and “extreme,” with no consistent definition used by authors in defining

these systems. In this review, our focus was to maintain the terminology used by the authors rather than standardize it.

Some rapid systems are used for regulatory reasons. According to Bowater (2001), in the EU, beef and lamb carcasses must reach internal temperatures of 7°C after 24 h or before moving the carcass to the boning room (pork must reach 4°C after 24 h or before moving the carcass). In the U.S., there are no regulatory requirements for specific temperature endpoints for chilling livestock carcasses; however, the U.S. poultry industry routinely uses rapid chilling because of the United States Department of Agriculture, Food Safety and Inspection Service regulatory requirement that poultry be chilled below 4.4°C before 4 h postmortem.

All frozen poultry begins the chilling process by being immersed in chilled or ice water (Brown and James, 1992). Ultra-rapid chilling consists of two stages, the first of which is a pre-chiller, such as an air-blast tunnel (Brown and James, 1992). This pre-chiller works to quickly reduce surface temperature in order to decrease the amount of evaporative weight loss. It also helps to absorb the initial heat load of the chiller (Brown and James, 1992).

When used in beef, rapid chilling has been shown to produce considerable toughening and severe consumer reaction, and tenderness has been identified as the consumer's number one concern (Joseph, 1996). However, Bowling, Dutson, Smith, and Savell (1987) have shown that it may be possible to chill beef carcasses at extremely rapid rates with less carcass shrinkage and with no detrimental effects on quality grade or cooked beef palatability. According to McGeehin, Sheridan, and Butler (2002), rapid chilling in the lamb industry could eliminate the need for overnight chilling, allowing lambs to be shipped on the day of slaughter in order to combat the relatively short shelf life of fresh lamb.

In the study mentioned above conducted by Bowling et al. (1987), rapidly chilled sides produced beef that was more tender than conventionally chilled sides, and loin steaks from rapidly chilled sides were more juicy and desirable in overall palatability. Rapidly chilled sides experienced 0.9% less shrinkage during the first 24 h postmortem than conventionally chilled sides, and received higher marbling scores. These sides also had darker and softer lean as compared to conventional sides (Bowling et al., 1987).

Very fast chilling (VFC) has been defined as the achievement of a carcass temperature of –1°C within 5 h of postmortem chilling (Aalhus, Robertson, Dugan, & Best, 2002; Joseph, 1996). It has been proposed that toughening in lamb could be prevented by freezing in less than 4 h postmortem to cause surface hardening that restrains cold shortening (Joseph, 1996; Davey & Garnett, 1980). A study by Brown and James (1992) revealed that pork loin chops from ultra-rapidly chilled sides stored for 2 d were tougher than those from conventionally chilled sides. Joseph (1996) also identified the problem of variability in VFC meat because of storage temperature gradients produced in muscle during the VFC process. The practice of freezing lamb carcasses pre-rigor was researched by Watt and Herring (1974). These authors determined that pre-rigor freezing is known to produce toughening, and this type of rapid chilling can result in crust frozen carcasses that require extensive thawing time before fabrication can begin (Watt and Herring, 1974).

In the study by Watt and Herring (1974) on lamb carcasses, those that were chilled more rapidly had brighter lean and whiter fat, and experienced shrink savings that were maintained through shipping. This method of chilling resulted in a toughening of the *M. longissimus* up to 16%, and up to 7% in the *M. semimembranosus*, *M. semitendinosus,* and *M. biceps femoris*. Rapid chilling results in toughening in pork, but that can be improved using electrical stimulation (Gigiel et al., 1989). Chilling pork to internal temperatures of 20 to 25°C within 2 to 3 h postmortem can reduce pale, soft, and exudative characteristics, but muscle should not be chilled below 10°C in the first 5 h postmortem (Reagan & Honikel, 1985). Kerth et al. (2001) reported a reduced incidence of PSE meat from pigs carrying the Halothane gene when pork was cooled using accelerated chilling. Liquid nitrogen chilling was shown by Borchert and Briskey (1964) to prevent PSE conditions without affecting 24 h pH. Accelerated air chilling has been shown by some to improve water-holding capacity (WHC) in pork (Kerth et al., 2001; Taylor, 1971; Taylor & Dant, 1971), and to have no effect on WHC by others (Gigiel & James, 1984; Jones, Jeremiah, & Roberts, 1993). Bertram, Donstrup, Karlsson, Anderson, and Stodkilde-Jorgensen (2001) found increased WHC when using rapid tunnel chilling versus conventional chilling.

Sheridan (1990) reported that an ultra-rapid lamb chilling system was capable of producing tender loins after 7 d of storage by chilling at air temperatures of -20ºC for 3 to 5 h with an air speed of 1.5 m/s. This work led to the recommendation that lamb carcasses should not be chilled less than 12 to 14 h at 3 to 4ºC in order to prevent cold shortening (Sheridan, 1990).

Some studies paired the use of electrical stimulation with blast chilling in order to further prevent cold shortening, and therefore ensure more tenderness. In a study conducted by Aalhus, Janz, Tong, Jones, and Robertson (2001) that employed blast chilling and electrical stimulation in combination, shear force values in the *M. longissimus lumborum* (LL) of beef sides were decreased by 9.5% when compared to conventionally chilled sides. The proportion of extremely tough LL steaks also was reduced by 1.3%. The authors reported that blast chilling had no effect on marbling score, and blast chilled/electrically stimulated carcasses had brighter lean color when compared to conventionally chilled carcasses. Also, leaner carcasses experienced approximately 0.3% less cooler shrink when blast chilling was applied, and blast chilling was able to reduce the increased drip losses normally associated with electrical stimulation. As a result of these findings, Aalhus et al. (2001) was able to recommend to the industry the combination of blast chilling and electrical stimulation in order to reduce postmortem chilling times and shrink losses while continuing to produce meat quality.

Rapid chilling regimes produced significantly lower percentages of cooler shrink (Bowater, 1997; Aalhus et al., 2002) along with an increased perception of marbling, darker meat color, and increased drip loss in research conducted by Aalhus et al. (2002). Aalhus et al. (2002) found that for –20°C and –35°C blast chilling temperatures, shrink losses in beef decreased as time in the blast chill tunnel increased. After 7 h of –35°C blast chilling, cooler shrink was totally eliminated, and sides began to gain weight after 10 h of this treatment. Very fast chilling in this experiment was achieved in the *M. longissimus thoracis* after 7 and 10 h of –35°C blast chilling. The best combination they found was 10 h blast chilling with 6 d aging to produce low shear values and a high proportion of tender and probably tender steaks. Aalhus et al. (2002) identified the advantage of VFC as a reduction in the aging time necessary to achieve an acceptable product. Blast chilling of pork before the start of conventional chilling is reported to decrease shrinkage and give pork a darker and firmer lean (Jones et al., 1988).

Conclusions

Methods of chilling carcasses greatly impact the quality and palatability of meat with the results of these methods being very species-specific. In beef and lamb, employing chilling systems that minimize cold shortening should be a goal. Processes such as delayed chilling can successfully prevent or reduce the negative influence of cold shortening, however, food safety concerns and the need for maximum throughput in commercial facilities makes this system less applicable today. Commercial uses of electrical stimulation and alternative carcass suspension programs offer meat processors the opportunity to negate most or all of the effects of cold shortening when traditional chilling systems are used.

For pork, because of the role of elevated muscle temperature and low pH on the development of PSE, more rapid chilling systems have been employed to remove heat and slow the pH decline in an attempt to reduce the incidence of PSE. However, there still can be quality issues as a result of the differential between the extreme cold temperatures on the outside of the carcass and warm muscle temperatures in the deep regions of the carcass causing two-toned muscles (i.e., muscles that are darker in color externally and lighter in color internally).

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COLOR STABILITY OF TRAY-PACKAGED FRESH PORK SAUSAGE LINKS IN LIGHTED DISPLAY

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Background

Fresh pork sausage is prepared with fresh or frozen pork, seasonings, and not more than 3% water or ice that may be added to facilitate chopping or mixing. Additionally, the finished product may not contain more than 50% fat (CFR, 2003). Manufacturers generally stuff the sausage in flexible films for linking as 0.45 kg chubs or in natural, synthetic, or collagen casings as small links that are typically utilized as a breakfast item. Fresh pork sausage also may be prepared in pattie form. An important step in preparation of this type sausage is maintaining a low temperature to prevent fat smearing when the fresh mix is coarsely ground or chopped and then stuffed. Particle definition of the lean and formation of oxymyoglobin are necessary to provide proper appearance in the finished sausage since it is marketed as a raw, uncooked product. With good sanitation programs and proper handling, low microbial counts are usually found (Surkiewicz *et al*., 1972). Most manufacturers ship the product frozen and thawing is conducted just prior to retail display. Shelf life expectations in display are generally in the range of 14-21 days (Anon., 2004, Sebranek *et al*., 2004).

Objectives

The purpose of this study was to evaluate the retention of fresh lean color of tray-packaged pork sausage links during lighted display as based on panel ratings and to provide an estimate of the range in shelf life when placed in display.

Materials and methods

Six fresh pork sausage mixes were prepared in a regional processing plant operating under USDA inspection. The mixes were stuffed in collagen casings yielding links of approximately 1.8 cm diameter and 9.3 cm in length. Individual links were then conveyed through a CO_2 freezer tunnel and frozen before being tray packaged. Fourteen links were arranged in a 7 link (column) x 2 link (row) array on foam trays and the trays were over-wrapped with film of high oxygen transmission rate (>10,000 cc/m^2/24 hr at 1 atm, 0% RH, 23^{o}C). Trays of frozen links from each mix were express shipped overnight from the plant to our laboratory, removed from the shipping container which contained dry ice, and placed in an onsite freezer (-16.2^{o}C) for 6 days. Each mix served as an experimental replicate (n = 6).

Two randomly selected trays from each coded sausage mix were placed in the dark at 2±1^{o}C and allowed to thaw for approximately 22 hr. The thaw period was designated day 0. After thawing, the 12 trays were placed in a display cabinet at 2±1^{o}C under 1615 lux of lighting provided by Cool White fluorescent lights. This day was designated as day 1. At 12 hr intervals, the trays were rotated between the lighted display and dark storage for the next 21 days. The last day of display was day 22.

Color evaluation was conducted by 9 panelists familiar with fresh pork sausage and prior evaluations for fresh meat color. Each panel member had previously been screened for color vision using the Farnsworth-Munsell 100 Hue test consisting of arranging 4 sets each of 25 colors in the correct hue sequence. Normal color vision observers make few mistakes in the arrangement. Two preliminary color evaluation sessions were also conducted using fresh and discoloured sausage link packages to familiarize panelists with the color rating system and display equipment. From day 1 through 22 within a 3 hr period (8 a.m. – 11 a.m.), each panelist rated the sausage color on an 8-point scale (8 = very bright red lean; 5 = slightly red lean; 4 = slightly dark red lean; 1 = extremely dark brown/grey of lean) and also selected the “best” 3 samples and “worst” 3 samples among the 12 trays. Color evaluations continued for the 22 day period excluding weekend days. Each day the 12 trays were randomly arranged in the display and assigned a new 3-digit numerical code, selected at random, to prevent panel members from associating any code with a particular sausage mix.

Preliminary statistical analyses involved general linear model analysis of variance (ANOV) (SAS, 1996). After establishing that the color rating fit a quadratic response over time as compared to a linear response, data were analyzed by least squares (LS) ANOV according to a randomized block split plot in time design. Polynomial regressions of rate on day were fitted for each sausage mix and the linear and quadratic coefficients (β_1 and β_2, respectively) were compared using linear contrasts. The regression used was as follows: $Y = y + \beta_1 (x - 11.022) + \beta_2 (x - 11.02)^2$ where Y = predicted color rating, y = LS mean of the sample color rating, β_1 = linear coefficient for "rate of change with time", x = day of sample display (from 1-22), β_2 = quadratic coefficient for "rate of change in the 'rate of change with time'", and 11.022 = day constant for days 1-22. LS means for the color rating for all sausages over all dates were also compared using linear contrasts.

Results and discussion

Preliminary analysis of the data by ANOV showed a significant ($P<0.001$) difference among panelists, sausage mixes, and days of display. Duplicate samples of the sausage mixes was not ($P>0.05$) a factor in color ratings over the evaluation period. Time (days of display) was the largest contributor to the sum of squares in the analysis. A number of interactions were significant (mixes*duplicates, mixes*days, mixes*duplicates*days), all involving the sausage mixes. Nonsignificant interactions were duplicates*days and panelists*mixes*duplicates, the latter serving as the error term for each component in the interaction.

The polynomial regressions coefficients for each mix are given in TABLE 1 with linear contrasts comparisons for significant differences determined using least significant difference (LSD) at $P = 0.05$. There was a significant difference ($P<0.05$) between the LS mean for color rating (y) for sausage mix 5 (highest LS mean of 6.10) compared to sausage mix 1 (lowest LS mean of 5.30). These extreme LS mean comparisons encompass the 22 day display period. Neither mix 5 nor 1 had a color rating different from the other mixes. This confirms that in following a sausage mix formulation involving different pork meat selections combined for mixing, seasoning, grinding, stuffing, packaging, storing frozen for shipping, thawing and then displaying for a 22 day period, five-of-six different production runs made for this study were not different in color ratings as judged by the panel members. The rate of change with time (β_1) shows that sausage mix 5 had the lowest rate of color loss ($\beta_1 = -0.151$) whereas mixes 1 and 4 had the highest rates ($\beta_1 = -0.242$ and -2.58, respectively). Other mixes (TABLE 1) were generally intermediate in rate in which color ratings decreased. No practical significance can be attached to the β_2 coefficient.

Data on panel selections for "best" and "worst" samples among the 12 packages evaluated over the 22 day period (TABLE 2) are consistent with the other findings in that sausage mix 5 was selected more frequently in the "best" category (29.9% of responses) and less frequently in the "worst" category (9.4% of responses). Opposite findings are present for sausage mix 1 which had the lowest LS mean for color rating and one of the highest rates of color change.

The average daily color ratings over all panelists from evaluations of the duplicate packages of sausage mixes 5 and 1, as examples of the extremes for fresh color retention, were plotted against the day of evaluation (FIGURE 1). The plot was then visually extrapolated to the approximate maximum number of days each sausage mix had an average color rating of 5.0 or higher (5 = slightly red lean). This provides an estimate of the range in shelf life for this product. Sausage mix 5 maintained a rating of 5.0 or higher for 16-17 days whereas mix 1 maintained the rating for 12-13 days. Other mixes would have expected color retention for time periods between the findings for these two mixes. Most manufacturers expect a display life of 14 days with a few additional days in the consumer's home for fresh pork sausage prepared and held as given in this study.

Conclusions

Fresh pork sausage links from 5 of 6 different production runs did not differ in rate of color loss over a 22-day lighted display period. Redness retention of the lean particles as rated by a trained panel indicated a product color life of 12-13 days to 16-17 days.

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TABLE 1. Polynomial regression coefficients for panel color ratings of fresh pork sausage links displayed in light for 12 hr each day over a 22 day display period.

Sausage Mix	Color Rating (LS Mean)	Coefficients	
		B_1	B_2
1	5.30b	-0.242ab	-0.0090bc
2	5.72ab	-0.224bc	-0.0119ab
3	6.10a	-0.193d	-0.0147a
4	5.46ab	-0.258a	-0.0089bc
5	6.14a	-0.151e	-0.0114ab
6	5.43ab	-0.214cd	-0.0068c
(LSD at P = 0.05)	(0.70)	(0.024)	(0.0041)

Regression model: $Y = y + \beta_1 (x - 11.022) + \beta_2 (x - 11.02)^2$ where Y = predicted color rating, y = LS mean of the sample color rating, β_1 = linear coefficient for "rate of change with time", x = day of sample display (from 1-22), β_2 = quadratic coefficient for "rate of change in the 'rate of change with time'", and 11.022 = day constant for days 1-22.

a-e Column means and coefficients having a common letter are not different (P>0.05).

TABLE 2. Selection of the "best three" and "worst" three packages for fresh sausage color by panel members among 12 packages displayed in light for 12 hr each day over a 22 day display period.

Sausage Mix	"Best" Color Choices		"Worst" Color Choices	
	Responses	(Percent of Responses)	Responses	(Percent of Responses)
1	40	(9.9)	108	(26.7)
2	48	(11.8)	49	(12.1)
3	89	(21.9)	46	(11.3)
4	61	(15.1)	92	(22.7)
5	121	(29.9)	38	(9.4)
6	46	(11.4)	72	(17.8)
(Total)	405	(100)	405	(100)

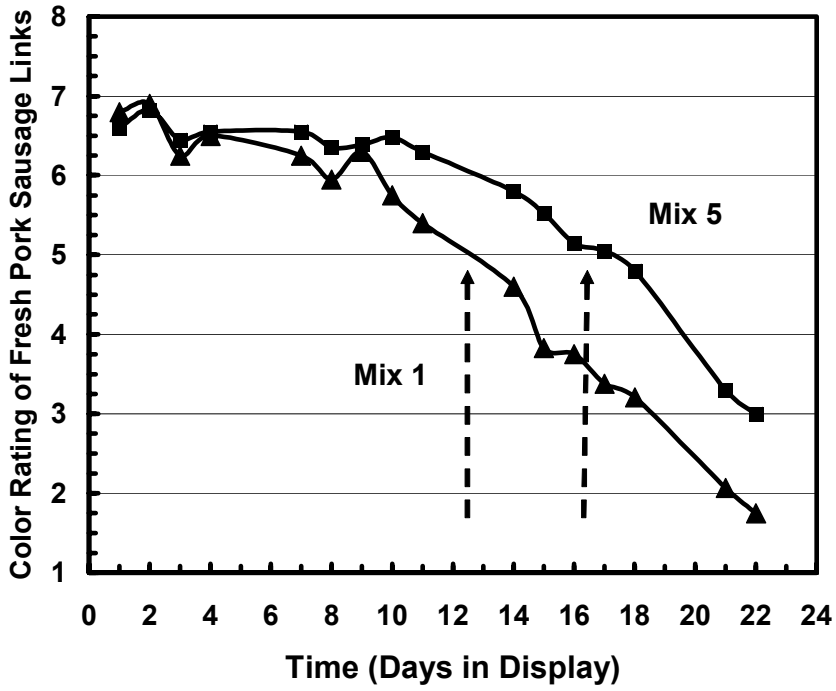

FIGURE 1. Panel color ratings of fresh pork sausage links from mixes with the highest (Mix 1) and lowest (Mix 5) rates of color change when displayed in light for 12 hr each day over a 22-day display period. The dotted arrow lines indicate the approximate days of shelf life for color retention based on panel ratings of 5.0 or higher.

BEEF CHARACTERISTICS OF THREE MUSCLES FROM COW AND YOUNG BULL

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Background

The social evolution has brought the consumer to prefer products of a high and uniform quality. This is also true for beef, whose characteristics are anyway conditioned by intrinsic and extrinsic factors which increase its variability. In the retail sale, beef is still a quite undifferentiated product and this does not agree with the needs of modern consumers. It is therefore of great importance to know in a more detailed manner the characteristics of meat in relation to the affecting factors so as to provide correct information to the consumer.

Objectives

To study beef characteristics in relation to the animal category and muscle type.

Materials and methods

A portion of *longissimus* (the lowest thoracic vertebrae), the *semitendinosus* and the *supraspinatus* were taken at commercial slaughterhouses from half-carcasses of cattle of various breeds and crosses (14 cows aged between 39 and 143 months and 10 young bulls between 12 and 19 months). The samples were analysed for chemical composition, water-holding capacity (drip losses during 48h; cooking losses in water bath until an internal temperature of 70°C was reached), Warner Bratzler shear force (maximum value in kg and work done in kg cm) on cylindrical cores 2.54 cm in diameter obtained from steaks used to determine cooking losses; measured by an Instron 1011 equipped with a Warner-Bratzler shear device.
The data were subjected to the variance analysis (ANOVA of SAS), considering as sources of variation the category, the muscle and their interaction.

Results and discussion

For each examined parameter, table 1 shows the R^2 value of the model and the factors which have a significant effect, as well as the least square means of the subgroups and of the groups.
Water content appears to be influenced by both the muscle type - higher in SS than in ST, which in turn contains more water than LD - and the category, the meat of cows being less humid than that of young bulls. Also in the experiment by Destefanis *et al*. (2003), carried out on Piemontese young bulls, LT contained less water than ST and SS. Also in Piemontese and Belgian Blue and White hypertrophied young bulls (Destefanis *et al*., 1996) the water content was the highest in SS (77.40%), followed by ST (75.96%) and by LT (75,27%). The present data confirm that the decreasing order for water content in beef is the following: SS, ST, LD. In the study of Gerhardy (1995), the meat of young bulls in average showed a higher humidity than that of cows, the difference being significant in ST, but not in LD.
Category does not influence the protein content, which appears very similar for all three muscles, in accordance with the results of the study by Fiems *et al*. (2003), in which the mean for cows (22.2%) was very close to that of young bulls (22.1%). On the other hand, muscle type influenced the protein content, the LD and ST being richer in proteins than SS (less than 20%). The low protein content in SS and the similar values for LD and ST are consistent with the data reported in the bibliographic survey on muscle type by Barge et al. (2001) and with the results of Barge *et al*. (1993) and Destefanis *et al*. (1996).
Unlike the previous parameters of composition, lipid content was influenced by both factors (P=0.0001), which also interacted each other. Both in cows and in young bulls, LD appeared to be the fattest and ST the leanest. In young bulls, however, LD contained almost twice as much lipids than ST, whereas in cows almost three times. In young bulls, Destefanis *et al*. (2003) reported that SS was the fattest of the three muscles and ST the leanest, while Barge *et al.* (1993) reported that ST was significantly different from SS, with LT in between. Both studies indicated ST as the muscle with the lowest lipid content. On the contrary, Destefanis *et al.* (1996) observed no significant difference between the muscles, the low fat content of hypertrophied young bulls being comprised between 0.39 in LD and 0.46 in SS, with ST in the middle.

For all three muscles and more clearly for LD, the lipid content was higher in cows than in young bulls (2.93 *vs.* 1.35% respectively). A higher fat content in cows than in young bulls (2.3 *vs.* 1.1%) has recently been reported by Fiems *et al*. (2003), whereas our data only partially agree with the results of Gerhardy (1995), which showed a difference between cows and young bulls significant for ST, but not for LD, indicating the relevance of the former muscle for meat analyses. In both muscles, cows contained less than twice as much fat as in young bulls. It should be noted that general mean of all our 72 meat samples is 2.1%, indicating that, even if belonging to commercial carcasses, beef is not as rich in fat as sometimes mass media tend to show. Berg *et al.* (1985) stated "The concept that meat is high in fat and cholesterol is widespread. The effect of this perception has created an atmosphere of negative attitudes which … continue to be reinforced by the usage of outdated data on the nutrient composition of meats".

As far as the haeminic iron is concerned, the adopted model accounts for 58% of the variance and its content is influenced both by category and the muscle type (P=0.0001). Regarding the latter factor, the decreasing order of iron content is: SS, LD, ST. Also Destefanis *et al.* (2003) found SS richer in iron than LT and ST. Concerning the category, cow meat appears remarkably richer, the iron content being one and a half times greater than in young bulls. This is true for all three muscles, but it is particularly relevant in SS. The observation on the difference between the categories seems related to the fact that the quantity of pigment also depend on the age of the animals. In Renerre (1982) the increase in iron with age varied considerably according to the muscle examined and, in the opinion of the Author, these differences were due to the different metabolic type of the muscle fibres.

When studying the haeminic iron content in two muscles of cows and young bulls, Dumont and Bousset (1990) indicated average value of 20.05 μg/g for *longissimus thoracis* and 20.50 for *pectoralis ascendens* of cows and 11.8 and 13.5 for the same muscles in young bulls. In particular, the values of LT ranged between 9.20 μg/g and 15.9 for young bulls and between 15.80 and 26.10 in cows. We observed a similar slight overlapping of categories in SS, ranging from 9 μg/g to 16 in young bulls and from 14 to 28 in cows.

Drip losses - The factors significantly influenced this parameter, for which the general mean indicated a loss of 2% in 48 hours. The losses appeared to be greater for young bulls in all three muscles and especially in ST. Similar results were found by other Authors. In Barnier (1995), drip losses of muscular portion were greater in Friesian young bulls than in cows of the same breed, both in *longissimus* and in *semimembranosus*. Also Fiems *et al.* (2003) found drip losses inferior in cows that in young bulls.

As far as the muscle type is concerned, ST showed greater losses than the other two muscles. The results perfectly agree with other experiments. In Honikel and Potthast (1991) the muscle significantly affected the percentage of drip loss: the highest was in ST, the lowest in SS, in between lied LD. In Destefanis *et al.* (1994) drip losses were greater in ST than in LT, which in turn were greater than in SS. Hypertrophied young bulls showed a significant interaction between muscle and breed (Destefanis *et al.*, 1996). Anyway, drip losses tended to be greater in ST (4.44%) compared to LT (2.64%) and SS (2.51%).

Cooking losses - This is the parameter for which the highest R^2 was obtained, indicating that the adopted model accounts for over two thirds of variability. The considered factors of the model influenced and interacted in a significant way. Also for this parameter, cows showed a lower loss than young bulls, particularly in SS. In ST cows loose less fluid, but the difference was not significant. In LD the situation tends to be reverted, with slightly higher values for cows, even if not statistically different. Gerhardy (1995) found not significant differences between six cattle categories for cooking losses at 75°C both for LD and for ST. However, the data indicated a tendency to slightly greater losses in young bulls than in cows (33.41 *vs.* 32.67% in LD); on the contrary, statistically significant differences between categories appeared for cooking losses at 55°C only in LD. Also in the study of Fiems *et al*. (2003) young bulls showed statistically higher cooking losses than cows (25.1 *vs.* 23.6%).

As for muscles, the order for losses in both categories was ST, SS, LD. In young bulls, however, ST and SS were very close and did not differ much from each other. Similar results were obtained by Destefanis *et al*. (1996), with LT showing significantly smaller losses than SS and ST (similar to each other).

WB max - The category had a great influence (P=0.0001), showing average values of 15.72 kg in cows *vs.* 10.66 in young bulls. The muscle did not affect the parameter nor did it interact with the other factor. It should be noted that the values of the muscles are near within the category: from 10.22 in LD to 11.28 kg in SS for young bulls, and from 14.83 in SS to 17.13 kg in ST for cows. On the contrary, a lower shear force for LT was found by Destefanis *et al*. (2003). If we consider that the three muscles of the present study belong to cuts of different economic value, due to the assumed tenderness of the product, the proximity of the values of these muscles is quite remarkable.

WBw - The two factors had an influence on this parameter and interacted. The model accounted for a great part of the variability (54%). The meat of cows required a greater work than that of young bulls for all muscles, but the difference varied from 27% in SS to 40% in LD, up to 70% in ST. Gerhardy (1995) had reported significant differences between the categories of cattle for maximum shear force and for shear force done in LD only for the cooking at 55°C (mean values higher in cows than in young bulls and in heifers), whereas significant differences were found in ST (higher value in cows *vs.* young bulls for two out of three cooking methods). As for the muscle type, in young bulls the LSM ranged from 20.82 kg cm in LD to 22.60 in SS, values statistically not different. In cows, instead, the work necessary to shear ST (36.66) was higher than that for the other two muscles (about 29.00 kg cm). In each of the three muscles, WB max and WBw were higher in cows than in young bulls, with a slight difference in SS (+31 and +27%), intermediate in LD (+31 and +40%), greater in ST (+64 and +67%).
Gerhardy (1995) found significant differences between LD and ST for max shear force, for extension and for shear work done, being the values always higher in ST.

Conclusions

The fact that cows had a protein content similar to that of young bulls in all the three muscles examined, indicates that also the meat of cows is a good source of proteins. However, in comparison with young bulls, meat from cows was fatter (about twice and even more in LD) and showed a shear force about 1.5 times higher. In return, it contained one a half times as much iron. Young bulls showed a water-holding capacity less good than cows, loosing more drip and more fluid during cooking (significant difference in SS).
The muscle type significantly influenced many parameters. Among the three muscles used, *longissimus* is the poorest in water but the richest in lipids; it is the muscle with the lowest cooking losses, but most of all tends to low shear force. The *semitendinosus* is the leanest (mean <1.7% even in cows), but contains less iron, shows a poor water-holding capacity and, at least in cows, the highest WB max. The *supraspinatus* contains more water and less protein than the other two muscles, but is richer in iron and tends to loose less drip.
As well as acting on many parameters, at times the muscle interacted with the category, confirming the need to work also on muscles other than *longissimus*, every time that the effect of some factor or of a new technological treatment is being studied. Apart of the interest at research level, a punctual knowledge of the characteristics of beef would allow the consumer to choose the category and the muscle which better suit him, considering that the most valuable products are not necessarily the best with respect to chemical composition or water-holding capacity or shear force.
Instead of pursuing the probably utopian idea of the uniform quality, while keeping on selling beef as an undifferentiated product, perhaps it would be better to study methodically the different characteristics of the muscles, of the categories, etc. and inform the consumer, enabling him to choose according to his objectives.

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Table 1 – R^2 values, significant effects (P<0,005) and least square means.

parameter	R^2; sign. effects	muscles	Cows	Young bulls	Cows + y.b.
Moisture (%)	0.49 category muscle	LD	74.066	75.583	74.824^{c}
		ST	76.097	76.529	76.313^{b}
		SS	76.979	77.432	77.205^{a}
		LD+ST+SS	75.714^{w}	76.515^{z}	**76.114**
Protein (%)	0.32 muscle	LD	20.922	21.203	21.063^{a}
		ST	21.125	21.088	21.107^{a}
		SS	19.810	19.960	19.885^{b}
		LD+ST+SS	20.619	20.750	**20.685**
Lipids (%)	0.45 category muscle interaction	LD	4.827^{a}	1.924^{bc}	3.376
		ST	1.679^{bd}	0.968^{cd}	1.323
		SS	2.284^{b}	1.148^{bc}	1.716
		LD+ST+SS	2.930	1.347	**2.138**
Haeminic iron (µg/g)	0.58 category muscle	LD	17.800	12.466	15.133^{b}
		ST	14.089	9.282	11.685^{c}
		SS	21.584	13.941	17.762^{a}
		LD+ST+SS	17.824^{w}	11.896^{z}	**14.860**
Drip losses (%) (DL)	0.44 category muscle	LD	1.730	2.321	2.026^{b}
		ST	1.806	2.937	2.371^{a}
		SS	1.379	2.121	1.750^{b}
		LD+ST+SS	1.638^{w}	2.460^{z}	**2.049**
Cooking losses (%) (CL)	0.69 category muscle interaction	LD	17.764^{c}	17.321^{c}	17.543
		ST	24.996^{a}	26.947^{a}	25.971
		SS	21.317^{b}	25.855^{a}	23.586
		LD+ST+SS	21.359	23.374	**22.367**
WB max (kg)	0.42 category	LD	15.183	10.223	12.703
		ST	17.134	10.463	13.799
		SS	14.831	11.280	13.056
		LD+ST+SS	15.716^{w}	10.655^{z}	**13.186**
WBw (kg cm)	0.54 category muscle interaction	LD	29.14^{b}	20.82^{c}	25.67
		ST	36.66^{a}	21.96^{c}	30.54
		SS	28.76^{b}	22.60^{c}	26.20
		LD+ST+SS	31.52	21.79	**26.66**

Within a beef parameter, significantly different values (P<0.05), are given with different superscripts (w, z for the category; a, b… for the muscle and for muscle x category).

LIPID OXIDATION AND 2-ALKYLCYCLOBUTANONESIN IRRADIATED TRADITIONAL PORK PRODUCTS

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Background

Irradiation is the treatment of food involving their exposure, either packaged or in bulk, to carefully controlled amount of ionizing radiation. Irradiation is considered a highly effective processing technology to achieve many technical objectives. Irradiation can kill harmful bacteria and other organisms in meat, poultry and seafood, disinfest spices, extend shelf-life of fresh fruits and vegetables killing insect eggs and larvae and control sprouting of tubers and bulbs such as potatoes and onions.
Similar to other food processes, irradiation has also technical and economic limitations that prevent its use on all foods under all circumstances. Low-dose irradiation treatments do not cause noticeable decreases in the nutritional quality of food, but the change in nutritional values depends on a number of factors: radiation dose, type of food, packaging and processing conditions such as temperature and oxygen exposure during irradiation and storage time (Crawford and Ruff, 1996). Irradiation causes certain chemical changes in food. Irradiation increased lipid oxidation in aerobically packaged meat and developed off-flavours (Ahn, *et al*., 2001b; Patterson and Stevenson, 1995).
Irradiation of fat-containing food generates also a family of molecules, namely 2-alkylcyclobutanones (2-ACB), that results from the radiation induced breakage of triglycerides. They are the only chemical compounds whose formation is thought to be specific for irradiation treatment of foodstuffs (Crone *et al*., 1992, 1993) and, for this reason they are useful for investigation of irradiated lipid-containing foods. These compounds contain the same number of carbon atoms as their precursor fatty acid and an alkyl chain of carbon atoms is attached to ring position 2. Due to the potential effects on human health the more investigated cyclobutanones are 2-dodecylcyclobutanone (2-DCB), 2-tetradecylcyclobutanone (2-TDCB) and 2-(5'-tetradecenyl) cyclobutanone (2-TdecCB). Their precursor fatty acids are listed in Table 1.
The Joint Expert Committee on the Wholesomeness of Irradiated Food of F.A.O., I.A.E.A. and W.H.O. concluded that irradiation of food up to an overall average dose of 10 kGy results in no toxicological hazard to humans. These organizations stated that irradiated foods are safe and nutritious (Joint F.A.O/W.H.O./I.A.E.A Expert Committee, 1981). Although irradiated foods have many known beneficial properties, the effect of long-term consumption of irradiated foods remains unknown. Lack of critical studies addressing the impact of long-term exposure to irradiated foods on human chronic diseases, such as cardiovascular disease, arthritis or various cancers, is considered a problem (Rao, 2003). Considering that some researchers underlined the problem about a potential toxicity of 2-ACB it is very important meanwhile to collect data concerning a wide variety of foods, wishing more investigations of this aspect.

Objectives

The aim of this investigation was to evaluate chemical effects of irradiation used as decontamination treatment on some typical Italian pork products. The research was focused in particular on the collection of preliminary data on the lipid oxidation and the formation of 2-ACB.

Materials and methods

The traditional Italian processed pork products investigated were: salame Milano, coppa and pancetta. Salame Milano is a typical minced, dry cured and fermented pork product; coppa is made up by the entire neck muscle, deboned, cured and afterwards matured in natural casing; pancetta is made up by adipose tissue of the ventral region of the pork and it looks layered alternatively fat and lean.
The products at the end of maturation were portioned and packed under vacuum in transparent plastic bags. For each kind of product the following groups were created: the control group (*Control*, n=5), the group irradiated at 2 kGy (*2kGy*, n=5) and the group irradiated at 5 kGy (*5kGy*, n=5). The treatment was performed using electron beam on refrigerated samples placed on cardboard boxes.

Proximate composition (AOAC, 1990) and fatty acid composition of total lipids (Zanardi et al., 2000) were determined on samples of *Control* groups. The samples belonging to *Control*, *2kGy* and *5kGy* groups were submitted to the following determinations:
- 2-thiobarbituric acid reactive substances test (TBARS test) as described by Tarladgis and Watts (1960), modified by Novelli et al. (1998). The procedure was based on the distillation of malondialdehyde and spectrophotometric determination at 534nm of the product of reaction between the distillate and 2-thiobarbituric acid;
- alkylcyclobutanones determination according the European Standard EN 1785: "Detection of irradiated food containing fat–Gas-chromatographic/mass spectrometric analysis of 2-alkylcyclobutanones".
Among the cyclobutanones, the 2-DCB, the 2-TDCB and the 2-TdecCB were considered in this investigation. The procedure involved a first step of lipid extraction, a second step of purification and concentration of alkylcyclobutanones and their analysis by gas chromatography-mass spectrometry (GC-MS). 12 g of well homogenized sample were mixed with 12 g of anhydrous sodium sulphate in a thimble placed in a Soxhlet extractor and the lipid was extracted by refluxing 160 ml of petroleum ether for a minimum of 6 hours. An aliquot of 200 mg of fat was then dissolved by 1 ml of internal standard (0.5 µg/ml of 2-cyclohexylcyclohexanone in n-heptane). It was applied to the column of 30 g of inactivated Florisil (PR 60/100 mesh, Supelco) and eluted with 150 ml of hexane followed by 150 ml of 1% diethylether in n-hexane. This latter fraction was collected separately, concentrated using a rotary vacuum evaporator and transferred in a test tube. The sample was further concentrated to dryness under a stream of nitrogen and resuspended immediately in 500 µl of n-heptane. GC-MS analysis was carried out on a gas chromatograph 6890N (Agilent Technologies) coupled with a Mass Selective Detector 5973 Network (Agilent Technologies). The column was a DB-5 (30mx0.25 i.d., 0.25 µm film thickness, Supelco). Mass spectrometric analysis was performed by electron impact mode and positive ions. Selected ion monitoring (SIM) was set for 2-DCB and 2-TDCB for ions m/z 98 and 112 and for 2-TdecCB for ions m/z 67, 81, 98 and 109. The retention time and ion ratio of the signals were confirmed to correspond to those of standards (Fluka and Alfa Aesar for 2-cyclohexylcyclohexanone). Quantitative determination of 2-ACB was performed by using the peak areas of ion m/z 98 because common to all compounds. Their concentration was reported as µg/g of sample. The limit of quantification (LOQ) was 0.01 µg/ml for 2-DCB and for 2-TDCB and 0.2 µg/ml for 2-TdecCB.
Analysis of variance (ANOVA) was carried out on the data and the Scheffé's test ($p=0.05$) was used for the evaluation of significant differences (SPSS 11.5 for Windows). Not detected values of 2-ACB, because under the LOQ, were considered as 0.

Results and discussion

General chemical parameters fall in a normal range for products of such a type (data not shown). Data on fatty acid composition of total fat are not reported because not complete.
The data of TBARS values are shown in Table 2. The lowest mean values were found in coppa and they ranged between 0.083 and 0.088 mgMDA/kg. Not significant differences were found between *Control* and treated groups. The highest concentration of TBARS has been found in pancetta. The samples irradiated at 2 kGy reached, indeed, average value of 0.311 mgMDA/kg whereas *Control* and *5kGy* groups scored 0.187 and 0.130 mgMDA/kg respectively. Because of the relatively high standard deviation, however, no significant effect can be attributed to the irradiation treatment. In Salame Milano the samples of *Control* group showed higher values (0.230 mgMDA/kg) compared to *2kGy* (0.135 mgMDA/kg) and *5kGy* (0.124 mgMDA/kg) groups. Despite the significance resulting by statistics analysis, the TBARS values can be considered similar also in the Salame Milano. The high variability of TBARS values among meat products, indeed, has been widely recorded in previous studies and seems to be linked to an intrinsic variability of raw meat. All the results, however, were lower than the suggested threshold for the appearance of rancidity off-flavours in fresh pork (0.5 mgMDA/kg) (Lanari *et al*., 1995). TBARS values were in line with the results of previous investigations (Novelli et al., 1998; Zanardi et al., 2000). The latter mentioned study reports, for example, TBARS values of Salame Milano and coppa around 0.280 and 0,110 mgMDA/kg, respectively, confirming the lower values in coppa. These values proved that, in this case, the irradiation does not accelerate lipid oxidation. This result could be explained by the vacuum packaging adopted in this research. Oxygen has a catalytic effect on irradiation-induced lipid oxidation (Lambert *et al.*, 1992). As has been suggested by some authors, the exclusion of oxygen should help reduce the extent of oxidation of fatty acid in vacuum packed cooked pork sausages compared to the same products packed in aerobic packaging

irradiated at 5kGy (Jo et al., 2003). The same effect of packaging has been observed on cholesterol oxidation in cooked pork (Ahn et al., 2001a). Moreover, the irradiation treatment was carried out on refrigerated pork products. Nawar and Balboni (1970) reported that the amounts of radiolytic products formed on irradiation are sensitive to the temperature at which the irradiation take place.
The data of 2-ACB content in salame Milano, pancetta and coppa are partially showed in Table 3. The three 2-ACB considered in this investigation were under the limit of detection (LOD) in all the pork products of *Control* groups. The irradiation dose 2kGy induced the formation of 2-ACB in all the three types of products. In particular the 2-DCB was observed in a range of values between 0.019 and 0.054 μg/g and the 2-TDCB between 0.029 and 0.039 μg/g in the samples of *2kGy* groups. The irradiation dose 5kGy did not further increase the content of both 2-ACB in salame Milano and coppa whereas induced a significant increase ($p=0.05$) of both 2-DCB and 2-TDCB in pancetta in which 2-DCB reached 0.123 μg/g and 2-TDCB 0.102 μg/g. When these concentrations of 2-ACB were plotted against irradiation doses, a linear response was observed both for 2-DCB and for 2-TDCB (Figure 1). These concentrations resulted also statistically higher ($p=0.05$) than those of 2-DCB and 2-TDCB in salame Milano and in coppa. Probably the difference among pancetta, salame Milano and coppa was due to the different fat content. The average percentage of fat in pancetta was, indeed, about 40%, whereas in salame Milano and in coppa was about 30%.
In Table 3, the values of 2-TdecCB were not included, because it was found occasionally. In all the samples of *Control* groups, in these ones irradiated at 2 kGy and in samples of coppa irradiated at 5 kGy the 2-TdecCB was not detected. These results could be explained by the high limit of quantification (0.2 μg/ml) due probably to the much greater fragmentation of 2-TdecCB in the ion source of the MS. 2-TdecCB was found only in salame Milano and in pancetta irradiated at 5 kGy. The average values were 0.355±0.224 and 0.969±0.422 μg/g respectively. These latter values were higher than the concentrations of both 2-DCB and 2-TDCB at the same dose of irradiation. This result was in line with Park *et al.* (2001) who detected the same 2-ACB in irradiated pork: 2-TdecCB showed the highest content for all irradiation treatments in the range of doses between 0.5 and 10 kGy. Compared to the data reported by the same author, the average content of 2-ACB on fat basis of the present study was lower. Due the absence of data on the use of irradiation of traditional pork products, the explanation of the results of the present investigation could be attributed also for 2-ACB to the packaging conditions. The absence of oxygen could reduce the induced radical species in the matrix.

Conclusions

The present study is a collection of preliminary data on the lipid oxidation and formation of 2-alkylcyclobutanones in some traditional pork products treated by ionising radiation as a decontamination process. The extent of lipid oxidation was not significantly influenced by ionising radiation at 2 and 5 kGy irradiation doses. 2-alkylcyclobutanones have been confirmed to be useful markers of identification of irradiated fat-containing food. 2-DCB and 2-TDCB can provide useful information to distinguish between treated and not irradiated food. 2-TdecCB could be used as an additional marker. Its high limit of quantification can not guarantee the distinction between treated and not irradiated food.
The conditions used during the treatment (vacuum packaging and refrigeration) seem influence both lipid oxidation and formation of 2-alkylcyclobutanones. The packaging can be considered a critical factor affecting the chemical quality of irradiated pork products.

Acknowledgments

The authors gratefully acknowledge the cooperation and the assistance of Dr. Alberto Butturini and Dr. Rita Tagliazucchi of Villani S.p.A. (Castelnuovo Rangone, Modena, Italy)

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Table 1: 2-ACB considered in this investigation and their precursor fatty acid.

Precursor fatty acid	2-ACB
Palmitic acid ($C_{16:0}$)	2-dodecylcyclobutanone
Oleic acid ($C_{18:1}$)	2-(5'-tetradecenyl)cyclobutanone
Stearic acid ($C_{18:0}$)	2-tetradecylcyclobutanone

Table 2: TBARS (mg malondialdehyde/kg) values (mean ± standard deviation) of salame Milano, pancetta and coppa.

Pork product	Control	2 kGy	5 kGy
Salame Milano	0.230 ± 0.069^{a}	0.135 ± 0.021^{b}	$0.124 \pm 0{,}007^{b}$
Pancetta	0.187 ± 0.080^{a}	0.311 ± 0.191^{a}	0.130 ± 0.027^{a}
Coppa	0.085 ± 0.009^{a}	0.088 ± 0.014^{a}	0.083 ± 0.020^{a}

[a,b]: different letters on the same raw stand for significant differences, p=0.05, Scheffé's test

Table 3: 2-dodecylcyclobutanone (2-DCB) and 2-tetradecylcyclobutanone (2-TDCB) content (µg 2-ACB/g) (mean ± standard deviation) of salame Milano, pancetta and coppa

Product	2-ACB	Control	2 kGy	5 kGy
Salame Milano	2-DCB	N.D.[a]	0.019 ± 0.008^{b}	$0.030 \pm 0.005^{b,e}$
	2-TDCB	N.D.[a]	0.029 ± 0.030^{ab}	$0.046 \pm 0.015^{b,e}$
Pancetta	2-DCB	N.D.[a]	0.054 ± 0.006^{b}	$0.123 \pm 0.029^{c,d}$
	2-TDCB	N.D.[a]	0.037 ± 0.005^{b}	$0.102 \pm 0.018^{c,d}$
Coppa	2-DCB	N.D.[a]	0.042 ± 0.011^{b}	$0.048 \pm 0.019^{b,e}$
	2-TDCB	N.D.[a]	0.039 ± 0.012^{b}	$0.061 \pm 0.027^{b,e}$

[a,b,c,]: different letters on the same raw stand for significant differences, p=0.05P<=0.05, Scheffé's test
[d,e]: different letters on the same column stand for significant differences, p=0.05, Scheffé's test
N.D.=not detected

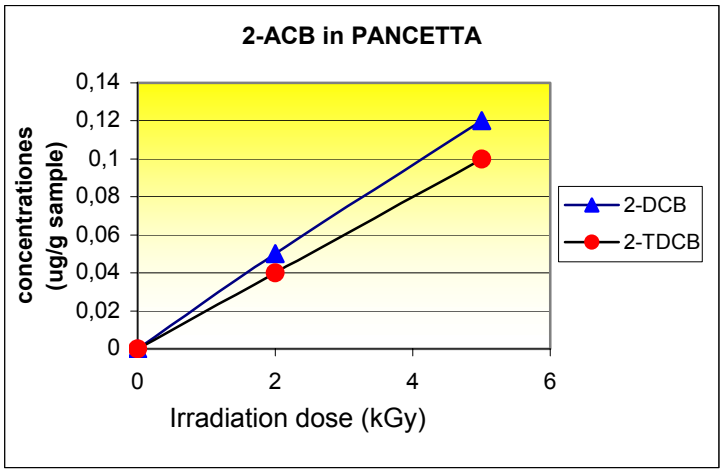

Figure 1: Effect of irradiation dose on 2-DCB and 2-TDCB produced in irradiated pancetta.

FAT CONTENT REDUCTION IN PIG-SKIN SHEETS BY EXTRACTION WITH SUPERCRITICAL CARBON DIOXIDE

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Background

Collagen is the most abundant protein in animals. This fibrous protein is widely used in the Food Industry for various applications. Most often, it is obtained from cattle skin. However, it is always advisable to count on some alternative sources to eliminate strong dependences on one of them. Pig skin could be such an alternative; however, it has a high fat content that needs to be reduced to meet the specifications required by some of the applications of the food industry. Although there exist some methods to eliminate or reduce the fat content of animal skin, we propose in this work a mild method that can achieve very high reductions or even total elimination of the fat contained in pig skin to achieve a protein concentrate that can be used for most industry processes that need these type of proteins as raw material. This method consist in using supercritical carbon dioxide as a solvent that can selectively extract the fat without affecting the protein.
Supercritical fluid extraction (SFE) is a clean technique that can use low temperatures. Fat and oil extraction with supercritical fluids is widely reported in the literature from a large variety of natural sources such as different types of seeds, spices, meat or marine products (King et al., 1989). The most commonly used supercritical fluid is carbon dioxide due to its mild supercritical temperature and pressure and because it is a gas under ambient conditions what allows an easy separation of the processed products. It is also cheap and achievable with high purity; it is non toxic, non corrosive, non inflammable etc.CO_2 presents a large number of nice features to be used as a solvent for extraction processes. Its main inconvenient is its lack of polarity that makes it a poor solvent of polar solutes. However, it is a good solvent of fat (Bamberger et al., 1988), which is the solute whose removal from pork skin is to be studied in this work.

Objectives

The objective of the work was the search of the process conditions that allowed the highest yield for reducing the fat content of pig skin by using supercritical carbon dioxide. The skin was defatted as part of the treatment for its conditioning to be used as raw material to obtain the collagen paste that is used as casing of Frankfurt-type sausages. Different experiments were carried out to identify the resistance controlling the extraction process and to search for the process conditions that overcame such resistance and gave a high extraction yield.

Materials and methods

The experimental study was made in a semi-pilot SFE-plant whose flowsheet is presented in Figure 1. Pig-skin sheets, around 5 mm thick, were placed in the extractor. The skin could not be ground since, by doing so, the later treatment necessary to obtain the desired collagen paste could not be carried out. The solvent, CO_2, was pressurized up to the extraction pressure and then, it was circulated with a certain flow (F) through the skin trying to achieve a good contact skin-solvent for the extraction to be faster. The solvent was recycled after removing the solute in a separator where the solvent power of CO_2 was reduced by reducing pressure and increasing temperature.
Two types of experiments were carried out. In a first set of experiments, the extraction was carried out continuously during a certain extraction time in order to evaluate the influence of extraction pressure, temperature and time, and initial water content on the extraction yield. In a second set of experiments the extractor was depressurized several times during the extraction experiment in order to take a sample of the raffinate whose analysis allowed to follow the course of the extraction.

Results and discussion

The first set of experiments were designed to study the influence of the extraction pressure (p), temperature (T) and time (t) on the extraction yield as well as the influence of the initial water content. The influence of

pressure and temperature on fat extraction is showed in Figure 2. It can be observed that fat extraction increases slightly with temperature and pressure, but a maximum yield of around 40% was obtained. The maximum temperature assayed was 40 ºC since collagens experience denaturational transitions at temperatures above 40ºC (Privalov et al., 1979). The large error bars in the data are a consequence of the heterogeneity of the fat content of the skin. The results of some additional experiments carried out to study the influence of the extraction time are presented in Figure 3, where it can be observed that even fairly large extraction times did not allow a limit of 60% yield to be overcame.

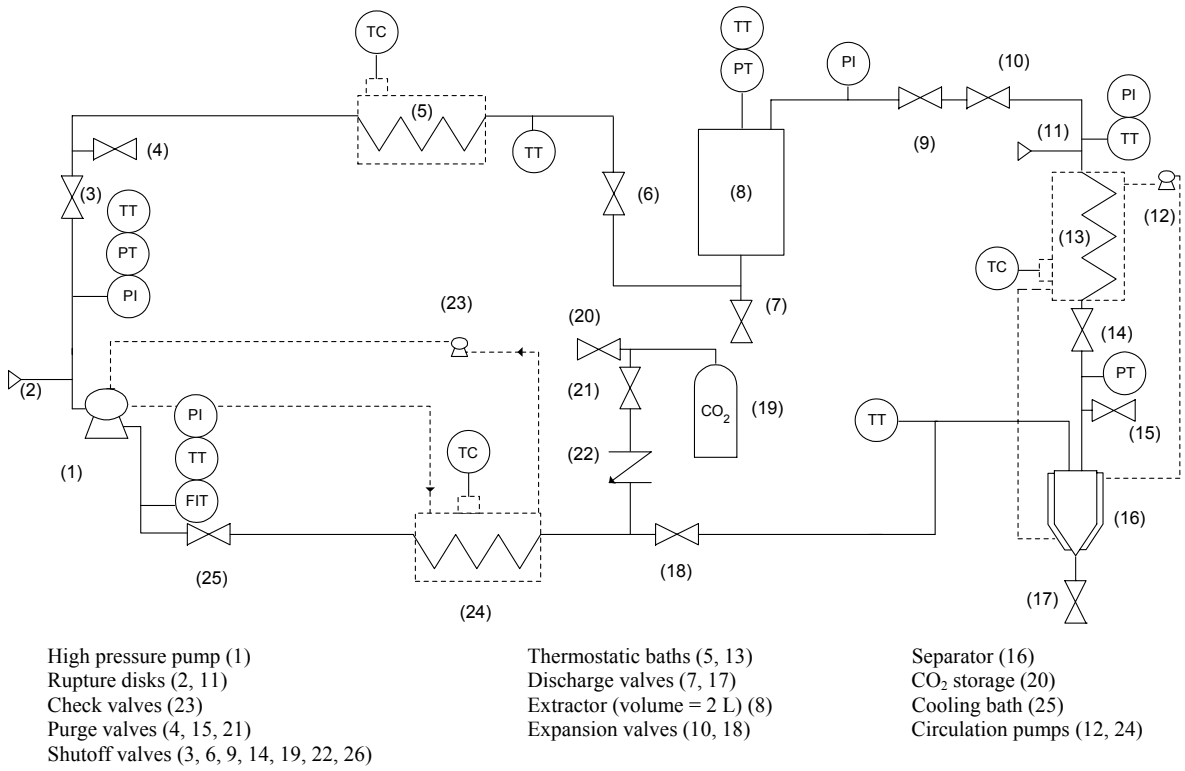

Figure 1. Flowsheet of the SFE semi-pilot plant of the Chemical Engineering Area of the University of Burgos. Maximum specifications: T= 200 ºC, p = 65 MPa and solvent flow, F = 20 kg/h.

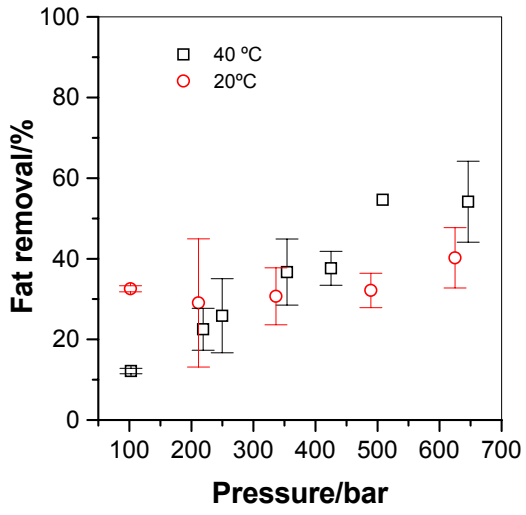

Figure 2. Fat extracted (%) versus pressure. F = 10 kg CO_2/h, skin sheets size = 2 cm × 14 cm, t = 60 min.

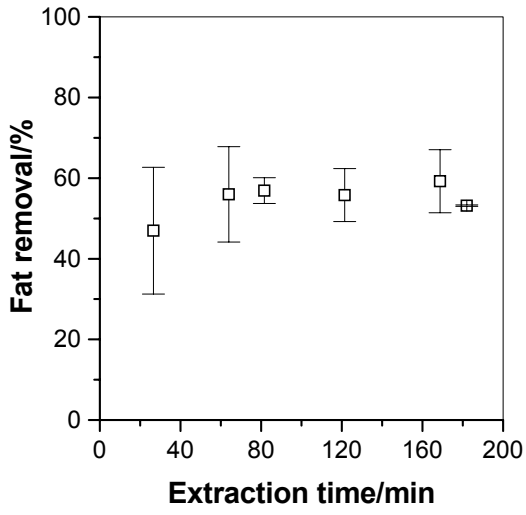

Figure 3. Fat extracted (%) versus extraction time. T = 40 ºC, p = 65 MPa, F = 10 kg CO_2/h, skin sheets size = 2 cm × 14 cm.

The influence of the skin water content on the fat extraction yield was also evaluated. The water content of the pig skin was reduced to different levels in a drier unit. The fat extraction yield obtained when using dried skin was also limited to an approximate value of 60%. Therefore, the study so far, proved that supercritical CO_2 removed the fat from pig skin, but the final concentration of fat was too high for this skin to be used to obtain the collagen-paste of interest. As an extraction temperature increase was not advisable and an extraction pressure or extraction time increase did not produce the required efficiency, a different extraction procedure needed to be assayed.

The fat is located all along the pig skin from the epidermis to the dermis and hypodermis (Compte, 1996). Therefore, it is reasonable to think that, when the skin is not ground, the external fat must be easily removed by the solvent but it must be much more difficult to extract the internal fat. In order to confirm or reject this assumption, some experiments were carried out to follow the course of the extraction process by determining the remaining amount of fat in the pig skin at different extraction times. In this experiments, the extractor was depressurized after some extraction time (called "cycle time") in order to take the skin sample necessary to determine its fat content.

The first observation made when analyzing the results obtained with this new experimental procedure was that the extraction yield of 60 %, that was the maximum obtained when working without depressurizing the extractor, was overcame and even total elimination of fat was achieved in some cases. That is, the successive and rapid depressurizations of the extractor seemed to be "exploding" the fat cell membranes and making the fat accessible to the solvent that extracted the fat much better. Therefore, two new variables that are going to influence the yield of the process have to be taken into account, i.e.: the number of cycles and the cycle time. Besides these variables, the influence of the initial fat content of the pig skin and of the solvent flow on fat removal was evaluated

Figure 4 shows the fat removal as a function of the number of cycles (a) and as a function of the total extraction time (b) for different cycle times. The experiments for cycle times of 10 minutes and 60 minutes were repeated to check for reproducibility of the tendencies. In the first 10 min cycle time experiment, sampling was made after every depressurization of the extractor and in the second experiment, after every three depressurizations. The two experiments carried out at a cycle time of 60 min differed on the initial amount of fat that is expressed in Fig, 4 as grams of fat per grams of protein. Figure 4a shows that fat removal increases when increasing the cycle time up to a cycle time of 60 minutes but larger cycle times do not improve fat removal. Therefore, a cycle time of 60 minutes was considered as the ideal time for the solvent to diffuse into the fat cells, so they explode with depressurization, and for extracting most of the fat accessible to the solvent after the previous rapid depressurizations performed in the extractor. A shorter cycle time required a larger number of depressurizations leading to a substantial loss of on-stream time, and a larger cycle time did not increase the extraction efficiency. Figure 4b shows that the tendencies were much more similar in all cases when the total extraction time was considered. Yields between 90 % and 100% were obtained in all cases after 4 hours of extraction.

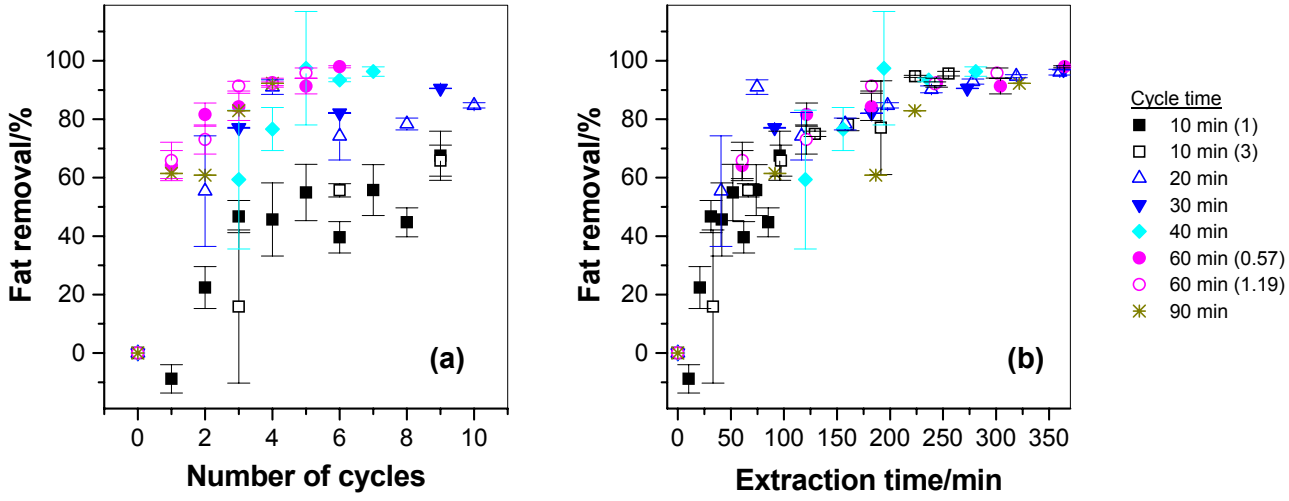

Figure 4. Percentage of fat removed against number of cycles for different cycle times (a) and as a function of total extraction time (b). T = 40 ºC, p = 35 MPa, F = 10 kg CO_2/h. skin sheet size = 15 cm × 14 cm.

Figure 5 presents the amount of fat in the skin, in a protein bases, as a function of the extraction time for different skin sheets with different initial fat contents. If fat removal was considered, there was no distinction between the two experiments indicating that the percentage of fat removed in a certain time is similar in both cases; however, Figure 5 shows that the fat content in the skin only reaches similar values for the two experiments after three hours of extraction. These experiments lead to the conclusion that the initial fat content in the skin is a variable to take into account. Therefore, the better the mechanical fat removal was performed, the lower the extraction time was for a given final fat content.

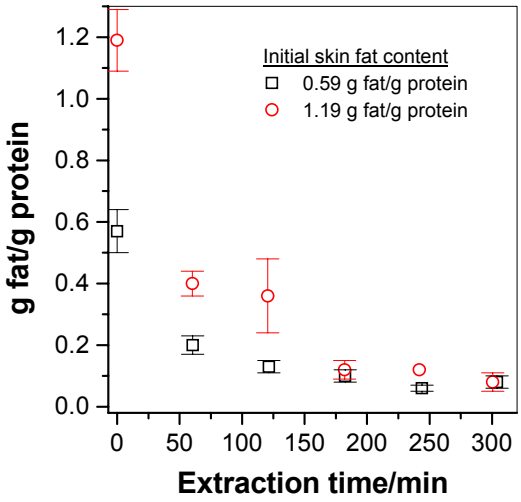

Figure 5. Fat content as a function of extraction time for different initial skin fat contents. T = 40 ºC, p = 35 MPa, F = 10 kg CO_2/h, skin sheet size = 15 cm × 14 cm, cycle time = 60 min

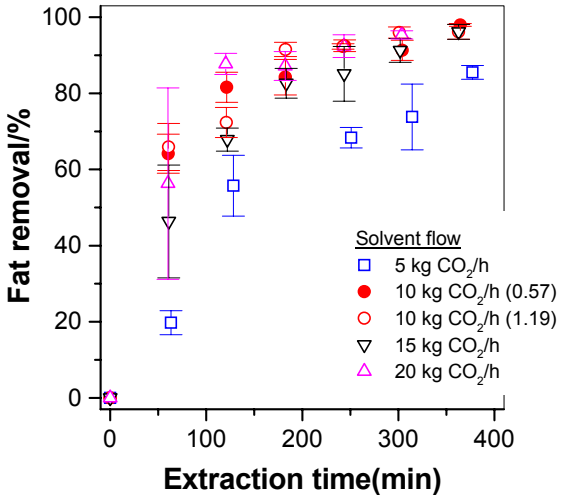

Figure 6. Fat removal as a function of extraction time for different solvent flows. T = 40 ºC, p = 35 MPa, skin sheet size = 15 cm × 14 cm, cycle time = 60 min

Figure 6 shows the fat removal as a function of extraction time for different solvent flows. It may be observed that when the solvent flow was low (5 kg/h) the extraction efficiency was also lower than in the rest of the cases indicating that the external matter transfer was important in the case. When the flow was equal or larger than 10 kg/h, the external matter transfer resistance seemed to be eliminated. Therefore it is recommended to work with a solvent flow of 10kg/h since using higher flows would only mean the use of a larger amount of solvent but not an improve of the extraction efficiency..

Conclusions

The results obtained showed that the internal matter transfer was the mechanism controlling the process; therefore, such resistance needs to be reduced or eliminated. A way of doing so, without grinding the skin, could be by "exploding" the fat cell membranes through rapid decompressions of the extractor where the skin sheets were previously swollen by the pressurized carbon dioxide. The optimum process conditions obtained after analyzing the influence of the process parameters were: extraction temperature 40 ºC, extraction pressure 350 bar; cycle time 60 min and solvent flow 10 kg/h.

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Acknowledgements: The authors gratefully acknowledge the Ministry of Science and Technology for financial support through grant PPQ2001-1024

QUALITY OF RABBIT MEAT UNDER MODIFIED ATMOSPHERES

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Background

Meat from rabbit is one of the healthiest meats because its dietetics properties (Dalle Zotte, 2002), which make it recommendable by the WHO to children, older and unhealthy people nutrition. In spite of these characteristics, rabbit meat consumption is very low (less than 3 kg/person in Europe) and is therefore in need of a publicity campaign and appropriate distribution channels. Distribution could be improved by conservation in modified atmospheres (MA) in order to prolong shelf-life for a time longer than normal refrigeration allows.

Only the effect of CO_2 and N_2 atmospheres (Gariepy *et al*., 1986), and vacuum (Fernández-Esplá and O'Neil, 1993) have been evaluated in rabbit meat, and a lack of studies on modified atmospheres packaging for fresh rabbit meat is perceived (Dalle Zotte, 2002).

Objectives

The aim of this study was to evaluate some parameters of rabbit meat when is packed in modified atmospheres with different gas compositions, to suggest a package atmosphere that best preserves the initial characteristics of rabbit meat and would have better acceptation.

Materials and methods

Sixty-eight rabbits from the Cunicultura-Villamalea S.A. Company (Albacete, Spain) were selected and slaughtered using standard commercial procedures. Carcasses were chilled at 1ºC for a day, and after that were cut in half-carcasses.

One hundred and thirty-six half-carcasses (550 g), without liver, were grouped in 8 half-carcasses to study initial meat quality (1 d post-slaughter), and 128 half-carcasses to study meat quality evolution under MA.

Samples were randomly assigned (32 half-carcasses per MA) to one of the following four types of MA: *Atmosphere A*: 30% CO_2 + 70% O_2; *Atmosphere B*: 30% CO_2 + 30% O_2 + 40% N_2; *Atmosphere C*: 40% CO_2 + 60% N_2; and A*tmosphere D*: 80% CO_2 + 20% O_2

A thermoforming atmosphere packaging machine ULMA Packaging model Smart (Guipuzcoa, Spain) was used for packing. The samples were places in clear rigid trays (LINPAC Plastic), having an oxygen permeability (OP) rate of 3.2 cm^3 /m^2 /24 h at 1 atm and 23°C ,and a cover film with an OP of 4.1 cm^3 /m^2 /24 h at 1 atm and 25°C) with the correspondent gas mixture.

Samples were chilled at 1°C for up to 21 days post-slaughter (i.e. 5, 10, 15 and 20 post-packing).

Eight samples were examined per atmosphere type (A, B, C, D) and per sampling time to determinate off-odour, instrumental and sensorial colour and rancidity evolution on meat.

Sample rancidity was determined in duplicate from 2 g of the *Longisimuss dorsi* (LD) muscle as described by Botsoglou *et al.* (1994) by determining 2-thiobarbituric acid-reactive sustances (TBARS). Absorbencies were measured with a spectrophotometer Perkin Elmer Lambda 20 (Norkwalk, USA) at 532 nm. Results were expressed as mg malondialdehyde Kg^{-1} meat.

Colour measurements (*L*, a*, b**) were taken immediately after that the packs were opened and the measurements were taken on the surface of the LD, at the level of the fourth lumbar vertebra. Measurements were recorded using a Minolta CR200 colorimeter (Osaka, Japan) calibrated against a standard white tile.

Sensorial colour and odour were also assessed by a panel of five experts. Colour was categorised in closed packs, and odour inmediatly after opened its. For both colour and odour, samples were scored as 1= not acceptable, 2= acceptable or 3= very acceptable.

The data were analysed using analysis of variance to determine the effects of atmosphere type on colour and rancidity. When the differences among types of modified atmospheres were significant ($p< 0.05$), Tukey's test was carried out to check the differences between pairs of groups. The effect of time of storage in refrigeration for each MA was analysed using Tukey's test at a significance level of $p< 0.05$. Data were analysed using the SPSS 11.0.1 statistical software.

Results and discussion

Acceptability of packs according to visual evaluation was higher and longer in atmospheres type A and B (Table 1). By contrasts, samples packed in atmosphere C were not accepted at any time during this experiment due to poor colour. In agreement with Gariepy *et al.* (1986) a faster discoloration of samples stored with higher proportions of CO_2 was appreciated, which was more marked in packs without oxygen in their initial composition.

Table 1. Percentage of acceptable packs according to sensorial colour[1] and odour[2].

	Time post-packing	Type A	Type B	Type C	Type D
Colour	5 days	100	100	0	85
	10 days	100	100	0	100
	15 days	100	96	0	0
	20 days	100	50	0	0
Odour	5 days	100	100	100	97
	10 days	100	100	100	100
	15 days	100	100	100	100
	20 days	25	25	70	70

[1]Closed packs, [2]When packs were opened
Modified Atmosphere: Type A (30% CO_2 +70% O_2), Type B (30% CO_2+30% O_2 + 40% N_2), Type C (40% CO_2 + 60% N_2), and Type D (80% CO_2+20% O_2).
Samples were categorised (for both colour and odour) as 1= not acceptable (strong off-odour), 2= acceptable (slight off-odour) or 3= very acceptable (no off-odour).

Off-odour was developed faster in samples with more oxygen (Table 1). The inhibitory effects of CO_2 on the spoilage bacteria could be the cause of the better odour quality of samples containing high proportions of CO_2. Unacceptable odour has been attributed to bacterial growth over 10^6 CFU/g (Gariepy *et al.,* 1986), and after 20 days none sample from any MA presented counts higher than 10^5CFU/cm^2 (data not showed). Also Gariepy *et al.*(1986) reported no objectionable odour in rabbit carcasses packed under CO_2 for more than 20 days.

Initial values for L^*, a^* and b^* were 54.92, 3.62 and – 4.23, respectively. A significant increase in L^* values was observed in all treatments (Table 2), and this is usually attributed to the oxidation of hem-pigments (Fernández *et al.*, 2000). However there were significant differences among groups only from 15 days onwards, then the values of this parameter were higher in samples of type A (more oxygen).

Redness (coordinate a^*) increased during the first 5 days and then decreased ($p< 0.05$) in all MA treatments wiht time. Similar evolution on a^* values was reported in beef (Insausti *et al*., 2001). The decrease was more pronunced in type A, and could be the result of metmyoglobin formation (Insausti *et al*., 2001). Also, an increase in yelowness was observed in all MAs along the first 5 days. After that time, coordinate b^* took negative increasing values (more blueness), which were higher in type C the first 15 days. After that differences between b^* values were higher in samples from type D. Increases in blueness has also been reported in frozen rabbit meat by Cabanes *et al.* (1994, 1996).

Slight rancidity increases in rabbit meat were appreciated in all treatments along time (Fig 1), but the increase was only significant in atmosphere A ($p< 0.05$). After ten days of storage significant differences were appreciated among treatments in all times. The atmospheres with higher oxygen concentrations (type A and B) showed the highest rancidity rates ($p< 0.05$), and the atmosphere without oxygen (type C) the lowest ($p< 0.05$). In addition, at the end of this study malondialdehyde levels in these atmospheres (type A: 1.11 ± 0.61 mg/kg, and type B: 0.89 ± 0.51 mg/kg) were similar or inferior than those reported by other authors for refrigerated fresh rabbit meat (Corino *et al*., 1999; Castellini *et al*., 2000). According to some authors, at least 5 mg malondialdehyde/kg are required for rancidity detection (Insausti *et al.*, 2001). After 20 days the MA types A and B were inferior than this value. Studies about rancidity evolution of rabbit meat were scarce

and focused on other storage systems like vacuum packaging (Fernández-Esplá and O'Neill, 1993). As in vacuum packaging, the use of modified atmospheres with carbon dioxide allows the extension of rabbit meat shelf-life. Besides, meat samples packed in non-oxygen-MA were more stable to lipid oxidation, because the absence or the minimal residual oxygen concentrations (O'Grady *et al*., 2000; Insausti *et al*., 2001; Jeremiah, 2001).

Table 2. Values (means ± e.s.) of colour (*L, *a**, *b**) parameters of rabbit meat preserved in modified atmospheres with different gas composition.**

Colour	Time post-packing	Type A	Type B	Type C	Type D	ANOVA
*L**	5 days	64.24 ± 1.08^{a}	62.23 ± 0.82^{a}	63.35 ± 1.00^{a}	65.57 ± 0.33^{a}	NS
	10 days	67.60 ± 0.75^{ab}	66.31 ± 1.50^{ab}	67.91 ± 0.63^{bc}	65.98 ± 1.03^{a}	NS
	15 days	$69.43 \pm 0.79^{b;x}$	$64.85 \pm 1.59^{a;y}$	$66.18 \pm 0.95^{ab;xy}$	$65.53 \pm 1.04^{a;xy}$	*
	20 days	$73.89 \pm 1.13^{c;x}$	$72.13 \pm 0.49^{b;xy}$	$71.05 \pm 0.62^{c;xy}$	$70.13 \pm 1.17^{b;y}$	*
*a**	5 days	6.99 ± 1.27^{a}	7.73 ± 0.82^{a}	5.37 ± 0.51^{a}	5.29 ± 0.44^{a}	NS
	10 days	$4.16 \pm 0.44^{ab;xy}$	$6.19 \pm 1.03^{ab;y}$	$3.38 \pm 0.30^{bc;x}$	$4.93 \pm 0.59^{a;xy}$	*
	15 days	$3.58 \pm 0.48^{ab;x}$	$5.50 \pm 0.66^{ab;y}$	$4.32 \pm 0.26^{ab;xy}$	$4.44 \pm 0.32^{a;xy}$	*
	20 days	1.32 ± 0.33^{b}	3.08 ± 1.06^{b}	2.47 ± 0.53^{c}	1.79 ± 0.49^{b}	NS
*b**	5 days	$1.55 \pm 1,28^{a;x}$	$1.56 \pm 0,94^{x}$	$-2.35 \pm 0,51^{y}$	$-1.59 \pm 0,68^{xy}$	**
	10 days	$-1.97 \pm 0.35^{ab;xy}$	-0.20 ± 0.95^{x}	-3.88 ± 0.42^{y}	-1.45 ± 0.75^{xy}	**
	15 days	-1.24 ± 0.97^{ab}	-2.02 ± 1.44	-4.49 ± 0.50	-4.24 ± 0.94	NS
	20 days	$-3.17 \pm 0.35^{b;xy}$	-1.60 ± 0.94^{x}	-3.60 ± 0.08^{xy}	-5.04 ± 0.46^{y}	*

Modified Atmosphere: Type A (30% CO_2 +70% O_2), Type B (30% CO_2+30% O_2 + 40% N_2), Type C (40% CO_2 + 60% N_2), and Type D (80% CO_2+20% O_2).
*,**: indicate significance levels at 0.05 and 0.01, respectively; NS: not significant
$^{a, b, c}$: values in the same column with different superscript are significantly different ($p < 0.05$); $^{x, y}$: values in the same row with different superscript are significantly different ($p < 0.05$).

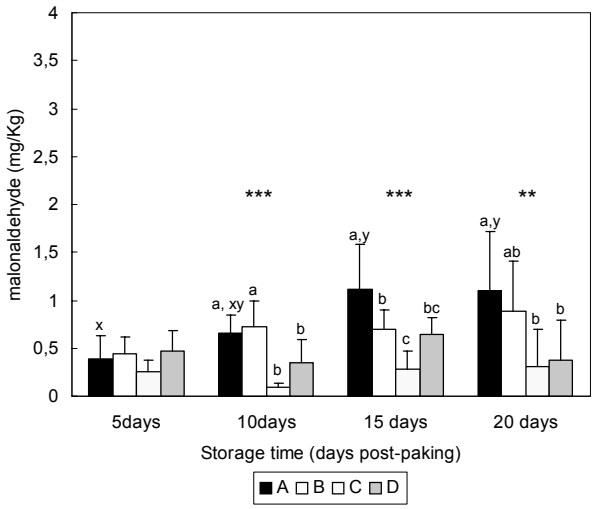

Figure 1. Rancidity levels (TBARS; mg malonaldehyde/Kg meat) in rabbit meat stored at 1°C in A: 30% CO_2 + 70% O_2 (black bars), B: 30% CO_2+30% O_2 + 40% N_2 (white bars), C: 40% CO_2 + 60% N_2 (pointed bars), and D: 80% CO_2+20% O_2 (cross bars). [**, ***: indicate significance levels at 0.01 and 0.001, respectively; $^{a, b, c}$: values in the same storage day with different superscript are significantly different ($p< 0.05$); ^{x,y}: values in the same colour bar with different superscript are significantly different ($p< 0.05$)].

Conclusions

According to colour samples from the atmosphere type A (30% CO_2 + 70% O_2) showed the best properties until the15th day; non off-odour were detected in any sample from this type, and although this atmosphere showed the highest levels of rancidity these numbers were always very inferior to the threshold level reported on the literature.

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CHANGES IN IMMUNOELECTRON MICROSCOPIC LOCALIZATION OF CALPAIN IN MUSCLE INDUCED BY CONDITIONING AND HIGH-PRESSURE TREATMENT

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Key word: calpain, immunoelectron microscopy, meat tenderization, meat conditioning, high-pressure treatment

Background

The most striking effect of postmortem aging is the improvement of meat tenderness, which is almost exclusively brought about by muscle endogenous protease that disrupts myofibrils. Especially, proteolysis of key myofibrillar proteins by calpain is the underlying mechanism of meat tenderization that occurs storage of meat at refrigerated temperatures. In general, calpain is widely distributed throughout the cytoplasm in a variety of cells. The calpains are present in two distinct forms, μ-calpain, a protease requiring 3-50 μM calcium ion for half-maximal activity; m-calpain, a protease requiring 400-800 μM calcium ion for half-maximal activity. The two calpain, μ- and m-calpain, are 110-kDa molecules each containing a 80-kDa and a 28-kDa polypeptide subunit. The 28-kDa subunit is identical in μ- and m-calpain; the 80-kDa subunits from two molecules differ, although they share 50 % sequence similarity.

It is well known that high hydrostatic pressurization is one of the new technology for reducing the conditioning period and improving the meat tenderness: there are many papers describing meat tenderization or acceleration of meat conditioning due to structural changes of the myofibrils caused by high pressure (Bouton et al., 1977; Locker & Wild, 1984; Macfarlane, 1985; Suzuki et al., 1992). Regarding calpain systems, Suzuki et al. (1993) provided direct evidence for the pressure-induced Ca^{2+} release from the sarcoplasmic reticulum from electron micrographs of the pyroanthimonate-fixed fiber bundles prepared from pressurized muscles. Ca^{2+} dispersion into myofibrils may cause the increase of activated calpain. Also Homma et al. (1995) indicated that the total activities of calpain in pressurized muscle appear to have been increased by the pressure and this may result in tenderization of meat.

There have been few reports describing the changes of calpain localization in muscle by high pressure treatment, and the behavior of the enzymes has not been clear. Many histological studies suggested that the calpain in the normal muscle localized primarily to Z-disk with smaller amounts in the I-band, A-band area, and between myofibrils. However, the changes in the localization of calpain in the muscle by conditioning or pressurization were not elucidated.

Objective

The objective of this paper was to investigate the changes in the localization of calpain in muscle exposed to high pressure by using immunoelectron microscopy, in comparison with those naturally observed in conditioned muscle.

Materials and methods

Tissue preparation: Lean meat was excised from the shoulder part of a culled-cow carcass 2 days after slaughter and stored at -25℃. As required, it was tempered overnight in a cold room (3 - 4℃) and divided into two parts, one for high pressure (100-400 MPa, 5min), and the other for conditioning at 2-4℃ 7 and 14 days. Muscle samples (1 mm^3) prepared from the pressurized and conditioned muscles were fixed with 0.25 % glutaraldehyde-PBS (0.1 M phosphate, pH.7.5, 8 % sucrose) for 30 min at room temperature, washed with PBS three times. After that the samples were dehydrated in graded alcohol (50 % and 75 %), and then were embedded in LR-White resin.

Immunoelectron microscopy: Ulrtrathin sections were cut onto 300-mesh grids and were incubated for 15 min in a PBS solution containing 5 % normal goat serum. After washing with PBS, the grids were

transferred onto drop of monoclonal antibody (Mouse anti-calpain small subunit (μ - or m-calpain) monoclonal antibody) (Chemicon) for 60 min. The monoclonal antibody was diluted 1:200 with PBS buffer. After incubation with the primary antibody, the grids were washed in PBS, and colloidal gold particle 10 nm in diameter conjugated with goat anti-mouse IgG+IgM (H+L) (Amersham) was added for 30 min at room temperature. The secondary antibody was diluted 1:25 (OD at 520nm of 0.1) just before use with PBS buffer. The grids were then washed in PBS, and the specimens were stained with 2 % uranyl acetate for 5 min. The stained specimens were examined with a CM 2000 Philips electron microscope at an operating voltage of 80 kV. Control sample was incubated with PBS in place of the primary antibody followed by incubation with the secondary antibody.

Results and discussion

To confirm the specific labeling for calpain, the muscle sample prepared immediately after thawing was first treated without anti-calpain antibody, then treated with colloidal gold conjugated anti-mouse IgG + IgM (H+L) (Fig.1). In which, non-specific labeling of the muscle was not observed. When the muscle sample was stained according to the procedure described in method, the immunogold particles were observed in Z-disk, I-band and A-band regions. This is proof that the anti-calpain small subunit (μ - or m-calpain) monoclonal antibody is specific to both μ - and m-calpain. But the immunogold particle seems to be concentrated primarily to Z-disk region than either I-band or A-band regions. This result agreed with those quantitative studies (Goll et al., 1983; Ishiura et al., 1980; Kumamoto et al., 1992) that calpain is located on the Z-disk with smaller amounts in the I-band and very little in the A-band area. When muscle was examined after postmortem, the calpain distribution was altered to being more diffuse throughout the sarcomere instead of concentrated at the Z-disk and between the myofibrils. As well as high density of immunogold particle was detected throughout the sarcomere, also disruption of myofibrillar structure was observed. The localization of the calpain may be involved in myofibril degradation and in membrane alterations of muscle cell.
When the muscle was exposed to pressure of 100 MPa or 200 MPa, a little more immunogold particle was detected in the sarcomere than that of unpressurizad muscle (Fig.2). With the increasing of the pressure up to 300 MPa applied to the muscle, detected high density immunogold particle throughout the sarcomere. Inversely, a few immunogold particle was detected in the sarcomere of the muscle exposed to pressure of 400 MPa. It seems to be due to the inactivation of calpain under high pressure. Also marked disruption in the regular structure of the myofibrils was observed in the muscle pressurized at 400 MPa as compared with the conditioned muscle. This disruption seems to be not due to the proteolytic degradation of myofibrils, but mainly due to the depolymerization of the F-actin, constituents of thin filament, as suggested by Macfarlane (1985)

Conclusions

From the results obtained in this experiment, it was clear that the changes in the localization of calpain in the muscle induced by high pressure were drastic in comparison with that in the muscle during conditioning.

Acknowledgments

This work was supported by Grant-in Aid for Japan Society for the Promotion of Science (JSPS) Fellows.

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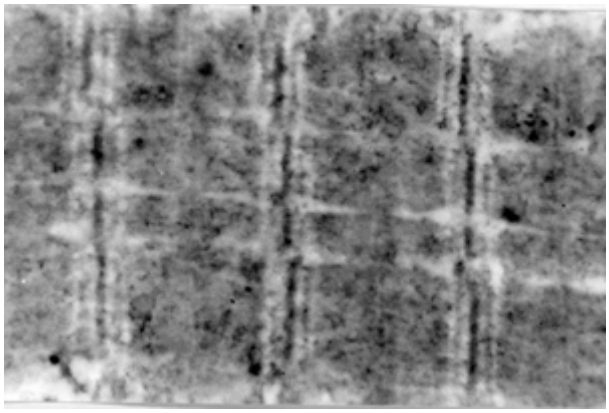

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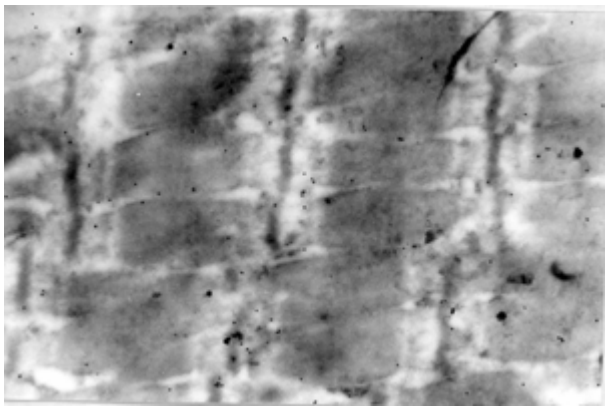
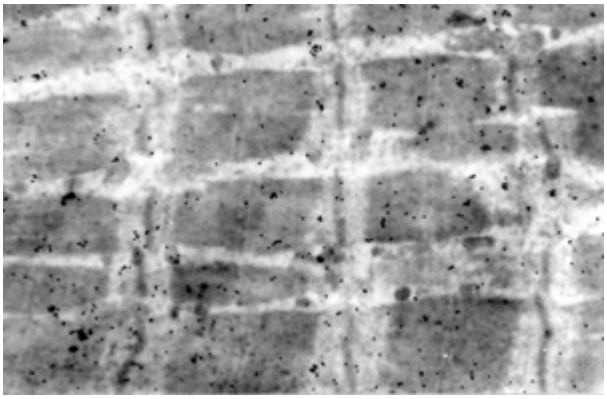


immediately after thawing



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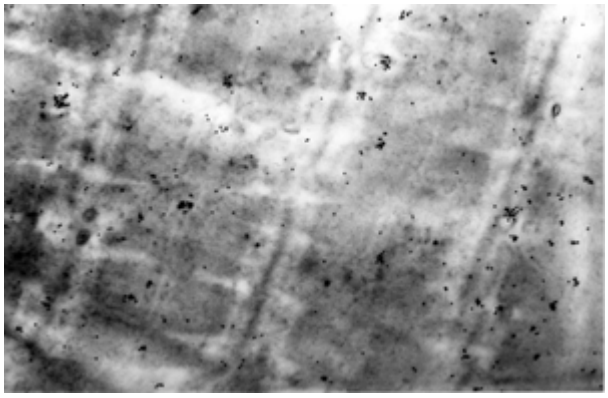

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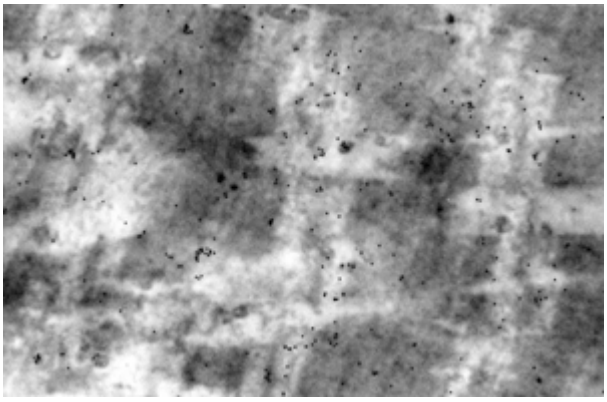

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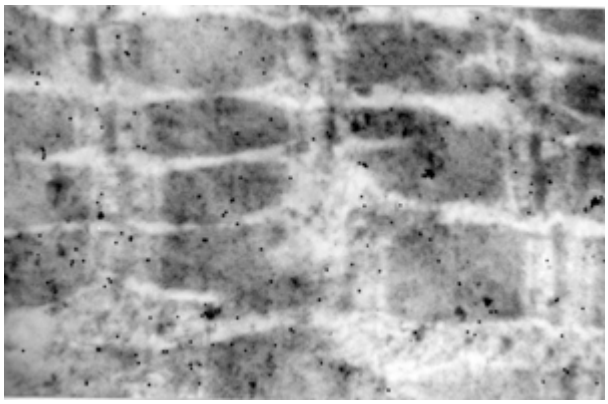
Fig.1. Immunoelectron micrographs showing localization of calpain during conditioning



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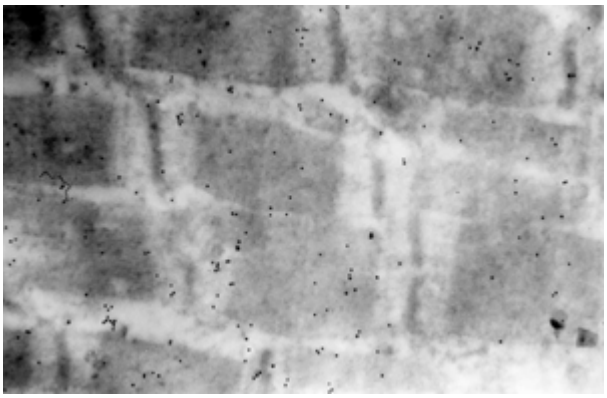

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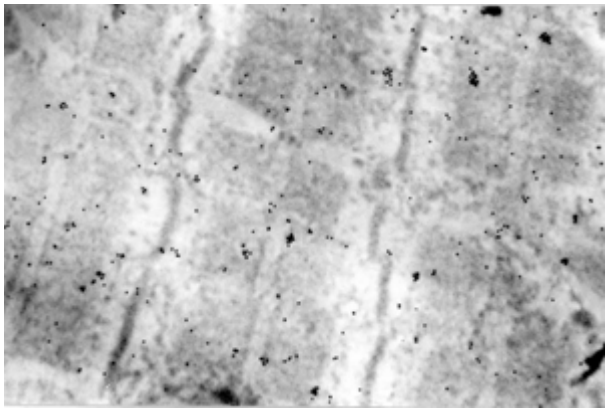
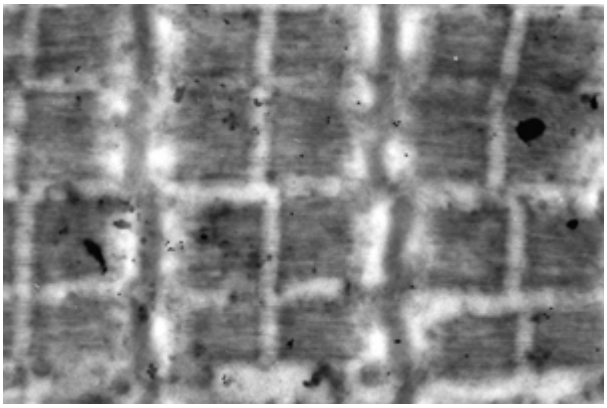


pressurized at 300 MPa



pressurized at 400 MPa

Fig.2. Immunoelectron micrographs showing the changes in localization of calpain caused by high pressure

THE EFFECT OF RIGOR-TEMPERATURE ON ISOMETRIC TENSION, SHORTENING, AND pH FOR OSTRICH *M. GASTROCNEMIUS, PARS INTERNA*

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Background

A major commercial attraction of hot-deboning is the considerable reduction in time, space and refrigeration capacity required (Taylor *et al*., 1980-1981). With regard to the export of ostrich meat, a reduction in time from slaughter to vacuum packaging of muscles could be beneficial towards increased shelflife since the temperature decline in hot-deboned muscles is faster and more uniform than in muscles left on the carcass (Van Laack *et al*., 1992). The former is more beneficial for controlling microbial spoilage (Lawrie, 1985). The good eating quality of ostrich might be reduced by the risk of cold-shortening if hot-deboning is performed (Taylor *et al*., 1980-1981). Fortunately, the increased risk of cold-shortening can be avoided by controlled chilling, but it is suggested that the maximum saving in time is achieved when hot-deboning is combined with electrical stimulation. To avoid cold-shortening, it has been recommended to debone at temperatures between 5º and 15ºC and keep the vacuum packaged meat cuts at this temperature for at least 10 hours *post-mortem* (Lawrie, 1985). By measuring the sarcomere length, Sales (1994) found that cold-shortening were absent in ostrich muscles. Sales & Mellet (1996) and Sales (1994) found ostrich *M. iliofibularis* to have a very rapid pH decline until two hours *post-mortem*, after which the pH increased. Morris *et al.* (1995) found that the lowest *post-mortem* pH value for ostrich *M. iliofibularis* and *M. gastrocnemius* occurred within 30 minutes after slaughter (not hot-deboned). As illustrated by the results found by Sales and Mellet (1996), the risk of cold-shortening would be reduced in the *M. iliofibularis* since it reached a pH $\leq$ 6.20, at approximately 30 minutes after slaughter. Sales and Mellett (1996) found that the apparent ultimate pH was reached rapidly at two hours *post-mortem* in the *M. iliofibularis* (6.00 ± 0.09), and at six hours *post-mortem* in the *M. gastrocnemius, pars interna* (6.12 ± 0.06). Therefore, it was suggested that there is a risk of cold-shortening in the *M. gastrocnemius, pars interna* if this muscle would be separated from the carcass at 30-45 minutes *post-mortem*, but not in the *M. iliofibularis*.

Objectives

The aim of this study was to investigate the development of isometric tension, *i.e.* measuring tension while the muscle is prevented from contracting, and shortening in ostrich *M. gastrocnemius, pars interna* during *rigor mortis* at 7 and 37ºC respectively, in an attempt to determine the time course of rigor, pH decline, and degree and extent of shortening, *i.e.* occurrence of cold-shortening. This will allow one to decide how soon after death it would be safe in terms of eating quality to hot-debone and vacuum pack whole ostrich muscles.

Materials and methods

Ten rested, about 12 hours lairage, and two stressed ostriches, were slaughtered during February to April at the same EU approved abattoir. The two stressed ostriches were slaughtered on arrival at the abattoir. The right *M. gastrocnemius, pars interna* muscle were removed from the rested ostriches within one hour after death, and within 20 minutes from two rested and the two stressed ostriches. From these muscles, strips (1x1x3 cm) weighing between 1.5 and 3 g were cut for measurement of isometric tension, expressed as force (mN) per unit area, and shortening, expressed as percentage decrease in muscle length, during *rigor mortis* using two separate rigometers (Rigotech®) at constant temperatures of 7 and 37ºC, respectively. A SenTix 41 probe, connected to a portable pH meter 340i (WTW, GmbH & Co. KG, Weilheim, Germany), was inserted into a larger portion of muscle, which was also placed into the rigometers, for continuous measurement of pH every 10 minutes during the rigor process for a 24 hour period.

Results and discussion

Muscle pieces from rested animals reached maximum tension after 50 minutes for both 7 and 37ºC and maximum shortening after 170 min for 7ºC and 150 min for 37ºC, while a shorter time was observed for stressed ostriches (Figure 1). Muscle strips from rested ostriches, going into rigor at 37ºC, reached maximum tension and shortening sooner ($P < 0.001$) than those at 7ºC (Table 1). In addition, although tension showed no lag phase for either of the two temperatures, the mean lag phase for shortening onset was longer at 7ºC (42.22 min) than at 37ºC (17.78 min), indicating that the time course of *rigor mortis* is temperature dependant. Devine *et al* (1999) demonstrated that the time for bovine muscle to reach maximum tension was highly negatively correlated with temperature, as was the time to reach maximum muscle shortening.

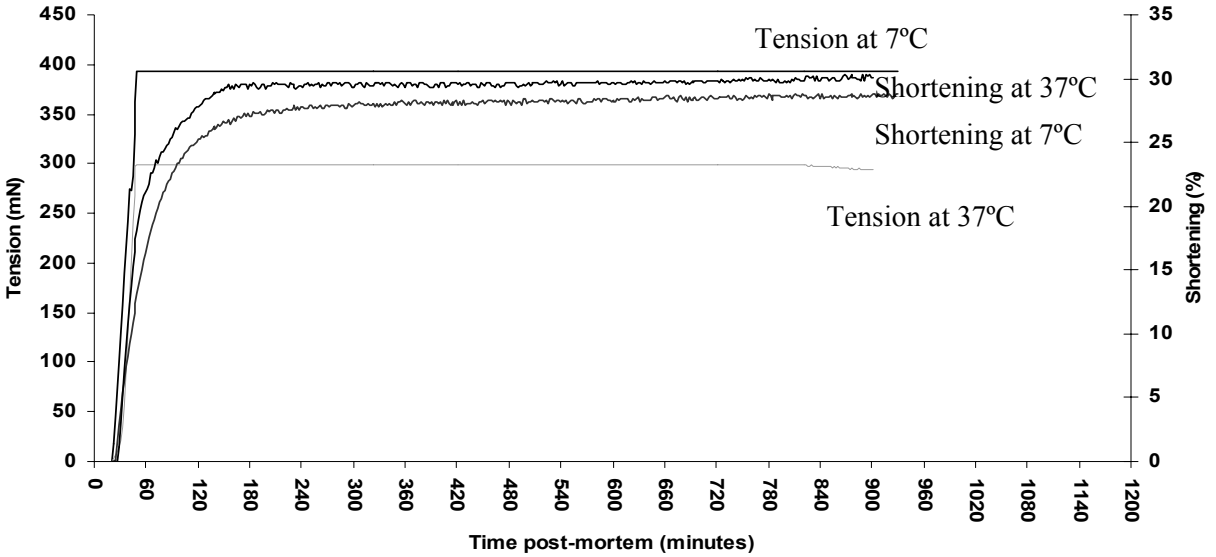

Figure 1. Measurement of isometric tension and shortening from the time (20 minutes after death) the muscle strips from the two stressed ostriches were placed in the rigometers and maintained at 7ºC and 37ºC, respectively.

Table 1. Mean times (minutes) for *M. gastrocnemius, pars interna* muscle from rested ostriches to reach maximum tension (mN) and maximum shortening (%)

Temperature (ºC)	Time (min) to maximum Tension (mN) (N = 8)	Time (min) to maximum Shortening (%) (N = 8)
7	679± 400	1400 ± 0.0*
37	183± 50	236 ± 40

*Shortening reached a high value at approximately 400 min, after which it continued to increased at a very slow rate up to 1400 min. without reaching a plateau.

The values for maximum tension and shortening were also temperature dependant, where maximum tension was higher ($P < 0.05$) at 7ºC (364 ± 50 mN) than at 37ºC (280 ± 70 mN), while in contrast, maximum shortening was higher ($P < 0.001$) at 37ºC (35.00 ± 5) than at 7ºC (11 ± 3%) (Table 2). This is in compliance with the findings of Devine *et al* (1999), demonstrating that muscle shortening decreases with decreasing temperature. Similarly, maximum tension was higher at 7ºC than at 37ºC, and maximum shortening was higher at 37ºC than at 7ºC in muscle strips from the two stressed ostriches. The occurrence of greater tension at low temperatures, with less shortening, than at high temperatures, could be explained by the release of calcium ions. Low temperatures (<15ºC) stimulate the release of Ca^{2+}-ions from the sarcotubular system, enhancing the contractile actomyosin ATP-ase (Lawrie, 1985). Actin and myosin interconnect but without the actin filament being pulled inwards, towards the centre of the sarcomere (causing the sarcomere to shorten), because of the lack of sufficient levels of ATP due to the decrease in enzyme activity at the low temperatures. There is thus tension due to the binding between actin and myosin, but less shortening since there is not enough energy to cause the sarcomere to shorten. The greater percentage of maximum shortening at higher temperatures could, on its turn, be explained by ATP levels (Hertzman *et al*., 1993).

Hertzman and co-workers found a high correlation between maximum shortening and the ATP level at the onset of the shortening rapid phase and explained the higher shortening at 37ºC compared to 15ºC due to the higher ATP level at this higher temperature. They further suggested that the release of Ca^{2+} at higher ATP levels (at 37ºC) could depend on the decreased stability of mitochondria to sequester Ca^{2+} under anaerobic conditions in conjunction with a faster decline in pH at 37ºC than at 15ºC.

Table 2. Maximum tension (mN) and maximum shortening (%) for individual *M. gastrocnemius, pars interna* muscle from rested and stressed ostriches at temperatures of 7 and 37ºC, respectively

Ostrich	Temperature of 7ºC		Temperature of 37ºC	
Rested	Tension (mN)	Shortening (%)	Tension (mN)	Shortening (%)
1	424.3	17.84	292.8	42.68
2	408.9	8.92	247.5	37.62
3	350.4	10.88	311.3	29.83
4	373.0	7.39	213.5	38.49
5	317.4	8.11	408.8	29.34
6	279.5	10.93	173.8	33.59
7	379.9	13.29	280.1	36.26
8	384.7	13.70	318.0	32.77
Mean ± Std.Dev.	364. ± 50	11 ± 3	280 ± 70	35.00 ± 5
Stressed				
11	393	29		
12			298	30

In muscle strips from rested ostriches, the tension peak was followed by a decline in tension when rigor developed at 37ºC, while at 7ºC the maximum tension remained constant. The decline in tension at 37ºC could be explained by the initial ageing process (proteolysis) of the muscle at high temperatures. It might also be explained as relaxation due to transport of Ca^{2+} back to the sarcoplasmic tubuli. At 7ºC, after the lag phase of about 120 minutes, shortening increased rapidly during the next 180 minutes of measurement, then continued to increase at a very slow rate, reaching maximum values at about 24 hours *post-mortem*, although, not yet reaching a constant value. In contrast, maximum shortening at 37ºC was reached within the first 120-180 minutes and remained constant at this maximum value (Figure 2). Muscle tension showed no lag phase before onset at either of the two temperatures, and the muscle strips from the rested and the two stressed ostriches reached maximum tension before reaching maximum shortening.

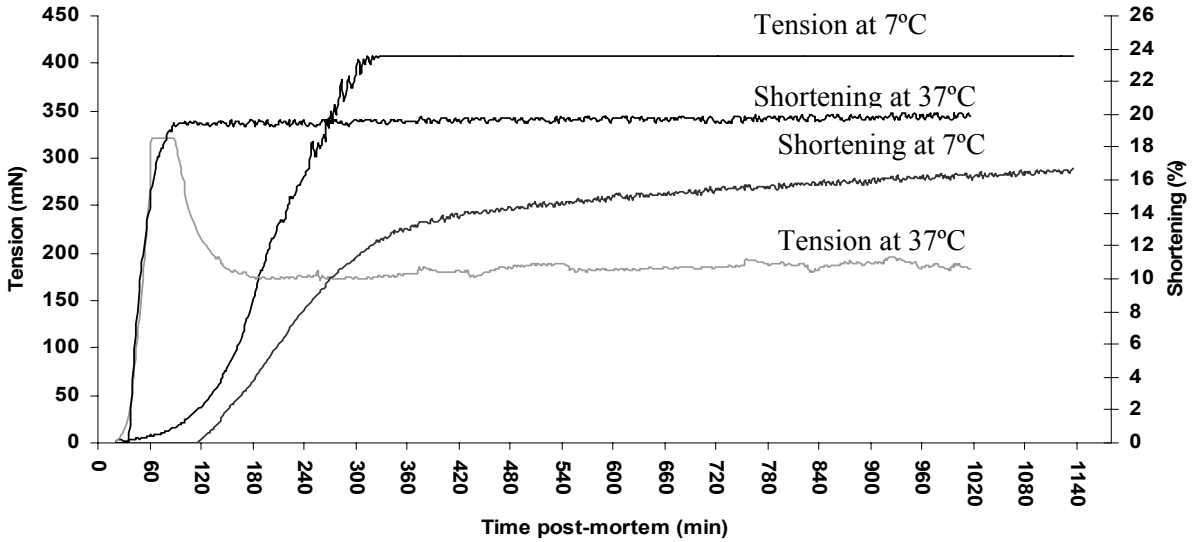

Figure 2. Measurement of isometric tension and shortening from the time (20 minutes after death) the muscle strips from two rested ostriches were placed in the rigometers maintained at 7ºC and 37ºC.

In general, the pH for intact *M. gastrocnemius, pars interna* from rested ostriches decreased from the start of pH-measurements (one hour after death) until about two hours *post-mortem* (reaching a minimum value of

6.0 ± 0.3), indicating a rapid fall in pH during the first 2-3 hours after death, after which the pH then increased (Figure 3). The change in pH for muscle strips from rested ostriches at 7ºC and 37ºC, showed a similar decrease in pH and subsequent increase. However, the time to reach the minimum pH at 7ºC was approximately four hours and approximately two hours at 37ºC.

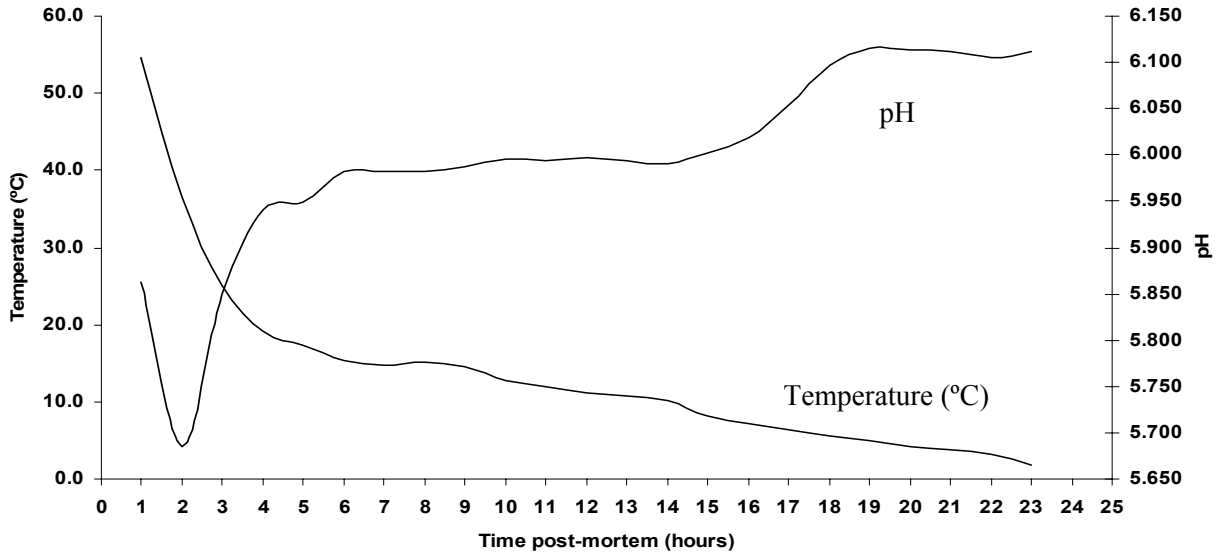

Figure 3. Representative measurements of temperature and pH change in intact *M. gastrocnemius, pars interna* from an individual rested ostrich.

Conclusions

The minimum pH obtained for ostrich *M. gastrocnemius, pars interna* seemed to be within 3-4 hours *post-mortem*, after which the pH increases. The reason for the increase in pH needs to be investigated. Cold-shortening occurs when muscles are exposed to low temperatures early *post-mortem* and when pH levels are still high. With the rapid fall in pH, the apparent absence of cold-shortening at low temperatures, and early onset of *rigor mortis* soon after death, it may be suggested that hot-deboning of ostrich muscles as early as 3-4 hours post-mortem would be without detrimental effects on the eating quality in terms of toughness.

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PREDICTING AND SEGREGATING BEEF TENDERNESS AMONG URUGUAYAN STEER CARCASSES UNDER COMMERCIAL CONDITIONS USING *POSMORTEM* CARCASS TRAITS, PH, TEMPERATURE AND COLORIMETER READINGS

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Background

In the food industry consumer-oriented quality approaches are more and more used if we consider the large development of marketing. The perception of the quality could be defined prior to purchase (beliefs and attitudes), at the point of purchase (intrinsic and extrinsic cues) and upon consumption (sensory attributes). The National Beef Tenderness Survey conducted in US (1990), documented a relative high incidence of toughness problems among different beef cuts for sale and identified the need to improve retail beef tenderness (George *et al*., 1999). The US beef industry has made it a priority to address the inconsistency in beef tenderness and has been developed strategies to ensure that all beef has acceptably tender. The development of tenderness based classification systems makes possible to identify carcasses with superior tenderness and add value to these carcasses that are undervalued in current systems. Studies have demonstrated that consumers recognized consistently differences in tenderness and they are willing to pay for this attribute (Boleman *et al*., 1997). Most of these systems evaluated by research are based on the relationship between muscle pH (Purchas, 1990; Wulf and Page, 2000), color (Wulf *et al*., 1997; Vote *et al*., 2003) and temperature (Jones and Tatum, 1994) with meat tenderness. This strategy to improve consistency of meat palatability –tenderness as main factor- is also followed by principal meat export countries, such as Australia (Guarantee tenderness) and New Zealand (NZ Beef and Lamb Quality Mark). Uruguay, as a meat export country in South America ought to follow this approach making a diagnostic of the variation of this attribute and according to the information gathered identifying the critical control points along the meat chain to ensure the consistency on it.

Objectives

This study was conducted to determine whether carcass traits and objective measures of muscle color, pH and temperature are useful to predict tenderness on selected beef steer carcasses of unknown origin, and to determine whether the application of USDA quality grading standards to Uruguayan beef carcasses could improve their segregation by tenderness.

Materials and Methods

One hundred seventy four beef steer carcasses of unknown origin were selected at one packing plant in Uruguay. Carcasses were selected at the time of grading by the Uruguayan official system without electric stimulation. Hot carcass weight (HCW) and fatness are associated with the age, for this reason it was decided to study whether within age could exist differences in quality traits, considering two HCWs and two backfat thickness (0-2 teeth carcasses: HCW < and ≥ 225 kg and Fat < and ≥ 5 mm; 4 teeth carcasses, HCW < and ≥ 240 kg and Fat < and ≥ 5mm; and 6-8 teeth carcasses, HCW < and ≥ 265 kg and Fat < and ≥ 7 mm). Carcasses were segregated into appropriate fat thickness categories based on actual carcass backfat measured at the 11th rib surface. Two official classification systems were applied, the American (USDA, 1997) and the Uruguayan (INAC, 1997) at different moments. The pH and temperature of the *longissimus lumborum* (LL) was determined at three different times: 1, 3 and 18-24 (ultimate) hs postmortem. The pH and temperature at 1 and 3 hs *postmortem* were measured between the 10 and 11th rib on the left side of the carcasses, at a depth of 2 cm. The measurement of ultimate pH was realized at the 11th rib cut surface in the pistol cut. The muscle pH was measured using a hand-held pH meter (Orion A 230) with a probe type electrode (BC 200, Hanna Instruments), standardized against two pH buffers (4 and 7). The probe was cleaned with alcohol and rinsed with water between uses. The temperature was determined by a thermometer (Barnant 115) with stainless steel thermocouple (type E). Muscle color measurements followed the CIE color convention (CIE, 1986).

Color measurements were made using a Minolta Colorimeter (model C-10). They were recorded in triplicate from the exposed LL muscle between the 10-11th rib immediately following ribbing (left side). A 8-cm portion of the LL was removed from the left side of carcasses, labeled, vacuum-packaged and transported to the Meat Science Laboratory at INIA Tacuarembó for shear force analysis considering 7 and 14 days of aging at 2 - 4 ^{o}C. The steaks were cooked by immersion within a plastic bag in a water bath at an internal temperature of 70^{o}C for 75 min. The internal temperature was monitored using type E thermocouples placed in the approximate geometric center of the steak. Six cores (1.27 cm diameter) parallel to the muscle fiber orientation were removed from each steak. A single peak WBSF measurement was obtained for each core using a WBSF machine (G-R Electric Manufacturing Co, Manhattan, KS). Individual-core peak shear force values were averaged to assign a mean peak WBSF value to each steak. *Statistical analysis* Descriptive statistics were computed in this experiment for selected carcass traits, pH, temperature, colorimeter readings and WBSF values. The model used in this experiment was $Y_{ijkl} = \mu + A_i + B_j + C_k + ABC_{ijk} + E_{ijkl}$ being Y_{ijkl} = ijklth Warner-Bratzler shear force at 7 and 14 days of aging, μ = overall mean, A_i = effect of the ith dentition, B_j = effect of the jth hot carcass weight, C_k= effect of the kth fat thickness, ABC_{ijk}= interaction effect of ijkth dentition * hot carcass weight*fat thickness, and E_{ijkl} = residual error. Least squares means were calculated using PDIFF procedure. To better understand the relationship between the independent and dependent (WBSF values) variables the information was analyzed by correlation and regression procedures (stepwise procedures, SAS, 1990).

Results and Discussion

In this study, it was not detected significant differences (P>0.05) in WBSF 7 and WBSF 14 explained by dentition, HCW and Fat. The WBSF mean obtained for 7 and 14 days of aging were 4.06 kg (CV: 35.3%) and 3.42 kg (CV: 30.7%). These results agree with Lawrence *et al* (2001) who did not find significant differences in WBSF and sensory panel tenderness among five dental classes. These authors supported the concept that carcass classification based on dentition should not be used in place of USDA carcass maturity to segregate carcasses for *longissimus* steak tenderness. The carcasses were majority classified as Traces (35.1%) and Slight (55.2%) according to USDA marbling score and as A (76.4%) to USDA overall maturity category. QG scores varied from Utility to Choice, where 88.5% of the carcasses were classified as Standard (44.3%) and Select (44.3%). As it was expected, the pH values (pH_1, pH_3 and pH_u) were lower (P<0.05) and the T records were higher (T_1, P<0.01, and T_3, P<0.05) for the fattest group in each age group. The positive relationship between muscle temperature and subcutaneous fat thickness resulted from the insulation effect of fat. Correlation Analyses. YG was the main variable negatively correlated (P<0.01) with both WBSF 7 and WBSF 14 (Table 1). This could be explained by subcutaneous fat thickness, where higher levels of this variable would be associated to lower WBSF values. All correlation coefficients between muscle color and WBSF were negative. These findings have also been reported by Wulf *et al*. (1997), Wulf and Page (2000) and Vote *et al*. (2003), showing that dark color muscles could be associated to tough steaks. Lean maturity presented a low correlation with WBSF 7 and WBSF 14 (r=0.17 and 0.19, respectively). Studying the relationship between color parameters with some of the measured variables, lean L*, a* and b* were moderately and highly correlated (P<0.01) with pH_u, presenting a* the highest correlation (r=-0.81). This suggests the importance of lean a* as indicator of meat quality and especially of tenderness in the present study. Ledward *et al*. (1992) reported that the negative correlations between colorimeter readings and pH_u could be explained by the oxygenation of the myoglobin and the reflectance of the light. Lean L* was most closely related with lean maturity (r=-0.61, P<0.01). This result is concordant with the information reported by Vote *et al*. (2003) who found correlation values (P<0.05) between both characteristics in the range of -0.52 and -0.69 in different experiments using the CVS BeefCam color output. *Regression analyses* The application of the USDA quality grade factors to Uruguayan steers (Table 2) explained in most of the cases 4 to 6 % of the WBSF 7 and WBSF 14 variation, with the exception of WBSF 14 in 6-8 teeth carcass group where lean maturity explained 21% of its variation. Vote *et al*. (2003) reported a partial R^2 for lean maturity of 6 and 8% in the WBSF 14 variation for 2 different experiments. Marbling showed low correlation (P>0.05) to WBSF 7 (r=-0.13) and WBSF 14 (r=-0.14). Wulf *et al*. (1996) in a research containing mostly carcasses classified as USDA Select reported no correlation between variables (P>0.05) and Vote *et al*. (2003) considered that marbling score did not explain WBSF variation in Select carcasses. When it was ran multiple linear regression procedures using all the measurements to predict WBSF values, the main single variable in predicting tenderness for all evaluated carcasses was lean a* (Table 3). This colorimeter reading explained by itself 16 and 24% of the WBSF 14 and WBSF 7 variation, respectively. Vote *et al*. (2003)

considering Choice and Select carcasses found that lean a* from the CVS BeefCam explained 16% of the WBSF 14 variation. The lean a* parameter was followed by pH_u and T (T_1 or T_3) in entering into the model for all steer carcasses. This tendency was also observed in 0-2 and 4 teeth carcasses. However, for the 6-8 teeth carcass group, variables associated to yield as REA ($r=-0.37$, $P<0.01$), YG and HCW contributed to explain the tenderness variation. REA singularly account for 30% of the WBSF 14 variation. The REA has been related to carcass yield, however Wyle *et al*. (2003) and Vote *et al*. (2003) had observed a limited relationship between this variable and palatability and the possibility to identify tough steaks using REA from CVS BeefCam adjusted by carcass weight. In this age category (6-8 teeth) QG explained an additional 24% of the WBSF 14 variation.

Conclusions

The population of cattle utilized in this study was representative of slaughtered steers in Uruguay according to age. For these animals, objective measures of muscle color, specifically lean a*, was the most effective in predicting WBSF, which explained 24 and 16% of WBSF 7 and WBSF 14 variation. However, analyzing the information by age category, the prediction of WBSF in 6-8 teeth carcasses was better accounted for other variables like pH_3 or REA (for WBSF 7 and WBSF 14, respectively). The addition of the USDA QG standards to the Uruguayan steer carcasses grading would seem do not contribute to improve the effectiveness to sort carcasses into tenderness group, with the exception of WBSF 14 prediction in 6-8 teeth carcass group. For this population, ultimate pH and lean a* measured at ribbing might be useful in sorting beef carcasses likely to yield tough or tender steaks depending on the defined WBSF critical point. Further research should be conducted to better understanding the relationship between pH/color and tenderness and how to improve it for Uruguayan conditions.

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Table 1 Main simple correlations between carcass variables and tenderness and color for all steer carcasses

Variables	WBSF7	WBSF14	Variables	L*	a*	b*
YG	- 0.20**	- 0.22**	Dentition	- 0.27**	0.14	0.04
Lean mat.	0.17	0.19*	Lean mat.	- 0.61**	- 0.43**	- 0.32**
MARB	- 0.13	- 0.14	QG	0.26**	0.35**	0.26**
QG	- 0.11	- 0.14	pH_1	- 0.52**	- 0.53**	- 0.44**
a*	- 0.19*	- 0.16*	pH_3	- 0.62**	- 0.63**	- 0.52**
b*	- 0.17*	- 0.13	pH_u	-0.67**	- 0.81**	- 0.66**

Note: ** Significative at 1% . * Significative at 5%.

Table 2. Multiple regression equations to predict WBSF 7 and WBSF 14 using USDA quality variables for all, 0-2, 4 and 6-8 teeth steer carcass group

WBSF 7			WBSF14		
Independent variables	**R^2**	**RMSE**	**Independent variables**	**R^2**	**RMSE**
All carcasses					
Lean maturity	0.03	1.33	Lean maturity	0.04	0.97
Lean maturity, Skeletal maturity	0.06	1.32	Lean maturity, Marbling	0.06	0.96
0-2 teeth carcasses					
No variables met 0.2 significance level for entry into the model for both dependent variables (WBSF7 and WBSF14)					
4 teeth carcasses					
Lean maturity	0.04	1.57	Skeletal maturity	0.04	1.08
6-8 teeth carcasses					
Lean maturity	0.04	0.85	Lean maturity	0.21	0.57

Note: Models were developed using stepwise procedure. R^2= coefficient of determination. RMSE=root mean square error

Table 3. Multiple regression equations to predict WBSF 7 and WBSF 14 using YG, QG, pH, temperature and color for all, 0-2, 4 and 6-8 teeth steer carcass group

WBSF 7			WBSF14		
Independent variables	**R^2**	**RMSE**	**Independent variables**	**R^2**	**RMSE**
All carcasses					
a*	0.24	1.60	a*	0.16	0.94
a*, pH_u	0.29	1.50	a*, T_1	0.20	0.90
a*, pH_u, T_3	0.32	1.45	a*, T_1, pH_u	0.24	0.87
0-2 teeth carcasses					
pH_u	0.11	1.32	a*	0.18	1.63
pH_u, T_3	0.27	1.14	a*, T_3	0.26	1.54
4 teeth carcasses					
a*	0.31	1.88	a*	0.23	1.00
a*, T_3	0.42	1.60	a*, T_1	0.38	0.82
a*, T_3, skeletal mat	0.50	1.43	a*, T_1, skeletal mat	0.43	0.77
a*, T_3, skeletal mat, pH_u	0.53	1.36			
6-8 teeth carcasses					
pH_3	0.17	0.63	REA	0.30	0.29
pH_3, YG	0.28	0.56	REA, QG	0.54	0.20
pH_3, YG, REA	0.34	0.54	REA, QG, HCW	0.58	0.19

Note: Models were developed using stepwise procedure. R^2= coeff. of determination. RMSE=root mean square error

CONSUMER'S PERCEPTION OF BEEF TENDERNESS

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Background

Tenderness is considered the most important palatability characteristic of beef and, consequently, the primary determinant of meat quality (Dikeman, 1987). It is also very variable, depending on several factors related to genetics, nutrition, *ante mortem* handling and *post mortem* technological treatments. As outlined by Koohmaraie (1996), tenderness variability is the main reason for consumer's dissatisfaction. In fact, today, the consumer requires food with quality characteristics standardized and adequate to the price.
Beef tenderness can be evaluated studying their intrinsic characteristics by instrumental analysis or by sensory analysis, using a trained panel. However, it is very important, even if more difficult, to know consumer liking of tenderness, considering that the consumer is the user of the product.

Objectives

The aim of this study was to determine consumer's ability to recognize differences in beef tenderness.

Materials and methods

A consumer panel of 220 people differing in sex and age was recruited from a broad range of socioeconomic background (table 1). The males represented about 58% of the panel. Concerning age, the individuals were allotted in quite a homogeneous way into the first three classes (28% 18 to 30 yrs old; 27% <45 yrs old; 31% < 60 yrs old), while the individuals over 60 yrs represented about 14% of the panel.
Tenderness evaluation was performed on 31 samples of *longissimus thoracis* taken between the 8th and 10th thoracic vertebra from the right side of the carcasse. The animals belonged to the most widespread commercial categories in Piedmont: milkfed calves (C; n = 10) and young bulls. The latter included dairy (DB; n = 10) and beef breeds (BB; n = 11). Meat samples were purchased at retail and transferred to the laboratory of Department of Animal Science of Turin, where they were divided in two further samples, vacuum packaged, frozen and stored at -25°C until of their utilization. The samples were thawed at 2°C and then cooked by roasting in an electric convection oven, preheated at 210°C, until they reached a final internal temperature of 70°C. The cooking temperature was monitored by an Iron/constantan thermocouple connected to a termometer and inserted into the geometric centre of the sample. The cooked meat was cut into 1.3 x 1.3 x 1.9 cm cubes for sensory analysis.
The tenderness sensory evaluation was carried out using a five point facial hedonic scale at the end-anchored by the words very tough/very tender (Cross *et al*., 1986; AMSA, 1995). The scale was: 1 = "very tough"; 2 = "tough"; 3 = "neither tough nor tender"; 4 = "tender"; 5 = "very tender".
The sensory analysis was performed at the laboratory of the Department and, in general, in each session the panelists evaluated three samples, one of each animal's groups for a total of 671 evaluation. Each consumer was involved in only one session.
The correspondence analysis was employed to study the sensory judgements distribution in relation to either the sex and age of the consumers or the category of the animals (SPSS, 1997).

Results and discussion

Table 2 shows that the distribution of the tenderness ratings was very similar in males and females (P = 0.75). Considering the consumers' age (table 3), a tendency of young people to give lower evaluations was observed, but the differences did not reach the level of significance (P = 0.49). Therefore the evaluation of beef tenderness seems not to be affected by consumer's sex and age. Similarly Huffman *et al*. (1996) did not observe significant effect of sex and age on sensory tenderness when the steaks were rated at restaurant, while reporting significant differences in tenderness rating across age groups in consumer's homes.
On the contrary, highly significant differences in rating distribution were observed according to the commercial categories of the animals (table 4; P< 0.001). Indeed, results show that meat of calves had scores

higher than meat of dairy (P<0.001) and beef (P<0.001) young bulls. The last two commercial categories did not differ in tenderness scores (P = 0.31).
Figure 1 shows that calves obtained more ratings in highest tenderness classes. In fact, 45% "tender" meat (class 4) and 55% "very tender" meat (class 5) belonged to calves, which also had lowest percentages in"very tough" (class 1) and "tough" (class 2) meat. Although meat from young bulls showed similar tenderness ratings, it must be, however pointed out that meat from young bulls of dairy breeds represented more than 50% "very tough" tenderness rating, whereas meat from young bulls of beef breed had a higher percentage in "very tender" rating (28% *vs* 16%).
The overall results can be represented in the correspondence analysis plot (figure 2). The dimension 1 explained the largest amount of inertia (95%). The calves and rating 5, showing the highest deviation from the origin, gave the main contribution to the inertia of this dimension. The calves placed to the right in the plot and near to ratings 4 and 5 differed from the young bulls placed on the left and near to ratings 1, 2 and 3. In particular, dairy young bulls were the closest to rating 1, while beef young bulls were the closest to rating 2.
These results are in agreement with the data by Boleman *et al*. (1995), Huffman *et al*. (1996), Miller *et al*. (1995), Wheeler *et al*. (2002) indicating that the consumers can detect differences in tenderness.

Conclusions

The results of this study show that, apart from sex and age, the consumers are able to consistently detect beef tenderness differences, when two commercial categories of animal are compared. In particular, ratings assigned by the consumers allowed to clearly discriminate the meat of calves from that of young bulls.
The large distribution of the meats of each commercial category of the animals in the tenderness ratings indicate that the meat sold at retail shows high variability. Consequently it is important for beef industry to promote the tender meat production.

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Acknowledgements

Research supported by University, "già quota 60%".

Table 1. Profile of consumer panelists for sex and age.

Sex	Age, yrs				Total
	18-30	<45	<60	>60	
Male	35	30	43	20	128
Female	27	29	26	10	92
Total	62	59	69	30	220

Table 2. Tenderness ratings by sex.

Sex	Tenderness ratings					Total
	1	2	3	4	5	
Male	32	89	94	116	59	390
Female	28	59	76	75	43	281
Total	60	148	170	191	102	671

Table 3. Tenderness ratings by age.

Age, yrs	Tenderness ratings					Total
	1	2	3	4	5	
18-30	19	48	52	43	26	188
<45	21	37	40	54	27	179
<60	14	48	51	65	34	212
>60	6	15	27	29	15	92
Total	60	148	170	191	102	671

Table 4. Tenderness ratings by commercial categories of animals.

Comm.Categories	Tenderness ratings					Total
	1	2	3	4	5	
C	4	27	36	86	56	209
DB	31	53	65	54	17	220
BB	25	68	69	51	29	242
Total	60	148	170	191	102	671

Figure 1: Tenderness profile

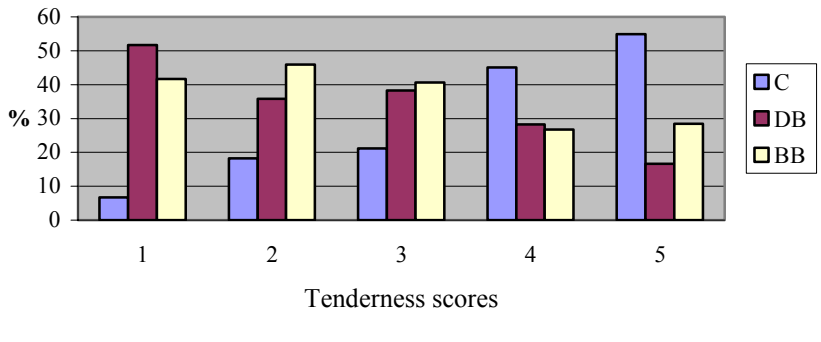
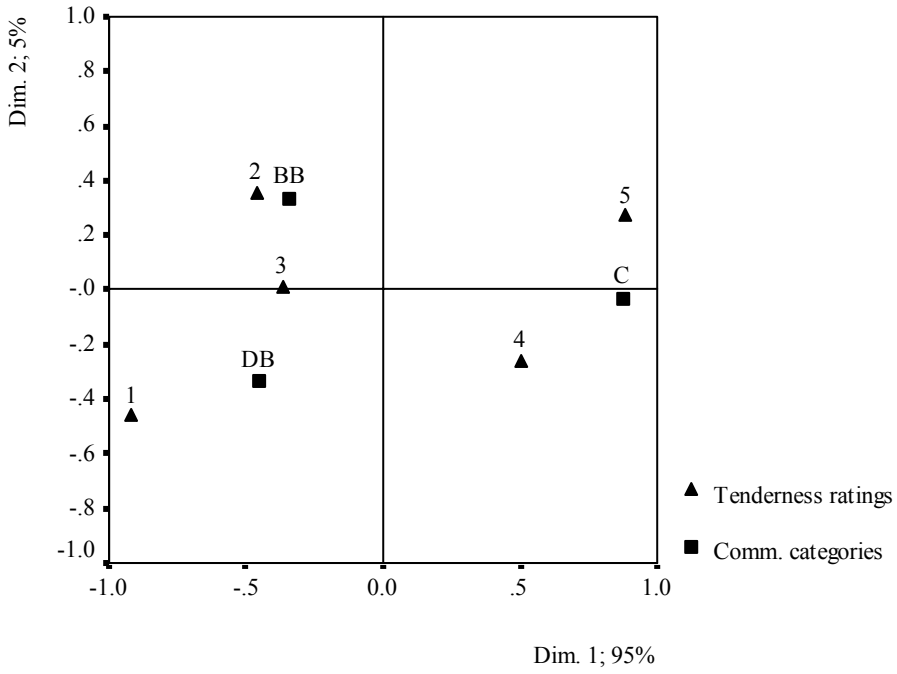


Figure 2: Plot of commercial categories and tenderness ratings



PHYSICAL CHARACTERISTICS OF *LONGISSIMUS DORSI* MUSCLE IN BOVINES FROM DIFFERENT GENETIC TYPES REARED IN SICILY (ITALY)

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Background

The marketing of meat in industrialised countries is conditioned more and more by the demand of quality on the part of the consumer, today, who are careful about a series of problems which, besides the aspects of the intrinsic surety of the food, finish up by involving all a togetherness of other aspects of the productive system. In the specific case of meat, it is known that the organoleptic qualities, i.e. smell, taste, colour, juiciness, texture and tenderness represent the principal factors able to condition drastically the choices of the consumers at the moment of purchase (Maltin *et al*., 2003; Sgoifo Rossi *et al*., 1999) therefore, the physical characteristics of the meat has a role of primary importance regarding the qualification of the product (Destefanis, 1988). The physical characteristics of the meat are influenced by different factors such as: age, sex, race, system of breeding, methodologies of transport, slaughtering and ripeness (Villaroel *et al.*,2002). In the light of these observations and given the importance and the vastness of the subject, the Agriculture and Forest Regional Assessorship of the Sicilian Region, with the project: Quality and traceability of the Sicilian beef meat, co-ordinated and carried out by the Consorzio di Ricerca Filiera Carni (CoRFilCarni), has in progress the realisation of a detailed map of the quality of the meat produced in Sicily, so as to individuate the eventual critical points and therefore to set up the adequate strategies of intervention.

Objectives

The specific objective of this part of research is to study the physical characteristics of the beef meat produced in Sicily, derived from different genetic types kept in ordinary breeding conditions.

Materials and methods

The research was carried out on 200 male yearlings, weaned in a natural way, belonging to the following genetic types : 20 Belgian Blu (BBL) x half-bred (MTT); 26 Charolaise (CHL) x half-bred (MTT); 45 Limousine (LMS) x half-bred (MTT); 29 half-bred (MTT); 20 Charolaise (CHL); 20 Limousine (LMS); 20 Charolaise (CHL) x Belgian Blu (BBL); 20 Limousine (LMS) x Belgian Blu (BBL).
The animals, up to age of 8±1 months were kept in extensive conditions with their mothers and they utilised the available pasture for feeding. Only 6±1 months before slaughter, at a body weight of 300±20 kg, they were bred in intensive conditions, in boxes provided with external paddocks, and fed with 1.3 kg/100 kg of body weight and with wheat straw *ad libitum*. The chemical composition of the feeding (table 1) was determined according to the A.O.A.C. (2000) official methods.
All the subjects at the age of 14-16 months, at a homogeneous fattening condition, were slaughtered after fasting for 12 hours, but not from water. On each half carcass, 45' (pH_1) and 24h (pH_u) after slaughter pH value of the *Longissimus dorsi* muscle was measured with pH meter (WTW).
A sample cut of meat carrying out of two perpendicular sections at the vertebral column in correspondence with the cranial margin of the X (T10) and the XI (T11) rib (Hankins & Howe, 1946) was taken from the right half carcass of each subject, after 48 hours of refrigeration at a temperature of 4°C and relative humidity of 80%.
After the dissection of the sample cut, a slice 2,5 cm thick of the *Longissimus thoracis* muscle was prepared for the determination of colorimetric parameters (CIE L*: Lightness; a*: redness index; b*: yellowness index) using a spectrometer of imagine Spectral Scanner (DV s.r.l. Tecnologie d'avanguardia-Italia) and successively for the measurement of the Water losses and of the tenderness (Warner Bratzler Shear Force-WBS) using the INSTRON 5542.

Data obtained were subjected to the analysis of variance using the GLM procedure of the SAS (Version 8.1, 2001).

Results and discussion

The results obtained in table 2, pointed out the uniformity of the physical characteristics of the meats of different genetic types; in particular, no statistically significant differences were observed among the parameters investigated.

The values of pH_u observed (Tab. 2) indicate a good progress of the process of acidification (Hoffmann 1994) even for the BBL x MTT, CHL x BBL, LMS x BBL genetic types that are characterised by a greater muscular hypertrophy and therefore more sensible to stress (Schakelford *et al.,* 1994; Destefanis *et al*., 1993).

The L* and a* parameters show a good lightness and red index (Lizaso *et al*. 1996). This could be due to a greater concentration of myoglobin at a muscular level for the breeding system which determines a greater physical activity of the animals.

The Water losses (Tab. 2) show normal values according to Bultot *et al*. (2002) and Offer and Knight, (1988).

The values of WBS (Tab. 2) permit us to classify the meat of these genetic types as tender showing values less than 4.54 kgf/cm2 (Shackelford *et al*., 1991).

Table 1. Chemical composition of feeding

	Concentrate	*Wheat straw*
Dry matter (%)	88.84	91.01
In DM (%)		
Crude Protein	17.97	2.81
Ether Extract	3.10	1.58
Non Structural Carbohydrates	53.43	10.40
Ash	5.29	7.19
Neutral Detergent Fibre	22.21	78.02
Acid Detergent Fibre	7.04	48.71
Acid Detergent Lignin	0.84	4.74

Table 2. Physical characteristics of *Longissimus dorsi* muscle (mean ± SE)

Genetic type	pH_1	pH_u	*L**	*a**	*b**	*Water losses (%)*	*WBS (kgf/cm²)*
BBL x MTT	6.65±0.08	5.86±0.10	42.0±0.8	25.8±0.8	8.8±0.7	27.1±0.8	3.9±0.4
CHL x MTT	6.56±0.10	5.62±0.09	42.9±0.6	25.7±0.6	8.3±0.5	26.5±0.6	4.3±0.3
LMS x MTT	6.50±0.09	5.60±0.11	42.4±0.4	24.3±0.4	8.4±0.4	26.8±0.4	4.1±0.2
MTT	6.62±0.11	5.61±0.07	42.6±0.5	24.2±0.5	7.8±0.5	26.4±0.5	3.8±0.2
CHL	6.48±0.06	5.54±0.06	44.0±0.8	23.4±0.8	8.3±0.7	25.7±0.8	3.6±0.4
LMS	6.61±0.09	5.59±0.08	42.9±1.1	22.7±1.1	7.6±0.9	26.4±1.0	3.9±0.5
CHL x BBL	6.62±0.11	5.74±0.10	42.0±0.8	23.6±0.9	7.3±0.7	27.7±0.8	3.9±0.4
LMS x BBL	6.51±0.09	5.77±0.09	42.0±0.9	24.9±0.9	8.3±0.8	26.1±0.9	3.8±0.4
P	*0.58*	*0.46*	*0.39*	*0.08*	*0.06*	*0.42*	*0.82*

Conclusions

Following the guidelines of a system for the qualifications of the zootechnical productions (CoRFilCarni), this preliminary study has pointed out that, the employment of bulls for meat (BBL, CHL, LMS) in the cross-breeding with Sicilian half-bred (MTT) has produced meat characterised by good physical characteristics, comparable to those of the best races specialised for meat.

Acknowledgements

Research financed by Agriculture and Forest Assessorship of the Sicilian Region.

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SENSORY EVALUATION OF BEEF LOIN STEAKS STORED IN DIFFERENT ATMOSPHERES

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Background

Case-ready fresh meat packaging is a fast growing segment (Zilbermann, 2003), and it is therefore important to optimise the packaging method to ensure a good eating quality including a long shelf life. In Denmark, most centrally wrapped consumer unit packages of fresh beef are Modified Atmosphere Packed (MAP; usually in 70-80% O_2 and 20-30% CO_2) or vacuum-skin packed (VSP). Although oxygen maintains the desirable red colour of the fresh meat it also promotes oxidation of lipids and may therefore be detrimental for the taste. A few studies have demonstrated that sensory properties decrease during MAP storage (Jayasingh, 2002; Toerngren, 2003). Furthermore, a few studies have demonstrated that beef stored in high oxygen atmosphere develops a well-done appearance at temperature much below than expected (Hunt et al., 1999; Toerngren, 2003).

Objectives

The purpose of the present study was to examine sensory quality of beef loin steaks packed with 6 different packing systems.

Whole cuts (longissimus dorsi) were 1) aged in vacuum, sliced to steaks and re-packed to 3 different atmospheres with different oxygen content, and 2) beef loin steaks were aged in 3 different atmospheres - O_2/CO_2, N_2 and under vacuum.

Materials and Methods

Source of meat: Six animals (cows and heifers) of Danish Friesian, Red Danish and Crossbreds, approx. 2-4½ years old, carcass weight (263-304 kg), were slaughtered at a Danish slaughterhouse, low voltage electrically stimulated and chilled so that no part of the carcass reached <10°C in the course of 12 hours after stunning. The carcasses were selected according to pH (5.5-5.6) and stored at 2°C for 3 days before boning. *Longissimus dorsi* (LD) was then excised from each carcass half and separated for different packaging systems, Table 1.

Table 1. *Packing systems and cut used during ageing and display*

Ageing conditions (2°C)			Display conditions, steaks (2°C)			Abbreviation
Cut	*Atmosphere*	*Days*	*Repacked*	*Atmosphere*	*Days*	
loin	Vacuum	19*	Yes	Air	2	Vac + Air
loin	Vacuum	14*	Yes	50% O_2/ 50% CO_2	6	Vac + 50%O_2
loin	Vacuum	14*	Yes	80% O_2/ 20% CO_2	6	Vac + 80%O_2
steaks	100% N_2	19*	Only top film	Air	2	N_2 + Air
steaks	50% O_2/ 50% CO_2		No		21*	50%O_2
steaks	Vacuum-skin pack		No		20*	VSP

* From packaging. Meat was packed 3 days after slaughter
Air capacity: approx. 79% N_2, approx. 21% O_2

Packaging and storage conditions: LD muscles (left and right side) were cut into 3 parts and randomised with respect to packing systems. LD was cut into steaks (2 cm thick) after boning or after aging. Storage temperature was 2 °C from boning to analysis. All steaks were moved to light surroundings (1,110-1,249 lux) for 2 days prior to the analysis (22-24 days after slaughter).

Vacuum bags for loins were made of transparent plastic with max. O_2 permeability: 4 $cm^3/m^2/d$, bar, (Bemis, Packagervej, Denmark APS). Vacuum: 5-10 mbar vacuum.

The *vacuum-skin packing (VSP)* consists of an upper co-extruded film with barrier properties and a bottom semi-rigid film usually in the form of a tray that maintains the original form of the product: Max. O_2 permeability (upper film and bottom): 2 cm^3/m^2/ x d x bar (Cryovac, Sealed Air Corporation). Vacuum: 5-10 mbar (Multicvac packing machine).

MAP (O_2): Tray (13x18x4 cm) covered with transparent film, O_2 permeability: 10 cm^3/m^2/d, bar (Cryovac. Sealed Air Corporation). Flowpack, BDF, Fuji.

MAP (N_2): Tray (12x16x3,5) in transparent bags (23x30 cm, NEN 40 HOB/LLDPE 75, AMCOR, oxygen permeability: 0,45 cm^3/m^2/ d, bar). One O_2 scavenger was placed in each pack (AGELESS, Mitsubishi Gas Chemical Company, INC). Multivac A300/16 packing machine (5 mbar vacuum, 750 mbar filling). Two days before the analysis, the bags were removed and the steaks exposed to air through O_2 permeable film.

Traditionally wrapped (Air): Tray (12x16x3.5) and transparent high O_2 permeable film (PE).

Atmosphere content was measured at time of packing and before analysis for MAP packed steaks (Check Mate 9900, BPI Dansensor)

Vacuum packed loin

MAP steak

VSP steak

Cooking and sensory evaluation: Steaks were equilibrated after storage at room temperature (approx. 20°C) to an internal temperature of max. 15°C prior to cooking on a preheated frying pan (155°C), turned every 2 minutes until an internal temperature of 62±1°C had been reached. Steaks were cut and served in pieces of 2½ x 3 cm. Samples were evaluated by 8 trained assessors using a 15-point non-structured line (0=slight and 15= intense). The attributes were tenderness, juiciness, Warmed Over Flavour (WOF), meat-flavour and doneness (internal colour).

Statistic: Data were analysed in an analysis of variance model (mixed procedure, SAS version 8.2). Fixed effect in the model were main effects.
Sensory data: $Y_{ijk} = \mu$ + packing systems $_i$ (fixed) + animal $_j$ (random) + assessors $_k$ (random) + e $_{ijk}$

Results and discussion
Results of the sensory evaluations are shown in table 2.

Tenderness: There were significant differences between packing systems ($P<0.0001$). Steaks aged and displayed in MAP (50% O_2/50% CO_2) gained the lowest score for tenderness: 6.5 point, which is 3 points lower than the traditionally aged and displayed steaks (vac+air: 9.5 point). Only steaks aged in N_2 (display packed in air) gained the same high levels of tenderness as the traditionally packing system. These results are in agreement with Jayasingh et al. (2002; ground beef) and Toerngren (2003; beef steak) who also found that MA-packing with O_2 and CO_2 decreased tenderness. It is expected that O_2 is responsible for the reduced tenderness in MAP, since Toerngren did not find that ageing in 50% CO_2/50% N_2 decreased tenderness. The

explanation for the negative effect of O_2 on tenderness could be protein oxidation. Rowe et al. 2004 found that increased oxidation of muscle proteins early postmortem could have negative effects on meat tenderness.

Beef steaks aged in vacuum-skin pack (VSP) were less tender than beef steaks aged in N_2. In an earlier study (Clausen et al. 2003) we also found that VSP steaks were less tender than the corresponding meat vacuum aged as a whole cut (app. 2 kg). The difference was significant at 4 and 11 days after packaging (meat was packed 3 days after slaughter). Vázquez et al. (2004) also found that the meat tenderisation process was slowed down in VSP beef steaks compared to traditional vacuum packed beef steaks.

Juiciness: There were significant differences between packing systems ($P<0.0001$). Beef steaks exposed to O_2 in higher concentration than normal air were less juicy. The reduced juiciness is probably caused by O_2 since Toerngren (2003) found that ageing in 50% CO_2/50% N_2 did not reduce juiciness compared to traditional packaging (vacuum aged and packed in air for two days). Jayasingh (2002) also found ground meat packed in 80% O_2/20% CO_2 tasting less juicy than the control.

Warmed Over Flavour (WOF): WOF varied between packing systems ($P<0.0001$). The highest degree of WOF was found in steaks aged and display packed in 50% O_2 for 20 days (8.4 point). Also steaks display packed in O_2 for 6 days scored high in WOF (50% O_2: 5.9 point 80% O_2: 5.7 point). As seen from table 2, development of WOF varied from 1.9 to 12.3 between animals (50% O_2 for 20 days). WOF is primary known from stored and reheated cooked meat and is caused by oxidation of fatty acid (Konopka and Grosch, 1991). However, in this experiment it appears that the oxidation already occurred in the raw meat stored in MAP with high oxygen content. Jakobsen and Bertelsen (2000) and Jayasingh (2002) also found increased lipid oxidation during storing in O_2 atmosphere. The degree of WOF probably depends of antioxidant content like tocopherol in fresh meat. Consumers sensitive to WOF will probably dislike O_2-MAP meat with high WOF.

Meat flavour: Meat flavour differed between packing systems ($P<0.0001$). Once again, steaks packed in MAP with O_2 deviated from the others and with less meat flavour, too.

Colour: Visual internal colour of the steaks (cooked to 62°C±1°C) varied between packing systems ($P<0.0001$). Steaks stored in MAP with O_2 for 6 or 20 days appeared well done (high score), and the VSP steaks scored the lowest points and had the most reddish-pink color. Hunt et al. (1999) demonstrated that patties containing the myoglobin forms oxymyoglobin and metmyoglobin turned brown at 55°C. These results indicate that oxygen penetrates beef steaks and oxidizes deoxymyoglobin in 6 days. Toerngren (2003) found that loin steaks looked nearly well done after two days' storage in MAP with 80% O_2 and well done after 18 days. MA-packed steaks with 80% O_2 were less brown than beef packed with 50% O_2. The explanation could be that CO_2 helps O_2 to penetrate into the meat as CO_2 is dissolved in meat (Jakobsen & Bertelsen 2002).

Conclusions

Beef steaks packed and stored in modified atmosphere containing O_2 (50 or 80%) for 6 and 20 days at 2°C were less tender and juicy, had less meat flavour and more warmed over flavour than steaks exposed to normal air for two days. Furthermore, steaks exposed to O_2 (50 or 80%) looked well done, even if they were cooked to an internal temperature of only 62°C.

After 20 days' of ageing, vacuum-skin packed beef steaks were less tender than steaks aged in N_2.

Table 2. Mean (incl. min. and max.) sensory score (8 trained assessors using a non-structured line scale, anchored to the extremes; 0= slight, 15=intense) of beef loin steaks stored in 6 different packaging systems (see table 1) (n=6).

	Tenderness	Juiciness	Warmed Over Flavour	Meat - flavour	Internal Colour Doneness
Vac + Air (Traditional)	9.5^{a} 6..8-12..0	10.6^{a} 9.2-11.2	1.4^{c} 0.6-3.3	8.8^{a} 7.4-10.6	7.1^{c} 6.5-7.4
Vac + 50% O_2	7.8^{b} 3..3-10..5	7.9^{c} 6.7-9.4	5.9^{b} 3.1-10.5	6.8^{b} 4.1-8.4	12.3^{a} 11.4-13.0
Vac + 80% O_2	8.4^{b} 4..5-10..4	9.6^{b} 8.1-10.7	5.7^{b} 2.2-10.4	6.8^{b} 4.4-9.2	11.0^{b} 8.3-12.8
N_2 + Air	9.6^{a} 5..2-12..9	10.8^{a} 8.9-11.9	0.7^{c} 0.1-1.4	9.3^{a} 7.9-10.9	5.9^{d} 4.2-7.7
50% O_2	6.5^{c} 3.6-9.7	8.8^{b} 6.9-11.2	8.4^{a} 1.9-12.3	5.3^{c} 3.7-8.4	12.4^{a} 10.7-13.2
VSP	8.5^{b} 5.9-12.1	11.2^{a} 9.0-12.0	0.5^{c} 0.1-1.4	9.0^{a} 7.4-10.1	3.9^{e} 2.7-6.1
Significance	***	***	***	***	***

Different letters in same row are significantly different ($P<0.05$)

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VOLATILE CHARACTERIZATION OF BLOOD SAUSAGE "MORCILLA" DE BURGOS

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Background

"*Morcilla de Burgos*" is a popular cooked blood sausage (black pudding) produced in the region of Burgos, in the north of Spain. It consists of a mixture of chopped onion, rice, animal fat, blood, salt and different spices such as black pepper, paprika and cumin. According to a general scheme, onion and frozen fat are chopped at low temperature; also rice (raw or pre-cooked according to local procedures), salt, spices and blood are added and mixed. Then, the mixture is stuffed in natural casings of around 35-45 mm of diameter, which have been preserved with salt and are rinsed in clean water prior to use. The product is boiled for around 1 h at 94-95 ºC, air cooled to 8-10 ºC and finally store chilled at 4 ºC. This product is commercialised in local markets as a fresh product with a shelf-life of around 8-10 days.

Previous studies have been made about composition, physic-chemical and sensory characterization of this product. Depending on the amount of onion (between 15 and 70%) and the mixture of spices used, it is possible to distinguish between three different types of *morcilla*: type I, around 15% of onion and little spiced; type II elaborated with around 40% of onion and high spiced, and type III with an amount of onion between 45 to 70% and half spiced. These three types of *morcilla* are distributed in different geographic zones of Burgos, type I in the North, type II in the South and type III in the centre (Santos 2001, Santos et al., 2003). The presence of onion could be an important and characteristic feature in the flavour profile of *morcilla de Burgos*, together with the spices mixture that can be the key point to distinguish this type of blood sausage from others made in other places.

Objectives

The purpose of this study was to determinate the volatile profile of the blood sausage *morcilla de Burgos* by Solid Phase Micro Extraction (SPME) and GC-MS and determinate the influence of the percentage of onion in the total volatile profile.

Materials and methods

Sample preparation: Seven blood sausages of different manufacturers, four of them belonging to *morcilla* type III, 2 from the type III, and 1 from the type I were used in this study. All samples were vacuum packaged and were purchased from different local supermarkets. Each analysis was performed twice for every producer and each blood sausage was analysed for duplicate.

After peeling the sausages, they were homogenised in a lab blender, and 2 g of this homogenised were introduced in a headspace vial and heated at 70ºC (optimal consumption temperature) in a water bath for 5 min and allowed to equilibrate for 10 min with a 2 cm x 50/30 µm divinylbenzene/carboxen/polidimethylsiloxane (DVB/Carboxen/PDMS) Stable-Flex SPME fibre (Supelco. Bellefonte, USA). The fiber was conditioned prior to sampling at 270 ºC for 1 h. The SPME fibre was then injected into the GC injection port, and thermally desorbed in the GC injection for five minutes.

GC-MS Conditions: Gas chromatographic analyses were performed with an Agilent Technologist 6890N Series GC System (Agilent Technologist, Palo Alto. CA. USA) coupled to a Waters Autospec mass spectrometer (Waters. Milford, USA). The SPME fiber was injected into the GC injection port, and thermally desorbed at 150ºC. Compounds were separated on 30 m length x 0.25 mm I.D fused silica capillary column coated with 0.25 µm film thickness of silphenylene polysiloxane (Quadrex Corporation. New Haven. USA). The temperature of the column was programmed starting at 30 ºC after injection for 5 min, afterwards temperature was increased at a rate of 3 ºC/min from 30 to 120 ºC and held there for 5 min. Helium gas with a flow of 0.9 L/min was used as the GC carrier gas. During desorption of the SPME fibre the injector split valve was closed. The effluent from the capillary column went directly into the mass spectrometer, operated in the electron impact (EI) mode with an ionisation voltage of 70 eV.

Compounds were identified by comparing their mass spectra with a NIST spectrum library. These compounds were related to the different ingredients used in the elaboration of *morcilla* de Burgos, by

comparing them with previous published spectra obtained from different *Allium cepa* L. varieties, and spices (see references on table 1), and also from samples analysed, in the same conditions mentioned above, and belonging to the same onion variety and the same spices used for the elaboration of the product taken from one of the producers factory (data not shown).

Results and discussion

The results obtained show 60 different volatile compounds found in *morcilla de Burgos*, taking into account all the compounds that appear in all different samples analysed. Forty six of these compounds have been tentatively identified using the NIST library, and they are listed according to their appearance time in Table 1. As it was expected, all seven samples of *morcilla* presented a different volatile profile due to the particular way each producer elaborates their product, although all samples belonging to the same geographical area show more similarities in their profiles than to the others. In table 1 is also shown that *morcilla* type III has the higher number of volatile compounds (33), followed by *morcilla* type II with 25 and finally *morcilla* type I with 22. Only 10 of the compounds found were common to all three types of *morcilla*, four of them have been related to the presence of onion (2-methyl 2-pentenal, dipropyl disulphide, 1-propenyl propyl disulphide and dipropyl trisulphide), 3 could be related with black pepper (β-myrcene, 3Δ carene and δ limonene), 2 with oregano (see table 1) and one has not been associated to any ingredient. Dealing with the number of compounds becoming from onion, it has been shown that their number increase with the percentage of onion in *morcilla* composition. In that sense, eleven compounds were found in *morcilla* type III, which have a percentage of onion between 50 and 70%, 6 of these compounds appear in blood sausages belonging to type II, and finally only 4 of these compounds are recognised in *morcilla* type I, the one with a low percentage of onion in their composition (around 15%). In general, the most abundant compounds are those related with some spices, as black pepper (δ limonene), oregano and so on, and those associated with onion, such as dipropyl disulphide, which is the more abundant in some *morcilla* type III, as it is shown in Figure 1. However, it is remarkable that only few volatile compounds are related to rice and fat, and none have been identified related to cooked-blood. This feature can be due to the high intensity and high number of compounds associated with spices and onion, that can mask other volatiles, as well as the extraction method and column used in the present work, which is very specific to detect sulphured compounds.

Besides these general features, it is possible to see that some specific and characteristic compounds appear in the three different *morcilla* types that seem to agree with the different spices mixture used in their manufacture. In *morcilla* type I apart from some compounds, related to oregano and black pepper, appears the volatile, 1,3,8 p-menthatriene, which is characteristic from parsley (Belitz and Grosch, 1999), this spice is typically used in the north zone of Burgos. Moreover, in this type of *morcilla* appears decanal, 2-propanol 1-butoxi, and hexanal that could become from rice (see table 1), which is more abundant than in blood sausages elaborated in the other geographic zones. In *morcilla* type II it has been identified the compound cuminaldehyde, which is the more abundant substance in the chromatograms and it is related to cumin that it is used in abundance in that southern zone of Burgos. Finally, *morcilla* type III present some compounds such as 2-butenal 2-methyl, 1,3-butanediol or benzene 1,2-dimethyl that several authors had associated to paprika (Mateo et al., 1997; Guadayol et al., 1997). Together with these compounds, the presence of volatile compounds derivate from onion is also characteristic of *morcilla* belonging to this zone, as it has been mentioned above.

All these results agree with those obtained by Santos et al. (2003), where a trained sensory panel characterised *morcilla* type I by the blood flavour, black pepper predominant taste and by the abundance of rice, *morcilla* type II where characterised by the strong spicy taste, mainly by the addition of cumin and other spices, together with a background flavour and taste of onion, and *morcilla* type III by their strong onion flavour, with some burn notes due to paprika.

Conclusions

According to the results obtained in this study, it can be conclude that *morcilla de Burgos* has a volatile profile characterized by the presence of flavour notes becoming from onion, black pepper and oregano, as common feature of all different *morcilla* types of the region, although each producer present a slightly different volatile profile according to the kind of ingredients and percentages of raw material employed. Volatile onion compounds are mainly sulphur derivates, and they are more abundant in *morcilla* type III (11 compounds found), followed by *morcilla* type II (6), and finally by *morcilla* type I (4), in accordance with the percentage of onion used for elaborating the blood sausage in the different zones of production described.

It is also possible distinguish the *morcilla* of each zone by some characteristic spices used. In that sense, *morcilla* type I is not so spicy and only it appears a slightly note of parsley. In *morcilla* II the characteristic spicy among others used to be cumin, and in *morcilla* type III paprika.
More studies should be done to obtain more evidence to this preliminary study and to compare the volatile profile of *morcilla de Burgos* with other blood sausages elaborated in Spain and other countries.

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Acknowledgements

We want to express our thanks to *Excma. Diputación Povincial de Burgos* for supporting with a grant this project. We also want to express to gratitude to the Junta de Castilla y León for supporting this research project.

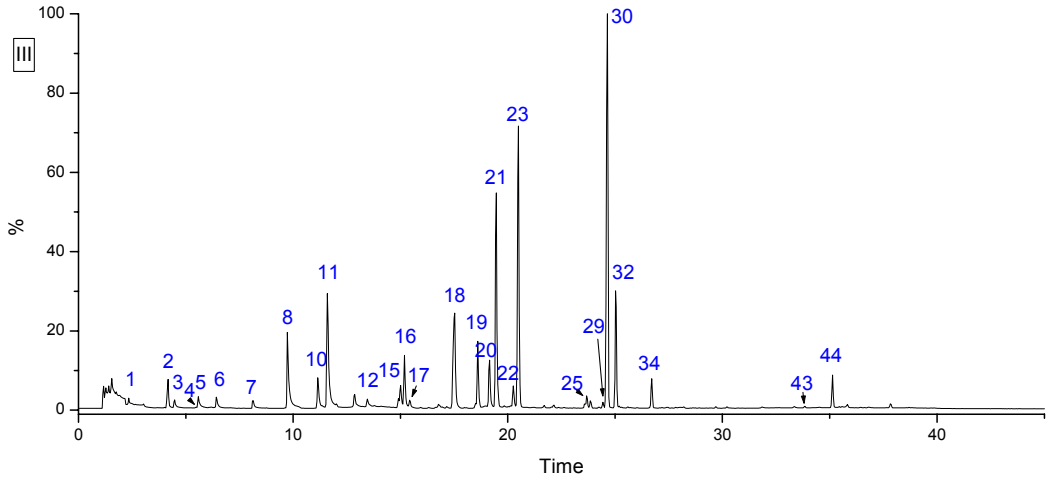

Figure 1. Chromatogram of volatile compounds of one sample of *morcilla de Burgos* type III.

Table 1. Volatile compounds identified in the different types of *morcilla*

Nº	COMPOUND	I	II	III	SOURCE	REFERENCE
1	Propanethiol			X	onion	Järvenpää, 1998
2	Furan 2,4dimethyl			X	onion	*
3	Acetic acid, anhydride with formic acid		X	X	paprika	*
4	Dimethyl disulfide			X	onion	Järvenpää, 1998
5	2 butenal 2 methyl			X	paprika	*
6	methylbenzene			X	rice	Mahatheeranont, 2001
7	hexanal	X		X	paprika/fat/rice	Mahatheeranont, 2001
8	2-methyl-2-pentenal	X	X	X	onion	Järvenpää, 1998
9	1,3 butanediol			X	paprika	Mateo, 1997
10	ethylbenzene			X	rice	Mahatheeranont, 2001
11	Benzene 1,2 dimethyl			X	paprika/rice	Guadayol, 1997
12	3,4 dimethyl thiophene			X	onion	Järvenpää, 1998
13	2 propanol 1 butoxi	X			Rice	Mahatheeranont, 2001
14	bicyclo(3.1.0)hex-2-ene-2-methyl-5(1-methylethyl)	X	X		oregano	*
15	Methyl isopropyl disulphide		X	X	onion	*
16	α pinene	X	X		black pepper	Belitz-Grosch, 1999
17	methyl 1 propenyl disulphide			X	onion	Järvenpää, 1998
18	bicyclo(3,1,1)heptane-6,6dimethyl-2-methylene	X	X	X	oregano	*
19	β myrcene	X	X	X	black pepper	Belitz-Grosch, 1999
20	α phellandrene	X	X	X	spices	*
21	3 Δ carene	X	X	X	black pepper	Belitz-Grosch, 1999
22	benzene 1 methyl 2 (1methylethyl)	X	X	X	oregano	*
23	δ limonene	X	X	X	black pepper	Belitz-Grosch, 1999
24	1,3,6-octatriene 3,7-dimethyl	X			spices	*
25	4-Carene		X		spices	*
26	cyclohexadiene 1 methyl 4(1methylethyl)	X			oregano	*
27	cyclohexene 1 methyl 4(1methylethylidene)	X			oregano	*
28	1-Terpinen-4-ol			X	oregano	Dudai, 2003
29	Linalool	X		X	spices	*
30	Dipropyl disulphide	X	X	X	onion	Järvenpää, 1998
31	1,3,8 p-menthatriene	X			parsley	Belitz-Grosch, 1999
32	1-propenyl propyl disulphide	X	X	X	onion	Järvenpää, 1998
33	cyclohexen-1ol-1-methyl-4(1-methylethyl)			X	oregano	*
34	Methyl propyl disulphide		X	X	onion	*
35	3 cyclohexen-1ol 4-metil-1(1-methylethyl)	X	X		oregano	*
36	cyclopentan-1-al 4-isopropylidene 2-methyl		X		spices	*
37	Decanal	X			rice	Mahatheeranont, 2001
38	Cuminaldehyde		X		cumin	Aldrich, 2000
39	benzene 1-metoxi 4-methyl 2-(1-methylethyl)			X	oregano	*
40	linalyl butyrate			X	oregano	*
41	1,3cyclohexadien 1-carboxaldehide 2,6,6-trimethyl		X		spices	*
42	α Thujenal		X		spices	*
43	Carvacrol			X	oregano	Belitz-Grosch, 1999
44	Dipropyl trisulphide	X	X	X	Onion	Järvenpää, 1998
45	cyclohexen 4-etenyl 4-methyl 3-(1-methyletenyl) 1-(1-methylethyl)		X		spices	*
46	Copaene	X			spices	*

I, II, III: m*orcilla* types; *: our own data;

FACTORS AFFECTING THE WATER CHANGES IN MEAT DURING TENDERISATION

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Background

Kristensen and Purslow (2001) showed that the water-holding of pork gradually decreased immediately post *rigor mortis* during ageing followed by an increase as ageing continued. They interpreted this as being due to degradation of the cytoskeletal proteins. They suggested that when the cytoskeletal proteins degrade, free water appears to be lost more readily during the first part of post mortem storage after which the water holding capacity increases to levels one day post mortem. It is not clear whether the water is directly associated with the cytoskeletal proteins or degradation of the protein assists with water appearance over time.

It is possible that the increase in water arises from connective tissue squeezing water as the structure of the meat is degraded through the breakdown of the cytoskeletal proteins. However an alternative view is that the water involved in the tertiary protein structures is released and this is the source of water rather than that from other compartments within the muscle. The degradative changes in the cytoskeletal proteins over time (ageing) results in increased drip and such changes are also likely to be those associated with meat tenderisation. In this study we explore the changes in drip and bound water associated with shear force changes as the meat ages.

It is now generally regarded that changes in the proteins holding the meat together termed structural or cytoskeletal proteins are responsible for shear force changes as the meat ages or tenderises. These proteins, desmin, titin, nebulin and others are affected by the action of endogenous proteases (calpain) in such a way to affect the tertiary structure altering water binding.

Objectives

To determine the relationships between centrifuge-expressed water, tightly-bound water, ultimate pH_u and meat tenderisation.

Materials and Methods

Animals

Lambs (n=24, approximately 12 months old), were processed in a commercial abattoir. The animals were electrically stunned followed by throat cut to sever carotid arteries and jugular veins and the *m. longissimus thoracis* (LT) from one side between 5th rib (T5) and 6th lumbar vertebra (L6) was removed immediately after stimulation and tightly wrapped by rolling in 4 layers of polythene cling film (11mm, GLAD wrap Clorox, New Zealand Ltd., Auckland New Zealand) and placed in a polythene bag (Devine et al, 2002). The wrapped muscle was chilled initially at 15°C until *rigor mortis* was complete.

Meat ageing

Following *rigor mortis* muscles were cut into four equal pieces along the length of the muscle, beginning at the lumbar end, placed in polythethene bags and held in air at 15°C and aged for 0, 5, 24 and 72hours as described previously (Lowe *et al.*, 2002). The meat samples were frozen at the specified times to stop further ageing. Thermocron iButton, Dallas Semiconductor Corp., Dallas Texas, USA) were placed both in the muscles to ensure the required temperatures were maintained. The ultimate pH (pH_u,) was measured with a Mettler Toledo pH meter with a combination puncture electrode (Mettler Toledo GmbH Process Switzerland) at approximately 24 hours post *rigor mortis*.

Cooking

Meat samples obtained for shear force measurement were stored at -20°C and cooked from the frozen state in an 85°C water bath to an internal temperatures of 75°C and then cooled rapidly in ice. The shear force was determined from six 1 cm x 1 cm samples sheared perpendicular to the orientation of the muscle fibres using an Instron with a tooth-shaped head to conform to the specification of a MIRINZ tenderometer (Graafhuis, *et al*., 1991).

Water binding measurements

At *rigor mortis* (0 ageing) a sample from the middle of the LT sample was weighed (approximately 2 g) and was cut so that the muscle fibres were vertical. The piece was transferred to a plastic centrifugation tube with the same orientation into which were placed 5 mm diameter polycarbonate beads and centrifuged for 15 min at 1800 G (Hettich Zentrifugen Tuttlingen, Germany). The duration of centrifugation was based on the extensive data from Kristensen and Purslow (2001) for pork. The same speed and duration of centrifugation was used throughout all experiments.

The centrifugation loss (free water) was the difference in weight before and after centrifugation. The remaining meat post-centrifugation, was then dried in a 105°C oven for 24 hours and the water loss determined. The water remaining was termed "bound water" for our purposes. The total water lost was the difference from original weight and final dried weight.

The procedure was undertaken at ageing times 0, 5, 24 and 72 hours. This gave shear force values, centrifuge loss (free water), a bound water compartment and total water changes over time.

Curves were fitted using the statistics function in Sigma Plot.

Results and Discussion

There was a large scatter in the results that appeared to be unrelated to measurement procedures and is in line with the observations of Honikel and Hamm (1984) showing that water binding of meat from different animals is highly variable. The pH_u over the range 5.4-6.16 was plotted against centrifuge-expressed water (analogous to free water or drip) at 72 hours (Fig 1 a) indicating that as the pH_u increased the centrifuge-expressed water decreased and this was in part responsible for the variability. The data was sorted into a low pH_u values (5.43-5.55) and normal to intermediate pH_u values (5.56-6.16) giving two sets of curves for each attribute (Fig 1b-1d). The two samples with pH_u values of 5.86 and 6.16 were left in the calculations, and form part of the discussion, although they were at the extreme edge of the relationships (Fig. 1 a) – with these included the significance between the low pH_u and normal pH_u attributes was not altered.

The centrifugation loss (Fig 1b) and total water loss (not shown) increased exponentially, whereas bound water (Fig. 1 d) and shear (Fig. 1 c) decreased exponentially. As the duration of ageing increased, the centrifugation loss increased (Fig 1 b) but changes with regard to the water content past this period were not considered in relation to factors such as re-uptake (Kristensen and Purslow, 2001). Final shear force values were achieved at 72 hours at 15°C (Fig 1 c).

There was a significantly ($p< 0.05$) greater (1.5 times) increase in free water 24 hours for meat of a low pH_u than for the normal to intermediate pH_u meat and a significant two-fold significant increase at 72 hours ($p< 0.05$).

For the shear force the curves for low pH_u meat were significantly more tender than for normal to intermediate pH_u meat at 24 hours and 72 hours ($p< 0.05$) (Fig. 1 c). The absence of a difference at 0 hours is expected as shear values start from the same point whatever the pH_u and in the case of sheep age to similar values but intermediate pH_u meat ages more slowly (Watanabe *et al*., 1995: Watanabe & Devine, 1996).

The bound water retained upon heating to 105°C also decreased over the ageing duration and there was significantly less water bound at 72 hours for the low pH_u group (Fig 1 d) than for the normal to intermediate pH_u group.

The total water (bound plus free) (range 75-90%) released also increased with ageing, with more being released for the low pH_u group.

When the two samples with pH_u values of 5.86 and 6.16 were removed from the calculations the relationships and significance between the low pH_u and normal pH_u attributes did not change.

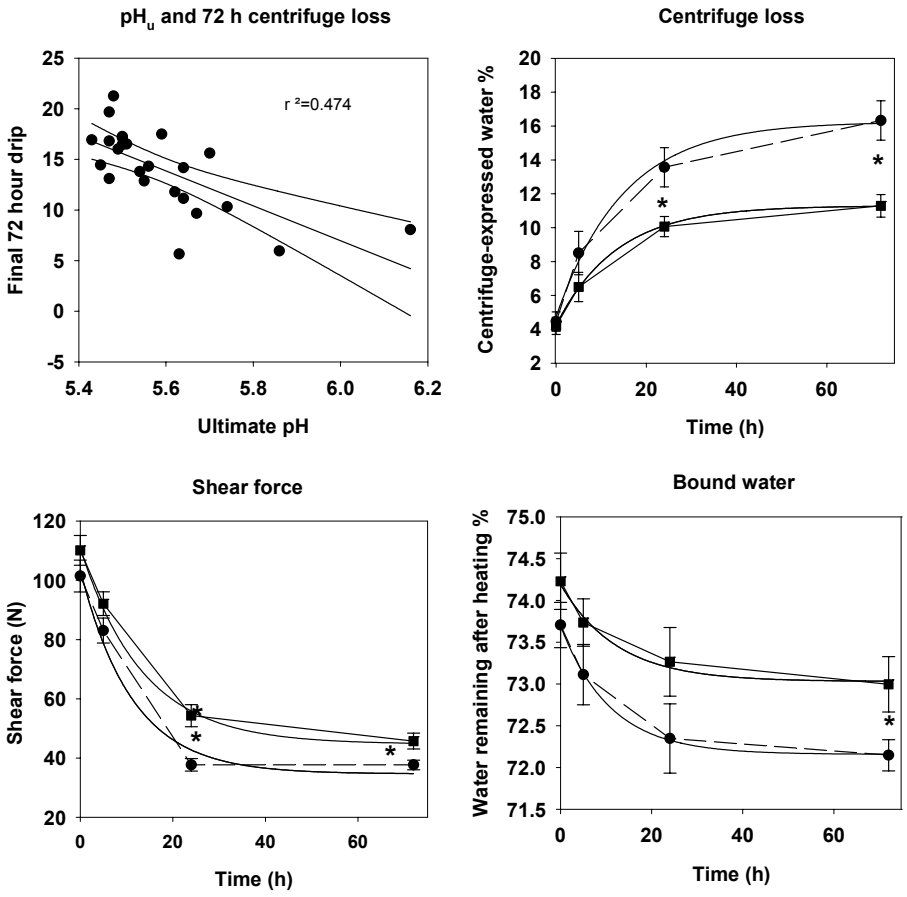

Figure 1. (**a**) The relationship between pH_u.and centrifuge-loss at 72 h ageing. The subsequent graphs are obtained from attributes sorted on the basis of low pH_u (5.43-5.55) (filled circles dashed line) and normal to intermediate pH_u values (5.56-6.16) (filled squares solid lines). (**b**) The increase in centrifuge-loss (free water) over time as the meat ages. (**c**) The reduction in shear force over time. (**d**) The decrease in the water retained in the meat after drying over time as the meat ages (bound water). The * indicates significant difference between the points on the curves at $p< 0.05$). The error bars are ± se and the fitted curves are derived from exponential functions.

The changes over time for free water, bound water and the shear force, are described by fitted exponential curves. It is difficult to establish causative interrelationships because such relationships could be fortuitous. However, the present experiments are consistent with changes in the water components being affected by degradation of cytoskeletal proteins. Free water expressed as drip, naturally increases over time, but the amount produced is also sensitive to various *prerigor* temperature effects (Offer *et al*., 1991) and as the temperatures were similar in this study, the relationship between centrifuge-expressible water and shear force is not unexpected.

The contractile proteins, actin and myosin, are components that are intimately bound to water in their tertiary structure and release of this water is a consequence of changes in interfilament spacing and protein denaturation related to the *prerigor* temperatures, sarcomere length and pH conditions (Honikel *et al.*, 1986; Offer *et al*., 1991). After *rigor mortis* is achieved, the myosin is protected to some extent through the formation of actomyosin (Offer *et al*., 1991), but the consequences persist while the meat ages. The characteristics of water binding by myosin, affected by temperature, pH_u and processing conditions, not only provides the initial drip (analogous but not identical to the centrifuge-expressed water) from meat, but this drip increases over time supplemented from the water associated with the breakdown of cytoskeletal

proteins. The bound water changes are still smaller that the changes that occur in free water. Previous studies by others have only been concerned with the water that comes out as drip rather than water associated with cytoskeletal proteins.

The water released over time includes the contribution from centrifuge-expressed water and bound water and suggests that the water content of meat known as drip is significantly affected by ageing duration. All sources of water are not known at this stage, while some is likely to be water located in the muscle fibres other water is likely to come from components of the extracellular matrix such as hyaluronan (Fraser & Laurent, 1996) that also bind water.

Conclusions

The increase of free water over time is an inevitable consequence of meat tenderisation, with low pH_u meat having more free water, less bound water, greater tenderisation than meat with an increasingly elevated pH_u. The water release during tenderising may be directly related to breakdown of the cytoskeletal proteins.

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PERFORMANCE DURING RETAIL DISPLAY OF BEEF AND BISON STEAKS AFTER STORAGE UNDER VACUUM AND MODIFIED ATMOSPHERE PACKAGING

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Background

The colour of meat is the single most important factor that influences the buying decision of the consumer. Myoglobin, sometimes referred to as dexoymyoblogin (DOMB), is the main pigment of meat and has a purplish red colour. When a freshly cut surface of meat comes in contact with air, DOMB is oxygenated and converted to oxymyoglobin (OMB); this gives beef a bright cherry red appearance and this colour is normally used as an indicator of freshness by the consumer. If only small quantities of oxygen are present, such as in a partial vacuum or a sealed semi-permeable package, DOMB is converted to metmyoglobin (MMB) via oxidation giving the meat a brown appearance. Development of this brown pigment is a serious problem in merchandising meat, because most consumers associate it with a product that has been stored too long. Bison meat is traditionally viewed as having a dark red colour. This colour is not as acceptable to consumers who are conditioned to the bright cherry red colour of beef. The colour of the meat can be controlled, if the factors influencing it are better understood. In the meat industry, vacuum packaging (VP) is employed to maximize the shelf-life of meat, whereas modified atmosphere packaging (MAP), containing high levels of oxygen, is widely used to attain the bright red colour of meat through oxygenation of DOMB.

Objectives

The objective of this study was to assess the influence of injection, packaging and storage conditions on the colour stability of beef and bison steaks during retail display.

Materials and methods

Fresh beef and bison loins (*longissimus lumborum*, LL - 4 each) were procured from local sources. Each LL was divided into two sections. One section was injected with brine containing NaCl and sodium tripolyphosphate (0.5% and 0.3%, respectively in the finished product) to achieve 20% extension by weight, while the other section was kept as a non-injected control. Then, each loin was divided into as many steaks (2.54 cm thick) as possible. These steaks were randomly allocated to storage atmospheres (MAP and VP), storage temperatures (-1 and +4°C) and storage interval subgroups (overnight, 1 and 2 weeks; and 1, 2 and 3 weeks for MAP and VP, respectively). The steaks (n=48) for VP treatment were individually packaged in ethylene/vinyl acetate copolymer polyvinylidene-chloride (PVDC) laminate bags. After making 2 holes through the over-wrap film for free exchange of gases, the steaks (n=48) for MAP were masterpacked in high oxygen using Cryovac B series bags. The headspace was evacuated, filled with a mixture of 70% O_2/30% CO_2 and then sealed. VP and MAP steaks were stored at designated temperatures for set periods. After removal from the main packaging following the designated storage interval, steaks were re-packaged on styrofoam trays over-wrapped with an oxygen permeable film and then placed in a retail display case (under 24 h fluorescent lighting with average light intensity of 975 lx) maintained at 3.0±1°C. Colour (L*, a* and b*) and absorbance was measured using a HunterLab Miniscan XE colorimeter daily for 5 days. Different forms of myoglobin (*i.e.*, OMB, MMB and DOMB) were calculated as described by Hunt *et al.* (1991).

Results and discussion

As is typical of most game animals, bison meat appeared darker than beef, yet was of similar initial pH (5.3). It has a negligible amount of intramuscular fat, which could be a possible reason for its darker appearance. In the present study, bison meat bloomed quickly with a dark red colour, but it tended to loose its brightness readily on storage compared to that of beef. The ability of different muscles to resist MMB formation during aerobic storage varies greatly and depends upon the species in question, anatomical location within the carcass and most importantly variations in the rate of DOMB oxidation and the ability of muscle to consume

oxygen; *i.e.*, muscles with high activities of oxygen-utilizing enzymes, that allow little penetration of oxygen into the tissue, tend to discolour more rapidly.

HunterLab colour values, particularly a* values, are a good indication of the redness of meat: the higher the a* value, the redder the meat. The change in colour of beef and bison steaks was monitored for 5 days. For beef, the decline in a* values was gradual and significant ($p<0.05$) between day 0 and day 5, whereas for bison the steaks held a bright red colour for only the first 2 days. After which, discolouration of bison steaks became evident; most of the steaks had to be discarded before the completion of the retail display study. As expected, steaks stored at the lower temperature (-1°C) held better colour than those stored at the higher temperature (+4°C). Because the maximum storage life of meat is attained at the lowest possible temperature, without freezing the meat (*i.e.*, between -1 and -1.5°C), any increase in this optimum storage temperature will result in a proportional decrease in the storage life (Gill and Shand, 1993). The rate of change in redness or discolouration increases with a rise in storage temperature (Jeremiah and Gibson, 2001). Injection had a beneficial effect on the colour stability of steaks during retail display; however, this positive effect was more pronounced for bison steaks compared to those of beef. Steaks stored overnight under MAP (MAP-OV) prior to retail display maintained the highest a* values (*i.e.*, a brighter red colour) for up to 5 days compared to those stored under vacuum (VP1, VP2 and VP3). Bison steaks stored for 2 weeks under MAP were grossly discoloured by the end of the storage period and were discarded from the retail display study (data not shown).

Figs. 1 and 2 depict the effects of injection treatment, temperature, packaging type and storage in the retail display case on the relative proportions of OMB. Beef steaks maintained significantly ($p<0.05$) higher proportions of OMB compared to bison steaks: darker and less redder bison steaks (*i.e.*, lower a* values) compared to beef ones complemented these results. Injected steaks and steaks stored at the lower temperature (-1°C) had significantly ($p<0.05$) higher OMB levels compared to non-injected steaks and those stored at the higher temperature (+4°C), respectively. Present results clearly show the detrimental effects of storage and display on OMB content: MMB formation increased, while OMB proportions decreased with increasing storage time in the retail display case and corresponded to the changes observed in HunterLab values. The decrease in proportions of OMB for steaks in the retail display case was dependant upon injection treatment and storage temperature, as injected steaks and those stored at -1°C were generally redder and contained more OMB. This clearly demonstrates the beneficial effects of a lower storage temperature and antioxidative properties of phosphates on muscle colour stability. MAP-OV steaks maintained the highest OMB content for up to 5 days during retail display compared to those stored under vacuum (VP1, VP2 and VP3). A continued supply of oxygen in the headspace was responsible for oxygenation of DOMB, hence, the bright red colour. Nevertheless, OMB levels were significantly ($p<0.05$) lower in bison steaks compared to those of beef irrespective of packaging treatments.

Conclusions

Marination had a beneficial effect on the colour stability of steaks during retail display, but this positive effect was more pronounced for bison steaks compared to those of beef. The results obtained from the present study support the view that MAP containing a high oxygen concentration is not suitable for long storage; however, such packaging is well suited for display applications when the time between packaging and display is short. High oxygen-containing modified atmospheres tend to stabilize the oxygenated state of DOMB for relatively short periods, whereas VP is a better option when prolonged storage is required.

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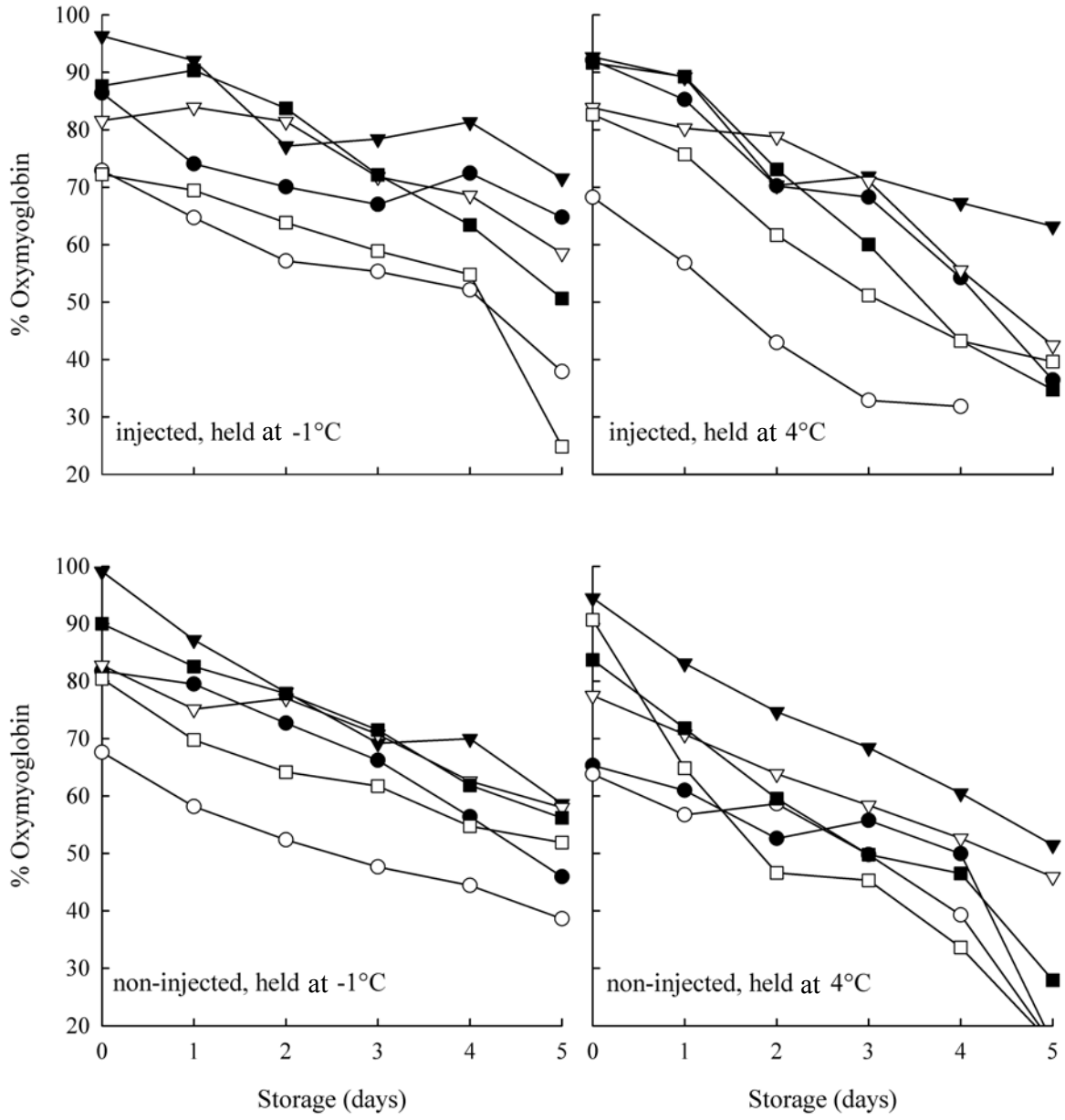

Fig. 1. Changes in surface oxymyoglobin content of beef steaks held under simulated retail display at 3°C following different conditions:

● MAP-1; ○ MAP-2; ▼ MAP-OV; ▽ VP-1; ■ VP-2; □ VP-3.

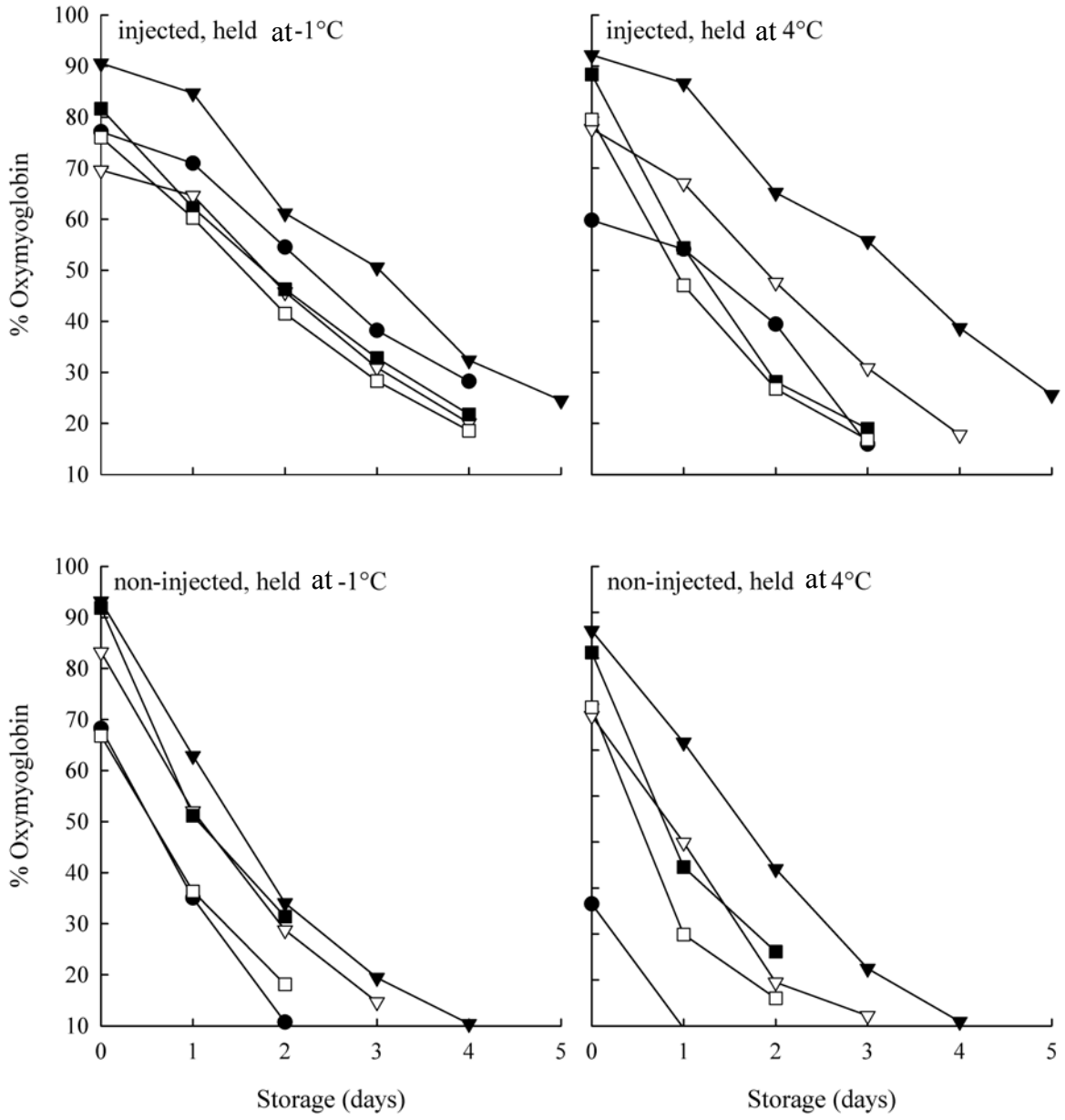

Fig. 2. Changes in surface oxymyoglobin content of bison steaks held under simulated retail display at 3°C following different conditions:

● MAP-1; ▼ MAP-OV; ▽ VP-1; ■ VP-2; □ VP-3.

CONSUMER PREFERENCES FOR LEANNESS IN BEEF OVER THE PAST 50 YEARS - SMITHFIELD REVISITED -

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Background

Beef is losing ground to sales of other meats and it is important therefore to try to determine the origins of this declining consumption. The origins can be found in both the intrinsic qualities of the beef on offer and consumer expectations. Concerns about healthier eating and the recommendation to reduce the consumption of saturated fats from animal origin that may be related to cardio-vascular disease could impact on meat consumption or on the choice of leaner meats. Changing life-styles, with smaller households and less time spent in meal preparation may also influence the type of food chosen with an increase in prepared meals and eating out. The knowledge of the importance of these factors could be crucial in predicting future trends in consumer consumption of beef and in providing a basis for future strategic planning in the beef industry.

Different approaches have been used to determine the intrinsic factors governing consumer choice. Surveys have been conducted on choice in relation to external fat cover and marbling in Canada (Jeremiah, 1981 and 1982) and, more recently by questionnaires, on Belgian consumers' perceptions of beef (Verbeke and Viaene, 1999) and the importance of characteristics of beef as quality indicators in the shop (Glitsch, 2000) in six European countries. These studies all show importance of various beef appearance characteristics, of which fat cover and marbling were included, however they do not investigate the actual preferred levels of fatness in beef.

In 1955, Pomeroy (1956) undertook a survey at the (now) Royal Smithfield Show in England to determine consumer preferences for beef in terms of fatness. Fatty beef rib joints (with 40% of the cut surface visible as fat) were preferred by 63% of consumers. In 1982, Dransfield (1983) repeated the survey using similar joints and observed that the preference had fallen to 24% and that most consumers preferred the leanest rib cut with only 30% visible fat. In 2002, the opportunity arose to restage the study on consumer preferences for fatness thus giving evidence of changes in preferences over half a century.

Objectives

- Determine British consumer preferences according to the level of fatness in beef.
- Determine the changes in consumer preferences over half a century by comparing the results with studies performed in 1982 and 1955.
- Relate consumer choice to socio-demographic categories.

Materials and methods

Both previous surveys were undertaken at the (now) Royal Smithfield Show, a renowned Agricultural show held every 2 years in London. As Dransfield believed in 1982, those attending the show might not statistically represent consumers in the whole UK population, however the opportunity to re-stage the surveys conducted in 1955 (Pomeroy, 1956) and 1982 (Dransfield, 1983) greatly outweighs any disadvantages of a restricted sample of consumers.

The procedures and analyses used here followed as closely as possible those used in 1955 (Pomeroy, 1956) and 1982 (Dransfield, 1983) to facilitate comparison of results. However, unlike the earlier studies, the fatter beef rib joints, with a cut surface of 40 and 48% visible fat, could not be obtained locally at supermarkets, butcheries or abattoirs. As an alternative, photographs of beef rib joints were made, following those published previously (Pomeroy, 1956; Dransfield, 1983). Digital images of these beef photographs were

computer modified to achieve the fatness levels (48, 40, 37 and 30% of the cut surface) and the lean to fat ratios (1.05, 1.36, 1.52 and 2.22, respectively for the given fatness levels) similar to those used in the two previous studies.

Four different beef rib cuts were photographed and each image was computer modified to give the four levels of fatness for each rib giving a total of 16 (4x4) photographs. Four A3 posters were made, each showing four images: the four different ribs of beef, each with a different level of fatness. The order of the level of fatness and the rib was randomly placed in the poster and each rib was randomly assigned a three digit number.

The posters of raw beef ribs were randomly presented to consumers at the Royal Smithfield Show in London, England in November 2002. Each poster was seen by approximately equal numbers of consumers on each of the four days of the show. The consumers were asked to rank the beef samples in order giving their first, second, third and fourth preferences. Place of residence, occupation, age, number of children and marital status were also asked of each consumer.

Significant differences in the ranking of the four fat levels were observed using Friedman test (SAS, 1999). Links between the consumers' first choices and socio-demographic information were determined using χ^2 test (SAS, 1996).

Results

1. Consumers

The survey was completed by 1064 consumers, all over 15 years old, of which the majority (987) were from Great Britain. This compares with 565 in 1955 and 1880 consumers in 1982.

Comparisons of the socio-demographic information about the consumers showed that a lower proportion of consumers from North East, London and Home Counties regions of England participated than had in the two previous studies and might be explained, at least in part, by changes in regional boundaries and the recent importance placed on attracting participation from all regions of the UK. Greater proportions of consumers were observed from Scotland and overseas.

The age distribution of participants was similar to that in 1982 but with a greater proportion of participants under 30 years of age than in 1955. A greater proportion of farmers and a lower proportion of butchers completed the survey in 2002 than in 1955 or in 1982. There were also a greater proportion of participants without children and fewer with 3 or more children than in 1982 (number of children was not available for the 1955 survey).

In the current survey, the proportion of single/divorced/widowed was similar to that of married/co-habiting people and there were almost four times as many men as women. These statistics were not available for the 1955 and 1982 surveys.

2. Consumer preferences

The consumer rankings of the four fatness levels are given in Table 1. The 2002 first choice rankings differed significantly (Friedman's test; $P<0.01$) from each other for all four fatness levels.

The leanest sample (with 30% of the surface as fat) was the most preferred cut, being the first choice for 53% of consumers. This was a slightly lower percentage than that (60%) obtained in 1982. In 1955, this leanest cut was ranked only third in preference.
The second most preferred cut in 2002 (placed first by 31% of consumers) was the second leanest (37% fat), followed by third leanest (40% fat chosen by 13% of consumers). In 1982, the inverse ranking of these two fatness levels was observed.

The biggest shifts in the first choice preferences from 1982 to 2002 were the increase in the percentage of people preferring the 37% fat and the decrease in those preferring the cut with 40% fat. Thus there had been a decrease in preference for fatness at these levels over the past 20 years.

In all three surveys, the fattest (48%) beef cut was the least popular selection, but, even at this low preference, a trend was evident. It was placed as first choice by fewer and fewer people, decreasing from 6 to 4 to 3% of consumers for the 1955, 1982 and 2002 surveys, respectively. The largest change over this half century was a decrease in first choice preference for the rib cuts with 40% fat. The percentage of consumers giving this as their first choice decreased from 63% in 1955 to 24 % in 1982 and to only 13% in 2002 (Table 1).

Table 1. Percentage of consumer ranking their preference for the four fat levels in surveys conducted in 1955, 1982 and 2002

Fat level (% cut surface)	First choice - preferred 1955	1982	2002	2nd choice 2002	3rd choice 2002	4th choice 2002
30	**12**	**60**	**53**	26	13	8
37	**17**	**12**	**31**	47	19	3
40	**63**	**24**	**13**	23	56	8
48	**6**	**4**	**3**	4	12	81

Second and third preferred choices, and by default the least preferred, were only available from 2002 survey (Table 1). In this, the most preferred second choice (for 47% consumers) was the 37% fatness level. The leanest (30% fat) and the 40% fatness level were selected each by a quarter of the consumers as their second choice. The third choice was most often (56% of consumers) the 40% fatness level and the last choice, or least preferred, for 81% of consumers, was the fattest rib (48% fat).

3. Preferences and socio-demographic data

From 1955 to 1982, and similarly from 1982 to 2002, the decrease in the proportions of consumers selecting either of the two fattiest beef cuts as their preferred was similar for all socio-demographic categories. A slightly higher proportion of consumers aged 50 years or over preferred one of the two fattest ribs in both 1955 and 1982 than the other age categories, although this tendency was not observed in the 2002 survey. Indeed there was a lower proportion of those 50 years and over for the fattest (48%) rib than for the other fatness levels. Of those choosing the fattest beef (48% fat), none of them were in the youngest (15-20 year old) age category.

There were few effects of gender on choice, except for the fattest beef which was placed as first choice by 30 people (3% of the whole consumer sample) of whom 28 were men.

Discussion

While fat is the only factor studied here, it is acknowledged that it is not the only factor considered in choosing beef by the consumer or in the purchase decision. However, fat content is, and clearly has been for the last 50 years, an important factor not only for consumer preference but also in purchase.

Considering only the surveys in 1982 and 2002, the proportion of consumers choosing the leanest rib had changed little, but fewer people chose the intermediate level of fatness in 2002. Considering all three surveys, the number of people choosing the two leanest cuts of beef has increased from 29% in 1955 to 72% in 1982 and 84% in 2002. The fattest rib cut (48% visible fat) was the least preferred in all 3 surveys. Although not unusual in 1955, it is unlikely that this high level of fatness in beef would be readily available nowadays.

Choice is likely to include other factors which may involve the consumer in a relatively extended problem solving and intensive evaluation of beef attributes in making the purchase (Verbeke, 2000). When purchasing meat in the UK in 1958 (see review by Jeremiah, 1982), 60% of consumers considered colour of lean, 36% leanness, 28% marbling, 25% quality of fat, 10% bone and 4% firmness. So, at that time, colour appeared to be more important although fatness played an important role. The reasons for this are unclear but

may be due to the variations on offer in the shop and the importance at that time placed on colour as an indicator of freshness and quality. The greater importance of fatness nowadays is supported by consumer studies conducted in different European countries (Glitsch, 2000) in which consumers were asked to rank "quality in the shop". Irish and UK consumers ranked leanness as one of the most helpful characteristics and marbling was one of the second choices. German and Spanish consumers ranked leanness as one of the second most helpful characteristics.

This study concerned only beef but other studies suggest similar tendencies in preference for leaner lamb and pork. However these other studies are punctual, whereas this survey on beef is the first time qualitative studies have been compared over such a long period. As far as we are aware no longitudinal studies have been performed on preferences for different types of meats.

Finally, it is interesting to note that the increasing preference for leaner meat since 1950s is in contrast to the trend of an increase in the proportion of over-weight people in the UK.

Conclusions

- Fatness in beef has been an important characteristic in the consumers' choice for the last half century, but the majority of today's British consumers preferring leaner meat to their predecessors.

- Since the first meat congress (now called The First European Meeting of Meat Research Workers) in 1955, the percentage of British choosing cuts of beef with less than 40% visible fat has increased from 29% to 84% today.

- The percentage of people choosing the leanest cut offered (30% of the surface area) has stabilised but fatter beef was chosen by fewer people than in previous surveys. The preference of UK consumers for lean cuts of beef is likely to continue in the future.

- Modern production methods should take into account this continued trend in consumer preference for lean beef.

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TENDERNESS IN *M. LONGISSIMUS DORSI* FROM COWS – EFFECT OF PELVIC SUSPENSION AND AGEING TIME

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Background

Tenderness is found to be the most important trait when meat is judged from a sensory point of view (Koohmaraie, *et al*., 1998). Meat from older cows is often rejected because it is considered tough. Madsen (1997) found that the age of the cow (2–9 years) did not substantially affect the tenderness in *M. longissimus dorsi* when it was aged for 14 days. Thus, it is of value to study the quality of meat from cows.

Most studies evaluating meat tenderness are done on meat aged for a standardized time, then frozen until heat treatment. However, in the fresh meat marketing of whole steaks, most meat is aged and sold fresh without freezing. Shanks *et al*. (2002) showed that freezing positively affected tenderness compared with meat stored fresh. This indicates the importance of a correct and consistent treatment when meat tenderness is evaluated and implies that results from studies on frozen meat might not be directly applicably to meat marketed/consumed fresh.

An alternative method to enhance tenderness in beef meat is the use of pelvic suspension, instead of Achilles suspension. Pelvic suspension has been reported to improve tenderness in young bulls (Lundesjö *et al*., 2001; Sørheim *et al*., 2001), steers (Hostetler *et al*., 1972) and heifers (Jeremiah *et al*., 1984; Lundesjö *et al*., 2002, Lundesjö Ahnström *et al.*, 2004). Moreover, pelvic suspension was also found to decrease the variation in tenderness. To our knowledge, no studies evaluate the effect of pelvic suspension on meat from cows and it is therefore of interest to evaluate pelvic suspension and different ageing times in cows.

Objectives

The aim of this study was to evaluate the effect of pelvic suspension and ageing time on shear force in fresh *M. longissimus dorsi* from cows. In addition, the effect of freezing on shear force was also studied for meat aged for 7 days.

Materials and methods

Animals. Twelve cows of the Swedish Red and White breed, selected at a commercial slaughter plant, were used. The background of the animals was unknown but only cows in good condition were included in the study. The average slaughter weight and age at slaughter was 315 kg (range 265 – 375) and 68 months (45 – 80), respectively. *M. longissimus dorsi* (LD) from both sides of the 12 carcasses were used.

Slaughter, cutting and preparation of samples. The animals were slaughtered according to the standard routines at the slaughter plant. All carcasses were electrically stimulated, 20 min *p.m*. Left sides were rehung in the pelvic bone, after splitting (app. 45 min *p.m*.), by attaching a rope in ilium. The right sides were used as control, and were kept hung in the Achilles tendon. The carcasses were chilled 30 min in a chilling tunnel (–2°C, –4°C, –2°C), and thereafter in a chilling room (+2-4°C). After 24 h chilling, the carcasses were divided in fore and hindquarter between the 8th and 9th rib from the cranial part. At 48 h *p.m*. LD from both sides were collected, packed in plastic bags and transported to the laboratory. pH was registered to avoid samples with DFD (pH>5.8) (pH-meter WTW pH 340, Germany; electrode Knick SE 104 Germany).

Length and weight of the muscles were registered and each LD was separated into six 10-cm pieces for texture analyses. The pieces were randomized for ageing at 4°C for 2, 4, 7, 14 or 21 days, and also for 7 days followed by freezing at –20°C. All samples were weighed and packed in vacuum bags. Within each carcass, samples were given the same treatment in both left and right side. The sample aged for 2 days was heat treated immediately at the laboratory. A 1-cm piece for analysis of intramuscular fat content (IMF) was

packed in vacuum and stored in –20°C until analysis. A separate 5-cm sample was also packed in vacuum for measurement of pH during storage and kept at 4°C.

Instrumental tenderness. Frozen samples (aged 7 days) were thawed overnight at 4°C. The samples were weighed before further preparation. All samples were kept in a room-tempered water bath for 120 min, before weighing, repacking and heat treatment in a water bath (70°C) during 120 min, until an internal temperature of 69.5°C was reached. Thereafter, the samples were chilled in cold running tap water for 20 min. The samples were stored at 4°C until shear force measurements were performed. Heat-treated samples were room tempered in a water bath for at least 2 h before shear force analysis. Maximal shear force values (WB_{max} in N) was registered on strips (10*10*40 mm) cut parallel with the fiber direction, using a Stable Micro Systems Texture Analyzer HD 100 (Godalning, UK), equipped with a Warner-Bratzler metal blade (1 mm thick; speed 0.83 mm/s) (Honikel, 1998).

IMF, pH and water losses. IMF was analyzed after hydrolysis, using petroleumether for extraction (Soxtec System H^+ equipment, Tecator AB, Höganäs). pH measurement at 2 days *p.m*. was performed when the LD were divided into the different treatments (pH-meter WTW pH 340, Germany; electrode Knick SE 104 Germany). The following pH registrations after 4, 7, 14 and 21 days of ageing were made on the same sample. Water loss was expressed in percentage of initial weight and was calculated as *purge* [(initial weight – weight before heat treatment)/initial weight *100] and *total loss* [(initial weight – weight after cooking)/initial weight *100]. Freezing loss for the frozen samples was calculated as [(initial weight – weight after freezing)/initial weight *100].

Statistical analysis. Data were analyzed with the MIXED procedure in Statistical Analysis System (Version 8e, SAS Institute Inc., Gary, NC, USA). The model used included the fixed effects of ageing time, suspension method, their interaction, and the random effect of animal.

Results and discussion

Pelvic suspension changed the shape of the cuts because loins from the pelvic suspended carcasses were 3.6 cm longer that those from carcasses suspended by the Achilles tendon ($p=0.001$), which equals to a 5.0% increase. This was also found by Lundesjö *et al*. (2001) in *M. semimembranosus* from young bulls. Both suspension method ($p=0.039$; least square means 47.9 and 50.9 for pelvic and Achilles, respectively) and ageing time ($p<0.001$) significantly affected meat tenderness measured as WB_{max}. The overall effect of pelvic suspension independent of aging time was 5.9%, which is lower than previous findings for heifers (12.5%) (Lundesjö *et al*., 2002). Young bulls of the same breed as the cows in this study had as much as 21% decrease in shear force in *M. semimembranosus* after pelvic suspension and 7 days of ageing (Lundesjö *et al*., 2001; 2002).

Suspension method did not significantly interact with ageing time ($p=0.818$). It was, however still interesting to evaluate the combined effect of the two treatments, and therefore, each subgroup is presented in Table 1. Despite the overall effect of suspension method, with a higher tenderness for pelvic vis-á-vis Achilles suspension, no obvious differences could be found between suspension methods within each ageing time. With 2 days of ageing, shear force values tended to be lower for the pelvic suspended group ($p=0.083$). This difference was reduced by ageing time, giving equal values between suspension methods after 14 and 21 days of ageing. A lowering of the shear force values as an effect of pelvic suspension tallies with earlier results in other sexes (Hostetler *et al*., 1972; Jeremiah *et al*., 1984; Sørheim *et al*., 2001; Lundesjö *et al*., 2001; Lundesjö *et al*., 2002; Lundesjö Ahnström *et al*., 2004).

Shear force values declined over time, indicating a higher tenderness with longer ageing time, independent of suspension method (Table 1). This is in agreement with the findings by Sañudo *et al.* (2004). In the present study, both suspension methods had significant lower WB_{max} values at 4 days than at 2 days, but no difference between 4 and 7 days of ageing. However, the difference between 4 and 14 days was significant, but not between 14 and 21 days. With Achilles suspension there are larger differences between 7 and 14 and 21 days compared with the pelvic suspension. It can thus be concluded that with pelvic suspension, tenderness after 21 days ageing is already reached within 7 days even though there is a slight, but not

significant increase in tenderness with time. On the other hand, with normal suspension, it takes 14 days of ageing to reach the same value as with 21 days of ageing.

By freezing the samples after 7 days of ageing, shear force values were significantly reduced (see Table 1). The same effect was found for both suspension methods. This reduction gives meat with the same tenderness as for the meat aged for 14 or 21 days. It is, however, not clear if the improved tenderness as a consequence of freezing is accompanied by a lower juiciness. This could be assumed, as a consequence of the finding of higher freezing loss compared with purge from fresh meat stored for the same time. Shanks *et al*. (2002) also found a significant effect of freezing on tenderness and concluded that results for frozen samples might not be transferable to meat stored fresh. This needs to be further investigated.

pH increased in the samples during ageing, from 5.39 at day 2 to 5.50 at day 21 ($p<0.001$). This might be explained by a degradation of proteins during storage, giving a buffering effect in the meat. The overall water loss increased significantly with ageing time ($p<0.001$) (Table 2), but was not influenced by suspension method ($p=0.436$). Purge also increased with ageing time ($p<0.001$) and was lower for pelvic suspension than Achilles suspension ($p=0.030$). Within subgroups, the only difference was found at 14 days of ageing, with a lower loss as a result of pelvic suspension ($p=0.015$). The small effects found on water loss by suspension method in cows found here are in contrast to a larger effect reported earlier in young bulls and heifers (Eikelenboom *et al*., 1998; Lundesjö *et al*., 2001; Lundesjö *et al*., 2002). The frozen samples had higher water loss compared with those stored fresh, both with pelvic ($p=0.011$) and Achilles suspension ($p=0.001$). Higher water loss as a result of freezing was also found by Shanks *et al.* (2002).

Conclusions

In conclusion pelvic suspension gave a more tender meat, measured as lower shear force values, at 2 days of ageing, compared with normal hanging (Achilles). This difference was not found at longer ageing times and thus, pelvic suspension, does not seem to have a great influence on meat tenderness in LD from cows. The tenderness increased during ageing, from 2 to 21 days, in the same way with both pelvic suspension and Achilles suspension. Freezing the meat after 7 days of ageing gave higher tenderness, compared to fresh stored, and gave the same tenderness as meat aged for 14 and 21 days.

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Tables

Table 1. Shear force values (WB_{max}) (least square means) at different ageing times and suspension methods

	WB_{max}[1]		
Ageing (days)	Pelvic	Achilles	P-value[2] Suspension
2	61.6^{a}	67.7^{A}	0.083
4	51.6^{b}	54.8^{B}	0.359
7	47.4^{bc}	51.2^{BC}	0.280
7 (frozen)	39.4^{d}	43.4^{D}	0.249
14	43.4^{cd}	44.7^{CD}	0.712
21	43.9^{cd}	43.4^{D}	0.872

[1] Least square means with same letter within column do not differ significantly (p>0.05).
[2] Comparison of WBmax for Achilles and pelvic suspension within each ageing time.

Table 2. Water loss (least square means) in *M. longissimus dorsi* with Pelvic or Achilles suspension and different ageing times

	Purge[1]			Total loss[1]		
Ageing (days)	Pelvic	Achilles	P-value[2] suspension	Pelvic	Achilles	P-value[2] suspension
2	-	-	-	14.0a	13.9a	0.912
4	1.1^{a}	1.3^{A}	0.684	15.9ab	15.6a	0.830
7	2.6^{b}	2.8^{B}	0.725	16.7bc	18.1b	0.241
7 (frozen)	4.2^{d}	4.9^{C}	0.268	20.9d	21.3c	0.738
14	3.0^{bd}	4.7^{C}	**0.015**	18.9cd	18.6b	0.785
21	5.9^{c}	6.3^{D}	0.456	21.0d	22.1c	0.320

[1] Least square means with same letter within column do not differ significantly (p>0.05).
[2] Comparison WBmax for Achilles and pelvic suspension within each ageing time.

PHYSICAL QUALITY OF SEVERAL EUROPEAN BEEF BREEDS: PRELIMINARY RESULTS

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Background

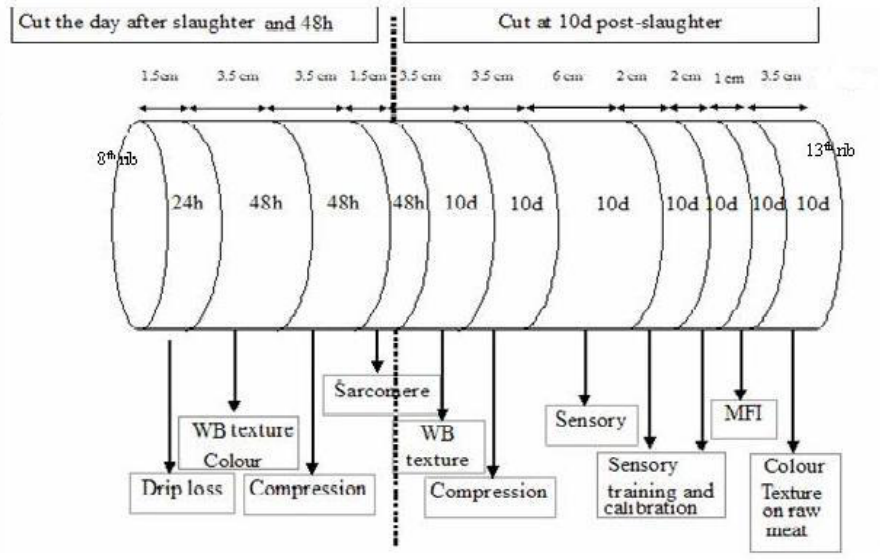
This research is a part of an European Union ongoing project aiming to compare carcass and meat quality of young bulls from several European cattle breeds (both dairy and beef), raised under standardized conditions to minimize environmental differences. *In vivo* measurements, covering production, slaughter data, meat and carcass quality parameters (both physical and chemical) and qualitative traits and their relationship with meat quality characters were investigated.
Some peculiarities of the different European cattle breeds are renowned, but we still need to improve our knowledge of their meat quality to understand the genetic background of these traits, to facilitate the design more efficient breeding programmes.
In particular, the extrinsic quality traits of meat, mainly tenderness and colour which are more appreciated by the consumer. These parameters are dependent on many *ante mortem* and *post mortem* factors, which need further study to understand the mechanisms determining their variability.

Objectives

Assess differences in instrumental meat quality traits (colour, water holding capacity and texture) in eight beef breeds from three European countries.

Materials and methods

The research was carried out on 243 young bulls from 8 different European beef breeds: Asturiana de los Valles, Asturiana de la Montaña, Avileña-Negra Ibérica and Pirenaica from Spain, Piemontese and Marchigiana from Italy, and Limousine and Charolaise from France. Diet and slaughtering methods were standardized for all the animals and are described in Sañudo *et al.* (2004).
At 24 hours after slaughter the *Longissimus thoracis* muscle was removed from carcass and stored at 3+/-1°C until 48 hours and then a section was subdivided in three slices and frozen, the other section was aged for 10 days and then was subdivided as shown in figure. The samples were frozen at -18°C. In this paper only the data performed at 10 days and water loss and pH at 24 hours are reported.



The water loss, by the drip method of Barton-Gade *et al.* (1994), and pH, by putting the probe into the muscle, were performed on fresh meat, then on samples aged 10 days thawing losses were recorded.
On the raw meat samples colour and compression tests were

performed. Colour coordinates were measured at ten points on the surface of slices, 3 cm thick according to Cassens *et al. (*1995). After exposure to oxygen for 1 hour, lightness (L*), redness (a*), yellowness (b*), chroma (C) and hue (H) were calculated with CIEL*a*b* System using D65 illuminant using spectrophotometer Minolta CM-2600d; reflectance spectra between 360 and 740 nm (by steps of 10 nm) were also measured.
On ten samples 1 cm^2 of cross section, with fiber direction paralleled to length (long axis), maximum load, stress at 20% and 80 %, maximum compression were measured with an Instron 4301 machine, using a modified device which avoids transverse elongation of the sample (Campo *et al.*, 2000).
Shear force was measured on raw and cooked meat. Samples were cooked in a waterbath at 80°C to an internal temperature of 78°C, then cooled in running tap water for 45' and stored in a refrigerator for 4 hours. Cooking losses were later calculated as percentage. Shear force was measured by a Warner Bratzler device mounted on an Instron 1011, on ten cores with section 1x1 cm for each animal, both of raw and cooked meat. Analysis of variance using breed as a factor was performed using GLM procedure of SAS package.

Results and discussion

pH and water losses
The pH at 24 hours (Table 1) showed that Limousine had significantly the higher values (5.90), than all other breeds. The Asturiana de la Montaña was significantly different from other breeds with mean pH values of 5.82. The lowest values were found in Charolaise, Pirenaica and both the Italian breeds (5.58 in average) which did not differ significantly, whilst the pH values of Avileña and Asturiana de los Valles were intermediate. A similar trend was found in the thawed meat aged 10 days, except for Limousine, which unlike the data at 24 hours, showed a lower pH, however close to the mean value of all breeds.
A low pH was generally found in the animals with a more marked beef aptitude, in particular Piemontese, while the more rustic breeds had a higher pH, even if within the norm, showing muscular fibres with a lower glycolytic power.
The drip losses (Table 1) showed a definitely lower value in Asturiana de la Montaña (1.11%) followed by Avileña (1.91%), while intermediate values were found in Marchigiana and Asturiana de los Valles, whilst Pirenaica, Piemontese and the French breeds had higher losses. Also with regard to thawing losses Asturiana de la Montaña gave the lowest value (6.85%), while the Avileña mean value did not differ from the other breeds. Cooking losses were generally lower than those reported in bibliography (Destefanis *et al.*, 1996) for the relevant losses caused by thawing, mainly in Limousine, Pirenaica and Asturiana de los Valles which showed the lowest value for cooking loss, while Charolaise and Italian breeds lost higher percentages of liquids. Water losses were higher in animals with a relevant muscular development and a slight adipose covering (Sañudo *et al*., 2004).
The pH and in particular its rate of fall in the first 48 hours after slaughter was highly correlated with water losses and shear force, this is in accord with Thompson (2002).

Colour
Analyzing colour (Table 2), a group of breeds, Italian and French, did not differ in lightness and hue, higher than the others, but were lower than other breeds in redness (a*), ranging from 14.74 to 15.84, except for Asturiana de los Valles which showed similar values (15.51). The opposite conditions (the lowest L* and H and the highest a*) were found in Asturiana de la Montaña and Avileña-Negra Ibérica. The remaining two Spanish breeds (Asturiana de los Valles and Pirenaica) showed intermediate values for L* and H and only Pirenaica for a*. The same group, which had lighter meat, showed a higher yellowness (b*): Charolaise was significantly different from Limousine (15.72 vs 15.03), and showed intermediate values with the Italian breeds and Pirenaica, with respect to the whole group. The remaining Spanish breed had lower b* values, particularly Asturiana de la Montaña compared to Asturiana de los Valles and Avileña-Negra Ibérica (13.51 vs 14.34 and 14.44 respectively). The latter had higher chroma being definitely different from the others, whilst Charolaise and Pirenaica had intermediate values, but different only from Limousine and Asturiana de Valles. Piemontese, Marchigiana and Asturiana de la Montaña had an intermediate chroma value and differed significantly only from Avileña-Negra Ibérica.

Texture
The Spanish rustic breeds and Charolaise presented a higher value of shear force on raw meat (Table 3), in particular compared with Piemontese. However, in these breeds a low shear force on cooked meat was

observed, together with Pirenaica, differing from Marchigiana, which was significantly tougher than the others on cooked meat, also if it showed intermediate value in raw meat.
With cooking Asturiana de los Valles and Piemontese meat became tougher, it is possible that excessive water losses and a marked shrinkage effect by the muscle contraction during cooking undervalued the Asturiana de los Valles and Piemontese, which had lean meat, with a greater development of muscle fibers, being hypertrophic animals. In particular Piemontese meat is commonly deemed as tender by the consumers and is consumed rare or raw (carpaccio).
Compression at 20% values, which can be related with myofibrillar resistance (Lepetit and Culioli, 1994), were in general low (near of 4 N/cm^2), showing an adequate tenderization through ageing (10 days in our study). The differences between breeds were not important, which showed the natural tendency of homogenizate myofibril resistance differences among breeds or individuals with ageing time. According to previous results (Sañudo *et al*., 2004), the highest values were found in Marchigiana breed, in correspondence with its shorter sarcomeres and its higher shear force values.
Compression at 80% values, which is probably due to the connective component resistance, showed important variability among breeds. This was expected since their differences in precocity and subsequent collagen characteristics (Campo *et al*., 2000), whereby the lowest values in Piemontese were related with the collagen composition of high muscularity animals. Conversely, two rustic type breeds and Charolaise gave the highest values (up to 40 N/cm^2), which are more characteristic of unimproved breeds.

Conclusions

Breed has an important effect on instrumental meat quality characteristics.
In particular, Asturiana de la Montaña shows very good water holding capacity and tenderness, while Italian and French breeds are characterized by their lightness and bright red appearance, appreciated by the consumer.
The data variability of these animals made us hope in the possibility of improving some quality traits, obtaining products appreciated by the consumer.

Acknowledgements

Authors greatly to the EU (QLK5-CT-2000-00147) for the financial support, to the Breeders Associations and the rest of people of the different groups for their technical assistance.

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Table 1. pH and water losses on *longissimus thoracis* muscle

	pH 24h	pH thawed meat at 10d	Drip losses (%)	Thawing losses (%)	Cooking losses (%)
ASV	5.73 ^{c}	5.57 ^{b}	2.28 ^{b}	7.33 ^{ab}	24.90 ^{cd}
ASM	5.82 ^{b}	5.59 ^{a}	1.11 ^{c}	6.85 ^{b}	25.44 ^{bc}
AV-NI	5.68 ^{c}	5.57 ^{b}	1.91 ^{bc}	7.52 ^{ab}	25.56 ^{bc}
PI	5.57 ^{d}	5.55 ^{c}	3.15 ^{a}	7.50 ^{ab}	24.02 ^{d}
PIE	5.58 ^{d}	5.51 ^{d}	3.02 ^{a}	7.10 ^{ab}	25.66 ^{ab}
MARC	5.58 ^{d}	5.52 ^{d}	2.47 ^{b}	7.45 ^{ab}	27.31 ^{a}
LIM	5.90 ^{a}	5.56 ^{bc}	3.58 ^{a}	7.77 ^{a}	24.74 ^{cd}
CHAR	5.57 ^{d}	5.57 ^{b}	3.29 ^{a}	7.06 ^{ab}	26.64 ^{ab}
Mean	5.67	5.56	2.61	7.31	25.52
Root MSE	0.137	0.046	1.602	1.837	2.92

Table 2. Colour determination on *longissimus thoracis* muscle, aged 10 day

	L*	a*	b*	C	H
ASV	41.88 ^{b}	15.51 ^{c}	14.34 ^{c}	21.21 ^{c}	42.88 ^{b}
ASM	39.06 ^{c}	17.00 ^{a}	13.51 ^{d}	21.73 ^{bc}	38.59 ^{c}
AV-NI	39.81 ^{c}	17.84 ^{a}	14.44 ^{c}	22.98 ^{a}	39.03 ^{c}
PI	42.84 ^{b}	16.60 ^{b}	14.87 ^{b}	22.34 ^{b}	42.07 ^{b}
PIE	44.39 ^{a}	15.42 ^{c}	15.32 ^{ab}	21.79 ^{bc}	45.01 ^{a}
MARC	44.75 ^{a}	15.84 ^{bc}	15.49 ^{ab}	22.19 ^{bc}	45.52 ^{a}
LIM	44.60 ^{a}	14.74 ^{c}	15.03 ^{b}	21.09 ^{c}	45.82 ^{a}
CHAR	45.67 ^{a}	15.63 ^{c}	15.72 ^{a}	22.20 ^{b}	45.27 ^{a}
Mean	42.86	16.07	14.83	21.94	42.89
Root MSE	2.941	2.095	1.289	2.047	3.661

Table 3. Texture determinations on *longissimus thoracis* muscle, aged 10 day

	Shear force		Compression test			
	raw (N)	cooked (N)	maximum load (N)	stress at 20% (N/cm^2)	stress at 80% (N/cm^2)	sarcomere length (µm)
ASV	40.44 ^{bc}	54.45 ^{b}	55.45 ^{c}	4.01 ^{b}	39.58 ^{c}	2.04 ^{b}
ASM	43.81 ^{ab}	47.36 ^{d}	58.89 ^{bc}	4.17 ^{b}	43.84 ^{a}	2.33 ^{a}
AV-NI	46.46 ^{a}	43.45 ^{d}	57.96 ^{bc}	4.10 ^{b}	41.60 ^{a}	2.08 ^{b}
PI	37.13 ^{c}	48.32 ^{cd}	54.14 ^{c}	4.12 ^{b}	37.06 ^{c}	2.11 ^{b}
PIE	32.38 ^{d}	54.67 ^{b}	39.71 ^{d}	4.25 ^{b}	27.29 ^{d}	2.04 ^{b}
MARC	38.76 ^{c}	61.84 ^{a}	63.69 ^{b}	5.19 ^{a}	39.95 ^{b}	1.95 ^{b}
LIM	37.57 ^{c}	53.08 ^{bc}	46.54 ^{d}	3.99 ^{b}	31.21 ^{d}	2.11 ^{b}
CHAR	45.67 ^{a}	47.09 ^{d}	64.28 ^{a}	4.23 ^{b}	42.95 ^{a}	2.13 ^{ab}
Mean	40.30	51.16	55.10	4.22	37.73	2.12
Root MSE	8.172	10.666	9.405	0.737	6.579	0.438

** data of Compression test and Sarcomere length are referred to only 15 animals for breed.*
ASV- Asturiana de los Valles; ASM- Asturiana de la Montaña; AV-NI- Avileña-Negra Ibérica; PI– Pirenaica; PIE- Piemontese; MARC- Marchigiana; LIM- Limousine; CHAR- Charolaise.
NOTE different letters mean significant differences with P<0.05.

EFFECT OF CARCASS WEIGHT ON SENSORY QUALITY OF YOUNG HOLSTEIN BULLS

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Background

A considerable proportion of beef produced in the UK is a byproduct of the dairy industry. In Northern Ireland, approximately 50% of beef animals slaughtered derive from the dairy herd while nearly 20% of animals are entire bulls. Young animals from this source are generally regarded as low in quality, especially at the lower weight ranges, receiving low EUROP grades for conformation and providing poor remuneration to the farmer. Meat from animals of this type is usually destined for the commodity minced (ground) beef market.

Previous research has studied the effect on the eating quality and other attributes of young bulls of diet and finishing, age at slaughter and genotype (e.g., Sinclair *et al.* 1998, Vestergaard *et al.* 2000). However, little of this research has focused on young dairy animals. This preliminary investigation forms part of a larger investigation on the rearing and use of young dairy bulls for beef production.

Objectives

This work aims to determine the effect of final live-weight of young dairy bulls on the eating quality of the beef from *biceps femoris* (silverside), chosen as it is a commonly used roasting joint of intermediate quality.

Materials and methods

Husbandry and slaughter

Ninety-three weaned Holstein bull calves were purchased from farms in Northern Ireland and housed at the Agricultural Research Institute for Northern Ireland. At approximately 15 weeks of age, calves were grouped into blocks of four animals according to similarity of live weight and age. One animal from each block was allocated at random to one of four treatments with different target slaughter weights, namely 400 (T3), 450 (T4), 500 (T5) and 550 (T6) kg. Animals were housed in pens accommodating between 4 and 9 animals within each slaughter weight treatment group. All animals were offered concentrates (maize meal, sugar beet pulp, vitamin/mineral premix, barley, and soyabean meal) *ad lib* with a restricted quantity of barley straw (nominally 0.5 kg/head/d). Of these animals, 48 were selected for assessment of eating quality, 15 in each of the heavier treatments and only three in T3. Data from this lightest group was excluded from statistical analysis due to the small number of animals.

The animals were slaughtered on seven different dates at the same plant with no electrical stimulation and the sides hung by the Achilles tendon. Treatments T4 to T6 were each slaughtered over four to six of these dates. The silverside joints were removed from the carcass at 24h *post mortem* and vacuum packed.

Preparation of meat

The joints were transported to the laboratory in a refrigerated van and aged at 2ºC for a total of 21 days from slaughter. After this time the joints were frozen and stored at -20ºC for a period of 3 to 4 months. Prior to sensory analysis the *biceps femoris* muscles were cut into 3 pieces cutting across the longitudinal axis of the muscles whilst still frozen, the joints were re-vacuum packed and returned to the freezer. The pieces were denoted A (proximal), B (centre), and C (distal). Only joints A and B were used in this study

Sensory Analysis

Joints were allowed to thaw in their vacuum packs at 4ºC for a period of 24h. Joints A and B (mean weights of 1720 and 2160kg) were prepared for roasting by removal of all fat and epimysium. The joints were browned on all sides before being placed in uncovered stainless steel dishes in an electric fan assisted oven (Falcon) at 180ºC for an estimated cooking time of 30 min per 500g, with start times adjusted to give similar finish times. All the joints were cooked to an internal temperature of 74 ºC. The outer surface was trimmed

from the cooked joints and the inner roast meat cut into portions (30-40g) for sensory analysis. The samples were held in a warm oven (95 to 100°C) before being served warm to the panellists within 5 minutes.
Panellists were volunteers from University and DARD departments who were untrained but had experience of participating in taste panels. The panellists assessed 96 joints from 48 animals over 36 sessions. Samples were allocated to the taste panels sessions using a balanced design (PSA Systems Version 3.3, Oliemans, Punter and Partners, Utrecht, The Netherlands) where each panellist was presented with a total of six samples. Panellists scored the first three samples (from three joints) for the subjective attributes, acceptability of aroma, flavour, texture, and overall acceptability, in this order, using a 10cm line scale anchored at each end (dislike extremely – like extremely) and at 50%. Panellists were advised that the central point differentiated between acceptable and unacceptable. Panellists were also asked to rate the sample on a four point category scale as being unacceptable (1), satisfactory everyday quality (2), better than everyday quality (3), premium quality (4), similar to that used in the Australian MSA system (Polkinghorne *et al.* 1999). Panellists then assessed three more samples from the same joints for the objective attributes, intensity of aroma, intensity of flavour, tenderness and juiciness, again on a 10 cm line scale. Each sample was tested by 10 consumers. Statistical analysis was conducted using a mixed model analysis of variance.

Results and discussion

Table 1 lists the live-weight of the animals used in this study together with those carcass characteristics that might be considered to influence eating quality. The groups of animals achieved their target mean live-weight at slaughter but there was some variation in the weights of individual animals. As expected, increasing live-weight and age gave a slight improvement in EUROP grade and an increase in fat grade and cover. These aspects will be discussed fully elsewhere. Table 2 shows the mean sensory scores for the subjective and objective sensory attributes of *biceps femoris* (silverside) from young Holstein bulls.

Table 1. Characteristics of animals belonging to the four groups used for eating quality assessment.

Treat-ment	No. anim-als	Mean live-weight (kg)	*SD*	Mean carcass weight (kg)	*SD*	Mean age (days)	*SD*	Conformation (EUROP grade)	Mean fat grade	*SD*	Mean fat cover (mm)	*SD*
T3	3	407	*12*	219	*11*	365	*21*	1P, 2O-	2.3	*0.5*	2.7	*1.6*
T4	15	458	*9*	239	*8*	375	*26*	6P, 8O-, 1O+	2.8	*0.4*	2.7	*1.2*
T5	15	502	*30*	267	*15*	389	*33*	4P, 4O-, 7O	2.8	*0.4*	2.3	*0.9*
T6	15	561	*20*	298	*10*	438	*26*	2P,6O-,6O,1O+	2.9	*0.3*	3.1	*0.8*

Effect of liveweight

Only small differences were observed between roast beef from animals reared to different live-weights. There were no significant differences in texture or flavour. Meat from the smaller animals (T4) was, apprently, significantly more juicy but had a less acceptable aroma and was a little less acceptable overall than that from the animals reared to 500 or 550kg (T5, T6). However, the three animals reared to 400kg (T3) did not follow the same trend and the observed effect may be related only to group T4. Sinclair *et al.* (1998) reported that age at slaughter had no significant effect on the tenderness of meat from several muscles, including *biceps femoris*, from young bulls.

Effect of position in muscle

The most significant effect on eating quality was that of joint or position within the *biceps femoris* muscle, with highly or very highly significant effects observed for all sensory attributes. The greatest differences were in aspects of texture. The joint from the proximal portion of the muscle (A) was much more tender than the centre portion (B) and also had more acceptable texture. Differences in overall acceptability reflected this texture difference. Significant but smaller differences in the same direction were also observed for juiciness, intensity of flavour, and acceptability of flavour. Sensory trials with untrained panellists frequently show

Table 2: Effect of carcass weight and position in joint on subjective and objective sensory scores for eating quality of roast beef from *biceps femoris* (silverside) from young Holstein bulls.

		Treatment					Significance[a] (SED)		
	Joint	(T3)[b]	T4	T5	T6	Mean	Live-weight	Joint	Inter-action
Subjective attributes									
Acceptability	A	(71)	68	69	68	**68**	**	**	*
of Aroma	B	(62)	60	67	68	**65**	(1.29)	(1.50)	(2.15)
	Mean	**(66)**	**64**	**68**	**68**				
Acceptability	A	(64)	64	65	61	**63**	ns	***	*
of Flavour	B	(47)	52	58	58	**56**	(1.41)	(1.75)	(2.41)
	Mean	**(56)**	**58**	**62**	**60**				
Acceptability	A	(68)	65	66	58	**63**	ns	***	***
of Texture	B	(35)	43	50	53	**48**	(1.92)	(2.50)	(3.35)
	Mean	**(52)**	**54**	**58**	**55**				
Overall	A	(65)	65	68	62	**65**	*	***	**
Acceptability	B	(50)	48	56	57	**54**	(1.60)	(1.88)	(2.69)
	Mean	**(58)**	**56**	**62**	**60**				
Objective attributes									
Intensity	A	(50)	54	50	47	**50**	ns	**	**
of Aroma	B	(44)	44	47	47	**46**	(1.42)	(1.64)	(2.35)
	Mean	**(47)**	**49**	**48**	**47**				
Intensity	A	(47)	52	50	46	**49**	ns	**	*
of Flavour	B	(34)	44	43	46	**44**	(1.54)	(1.79)	(2.56)
	Mean	**(41)**	**48**	**46**	**46**				
Tenderness	A	(64)	62	65	57	**62**	ns	***	ns
	B	(28)	49	48	51	**49**	(2.42)	(2.88)	(4.10)
	Mean	**(46)**	**55**	**56**	**54**				
Juiciness	A	(48)	56	51	44	**51**	***	**	*
	B	(33)	50	41	46	**46**	(1.79)	(2.18)	(3.05)
	Mean	**(41)**	**53**	**46**	**45**				

[a] SED = standard error of difference, shown in brackets. *, **, *** = significant at P<0.05, 0.01, 0.001 respectively. ns = P≥0.05

[b] Data from 400kg group were not included in statistics as comprises only 3 animals.

correlation between flavour, juiciness and texture, perhaps due to the difficulty of separating these attributes completely and possibly due to inter-relationships between, for example, juiciness and flavour release. In this study, many of the attributes were highly correlated (P<0.001) with one another even though assessments were conducted on two separate samples. It is interesting that the panellists were able to differentiate between these joints for intensity of aroma and acceptability of aroma. As these attributes were assessed first, before the panellists had taken the sample into their mouths, these differences appear to be small but real and suggest that joint A gave a greater concentration of key odour volatiles when roasted, probably also contributing to the observed difference in flavour.

Live-weight x joint interactions

Seven of the eight attributes showed significant joint x live-weight interactions, suggesting that the size of the animal was important for the eating quality differences observed between the joints. Most affected was

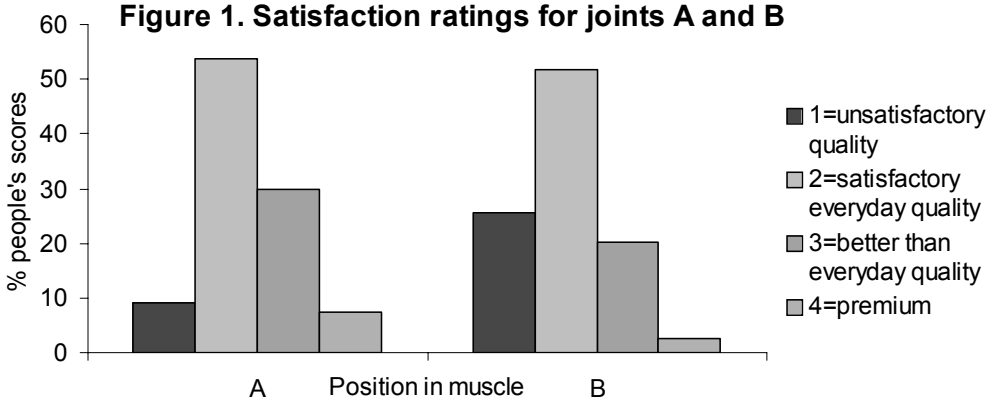
acceptability of texture, together with overall acceptability and intensity of odour. However, all the attributes followed a similar trend; joint A scored higher than joint B for the 450kg and 500kg animals but this difference was considerably reduced or removed in the 550kg animals. The data from the three 400kg animals also followed the same trend. Overall, this increased difference between the joints at the lower weights represented both a slight decrease in quality of joint B and a slight increase in the quality of joint A at these lower weights. As the muscles were aged to 21 days, the observed tenderness differences brtween joints may be due to differences in collagen content or sarcomere length. Such differences may be caused by differences in pH/temperature fall and size or fat cover of the muscle, or by differences in muscle development. Table 1 shows that the difference in fat class and cover between treatments T4 to T6 is small and would not appear to explain these results. Further work is required to determine the reason for these effects.

An exaggeration of sensory differences at the lower live-weights was also observed by Vestergard *et al.* (2000). Beef from young bulls reared to 360kg showed a greater difference in eating quality between beef from extensively and intensively reared animals than that from animals reared to 460kg. However, in this case, this effect was attributed to an intensive finishing period given to the older 'extensive' animals.

Satisfaction scores

The satisfaction ratings provide an indication of how the panellists in this trial assessed the overall quality of the *biceps femoris* roast beef presented to them (Figure 1). For both joints, most samples assessed to be 'satisfactory everyday quality' but, for joint A, a higher proportion of assessments were at the higher grades.

Figure 1. Satisfaction ratings for joints A and B



These preliminary results suggest that the eating quality of aged *biceps femoris* from these young dairy bulls is not poorer than that of roast beef joints purchased at retail and assessed previously. This observation concurs with the conclusion of Sinclair et al. (1998) that beef from young bulls, in their case of beef breeds, can be at least as good as the UK standard product. Further studies will be conducted to study the eating quality of a range of muscles from young dairy animals and to determine whether extensive aging is necessary to achieve acceptable eating quality.

Conclusions

This preliminary study indicates that the eating quality of *biceps femoris* (silverside) is affected significantly by the position within the joint but only slightly by live-weight. However, the sensory differences between joints are largely non-existent at 550kg but are exaggerated at the lower live-weights. Further research is needed to establish why this effect occurs and whether it can be managed to enable high quality beef to be obtained from young dairy bulls.

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CONSEQUENCES OF USING DENTAL CLASSIFICATION AS INDICATOR OF MATURITY AND BEEF TENDERNESS

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Background

Usually decreased meat tenderness has been associated with increased cattle age (Tuma *et al*., 1963 and Wulf *et al*., 1996), this fact has led to include dental classification in current grading systems (Aus-Meat, 1998). Lawrence et al (2001 a) suggested that it was more accurate to assess maturity by sorting carcasses through the number of permanent incisors present at slaughter rather than by visual appreciation of ossification. However, Wythes and Shorthose (1991) and Lawrence et al. (2001 b) found that the dentition status of steers had no effect on Warner-Bratzler shear force and tenderness of *M. longissimus* assessed by sensory panel . Therefore, there exists some contradictory conclusions referred to the relationship between beef cattle maturity and meat tenderness.

Objectives

Our objective was to determine the relationship between tenderness and age, assessed by dentition status, of steers commercially produced in Argentina.

Materials and methods

Fifty five Angus steers were used, the number of permanent incisors were recorded previous to slaughter. Twenty animals had no permanent incisors, twenty had two and fifteen had four. Animals fell into two fat classes, having respectively 4 to 5 mm (class 1) and 6 to 7 mm (class 2) subcutaneous fat depth between the 12^{th} and 13^{th} rib, measured at the abattoir. Samples of *M. longissimus dorsi* were removed from each left carcass. Half of each sample was kept for 24 hours at $3\ ^0$ C previous tenderness determination and the other half, also kept at 3^0 C, was analyzed after 96 hours. Tenderness was measured with an Instron 4442 Universal Testing Machine (Canton, MA, USA) with a Warner-Bratzler shearing attachment on samples boiled for 50 minutes, in a water bath, to an internal temperature of $70\ ^0$C. Data were analyzed using GLM procedure of SAS (SAS Inst. Inc,. Cary, NC, USA).

Results and discussion

After 24 hrs in cold storage, meat from steers with two incisors was more tender ($p < 0.05$) (Table 1), than from those with no permanent incisors, differences were not detected ($p > 0.05$) neither between two and four nor between four and zero permanent incisors. After 96 hrs ageing, meat from all the dentition groups showed enhanced tenderness, meat from animals with two and four permanent incisors was more tender ($p < 0.01$) than meat from less mature steers. These results partially agree with Wythes and Shorthose (1991), who reported no differences among dentition groups attributed to contraction of myofibrils in unstimulated muscles. In our case, carcasses underwent electrical stimulation, but ageing period was shorter, it was at the very most 96 hours. Lawrence *et al*. (2001) in a very accurate experiment did not find, among five dentition groups, differences in tenderness of muscle *longissimus* aged for 14 days. They do not explain the possible causes of such finding. Our results contradict those reports and an indirect relationship is established between maturity and improved tenderness (Tuma *et al*., 1963, Shorthose and Harris, 1990 and Wulf *et al*., 1996). This leads to suggest that more research is needed to determine if dentition can be used as a reliable predictor of tenderness. Correlation between fatness and shear force was significant ($r = - 0.34$, $p < 0.05$).

Conclusions

Ageing improved tenderness irrespective of dentition group. After a short ageing period, meat from steers with two and four permanent incisors proved to be more tender than meat from those animals showing no permanent incisors.

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Table 1 Shear force (kg) at 24 and 96 hours, mean values followed by the standard error of the mean

	Incisors		
Ageing, hours	0	2	4
24	12.7^{a} (2.2)	10.4^{b} (2.6)	11.6^{ab} (2.7)
96	10.1^{a} (1.8)	8.0^{b} (1.7)	8.8^{b} (1.9)

Within a row, means lacking a common superscript differ ($p < 0.05$) for 24 hrs, and ($p < 0.01$) for 96 hrs.

EFFECTS OF VARIOUS PRESLAUGHTER HANDLING TREATMENTS ON THE COLOUR OF DIFFERENT PORK MUSCLES

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Background

Colour is one of the most important quality attributes of pork because colour can be easily assessed by both producers and consumers. In addition, colour is related to other important quality aspects. Various phases during preslaughter handling, such as transport (Leheska et al., 2003), lairage (Pérez et al., 2002) and stress immediately before slaughter (Hambrecht et al., 2004b) may affect pork colour. Due to its size and accessibility, the LM is the most frequently assessed muscle. However, muscles consist of various fibre types that determine the metabolic properties of a muscle. It can be expected that muscles, depending on their anatomical location and metabolic profile, react differently to the physical and psychological stressors associated with preslaughter handling. In agreement, Warner et al. (1993) found that the longissimus, a predominantly glycolytic muscle, was a reliable pork quality indicator for other muscles when its colour was dark and exudate low. However, when its colour was pale and exudate high, quality of the longissimus muscle was only related to the major ham muscles and not to the shoulder muscles that have a more oxidative metabolism.

Objectives

The objective of the present experiment was to investigate whether the effects of transport, lairage, and preslaughter stress on colour are different in various, commercially important pork muscles.

Materials and methods

All pigs were commercial halothane-free end products of the *Hypor* pig breeding company. Pigs (n= 384) were assigned to one of eight treatments in a 2 × 2 × 2 factorial arrangement, with two types of transport (short (50 min) and smooth or long (3 h) and rough), two lairage durations (long (3 h; considered as optimal) or short (<45 min; considered as sub-optimal)) and two stress levels immediately before slaughter (minimal or high). Eight groups of 48 pigs, all originating from the same commercial farm, were processed during eight weeks on various days. Long and short lairage alternated between consecutive weeks. Transport types and preslaughter stress levels were varied within the same slaughter day. Pigs were electrically stunned in a fully automated, head-to-heart stunning system at a commercial plant. At 23 h post-mortem pork cuts were harvested for colour measurements. All measurements were done after a 10-min blooming period with a Minolta Portable Chroma Meter (Model CR 210). L* (lightness), a* (redness), and b* (yellowness) values were assessed in the *longissimus lumborum* muscle (**LL**) at the level of the third lumbar vertebra, in the *longissimus thoracic* muscle (**LT**) at the level of the 6th thoracic vertebra, in the *serratus ventralis* muscle (**SV**), and in the *semimembranosus* muscle (**SM**). Data were analysed by the mixed-model procedure (PROC MIXED) of SAS. Tests of multiple comparisons of least squares means were adjusted according to the TUKEY-KRAMER method to ensure the overall significance level of $P = 0.05$. The model applied included the fixed effects of muscle type, transport conditions, lairage duration, and stressor level, as well as their 2-way interactions, and the random effect of slaughter day nested within lairage.

Results and discussion

Effects of transport

Results are presented in Table 1. Long and rough compared with short and smooth transport decreased ($P < 0.05$) the yellowness of the meat. No other main effects or interactions between the transport treatment and muscle type were observed. The meat was darker (i.e. lower L* values) when transport was long and rough and pigs were additionally subjected to the short lairage treatment (transport × lairage interaction; $P < 0.05$; results not shown). This effect was similar for all muscles but the largest effect was observed in the SV muscle. Transport had no effect on the redness in the minimal preslaughter stress group. In the high stress group, however, long as opposed to short transport increased the redness in the LT and SM muscles but

decreased the redness in the SV muscle ($P < 0.05$; results not shown). LL redness was not affected ($P > 0.05$). These effects on redness are probably related to differences in the relative proportions of the various myoglobin forms (Lindahl et al., 2001) but remain difficult to explain. The physical exercise and psychological stress that is associated with transport varies depending on the type of transport. Transport in general was shown to be stressful for pigs (Geverink et al., 1998) and even more so in rough as opposed to smooth transports (Bradshaw et al., 1996). As a consequence, both the physical exercise and the psychological stress level was probably higher in the long and rough compared with the short and smooth transport treatment. Oxidative muscle fibres are preferentially recruited at low intensity exercise levels (Køpke et al., 1984; Lacourt and Tarrant, 1985) and were shown to be more sensitive to adrenaline (Górski, 1978; Fernandez et al., 1995) which is secreted in response to psychological stress. Consequently, it was expected that the SV, a more oxidative muscle compared with the other muscles, would show a larger response. This was only partly true, which is probably related to the small differences in exercise and stress level between the two transport treatments.

Effects of lairage

For the lairage treatments there was for all colour values an interaction between lairage and muscle type noted ($P < 0.05$). For the L* value, this was probably due to the relatively large decrease ($P < 0.05$) in lightness in the SV muscle, whereas the response to the short lairage duration was small in the LT and intermediate in the SM and LL muscles ($P > 0.05$). Regarding the a* value, the SV was again the only muscle that showed a difference ($P < 0.05$) between long and short lairage with a decreased redness after a short lairage period. None of the pair wise comparisons were significant for the b* values, but both the LL, SM and SV muscles appeared to exhibit a, numerically, lower yellowness for the short lairage treatment whereas there was no difference in b* values noted for the LT. Lairage is not a stress factor itself but is meant to provide a rest for pigs to recover from previous transport stress. Lairage times shorter than 45 min are usually considered as too short for recovery (Milligan et al., 1998; Pérez et al., 2002) whereas 3 h is often regarded as optimal for pigs (Warriss et al. 1998; Pérez et al., 2002). In agreement with the above mentioned higher adrenaline sensitivity and recruitment at low exercise intensity of oxidative muscle fibres, the oxidative SV muscle showed a somewhat larger response to the short lairage treatment. Another reason may be the heavier involvement of the shoulder and neck muscles in activities such as posture holding during transport, exploring and fighting due to mixing of foreign pigs and, as a consequence, an increased need for rest compared with other muscles.

Effects of stress

For all colour values, there were interactions between the stressor treatment and muscle ($P < 0.001$). High stress compared with minimal stress resulted in paler meat in the LT ($P < 0.05$) whereas the SV muscle became darker in response to high stress ($P > 0.05$). Meat was less red ($P < 0.05$) in both the LT and the SV for the high stressor treatment while SM redness seemed to be increased ($P > 0.05$). Whereas LL lightness and redness appeared to be hardly affected by preslaughter stress, the yellowness of the LL was, similar to SV yellowness, decreased ($P < 0.05$). No effect on yellowness was seen in the SM but LT yellowness was increased ($P < 0.05$) in response to high stress. The high stressor treatment was previously tested and shown to be associated with a high level of both physical exercise and psychological stress as well as large effects on pork quality (Hambrecht et al. 2004a,b). In agreement with the previously mentioned differences in adrenaline sensitivity, response to exercise intensity and involvement in physical activity, effects of the stressor treatment depended on muscle type. Barton-Gade and Olsen (1987) compared stress-susceptible pigs with stress-resistant pigs and found an increased incidence of PSE in glycolytic muscles such as the LM whereas oxidative muscles, including the SV, showed an increased incidence of the DFD condition. These results are supported by the effects of the high stressor treatment in the present experiment. Additionally, the present study shows that considerable variation exists for pork colour measured within the longissimus muscle. The high stressor treatment produced different and larger effects in the LT compared with the LL muscle. The reasons for these differences within the longissimus muscle are unknown. But results are in agreement with Lundström and Malmfors (1985) who showed a higher incidence of PSE in the shoulder part of the loin, compared with the mid-loin and the ham site of the loin.

Conclusions

Effects of preslaughter treatments depend on the muscle that is studied. The metabolic profile but also the location of a muscle play a crucial role. High stress levels associated with preslaughter handling may promote PSE development in some glycolytic muscles whereas more oxidative muscles will rather develop DFD pork. Effects of preslaughter handling on pork quality should be assessed not only in one but in several muscles.

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Table 1. Effect of transport, lairage, and preslaughter stress on pork colour in the longissimus lumborum (LL), the longissimus thoracic (LT), the semimembranosus (SM), and the serratus ventralis (SV) muscles[a]

		L* value		a* value		b* value	
Transport × muscle		Short transport (n=174)	Long transport (n=184)	Short transport (n=174)	Long transport (n=184)	Short transport (n=174)	Long transport (n=184)
	LL	53.9	53.7	19.4	19.1	5.5	5.3
	LT	58.9	59.1	19.2	19.2	5.6	5.6
	SM	54.4	54.0	19.7	19.9	6.5	6.5
	SV	38.9	38.5	24.3	24.1	4.3	4.1
	Pooled SE	0.23	0.23	0.09	0.09	0.10	0.10
Lairage × muscle		Long lairage (n=179)	Short lairage (n=179)	Long lairage (n=179)	Short lairage (n=179)	Long lairage (n=179)	Short lairage (n=179)
	LL	54.4	53.0	19.4	19.1	5.5	5.2
	LT	59.2	58.9	19.3	19.1	5.6	5.6
	SM	54.5	53.6	19.6	19.9	6.7	6.3
	SV	39.4^{y}	37.7^{x}	24.5^{y}	23.9^{x}	4.3	4.0
	Pooled SE	0.25	0.25	0.09	0.09	0.12	0.12
Stress × muscle		Minimal stress (n=177)	High stress (n=181)	Minimal stress (n=177)	High stress (n=181)	Minimal stress (n=177)	High stress (n=181)
	LL	53.9	53.7	19.2	19.3	5.5^{y}	5.2^{x}
	LT	57.1^{x}	60.8^{y}	19.5^{y}	18.9^{x}	5.3^{y}	5.9^{x}
	SM	54.2	54.2	19.6	20.0	6.5	6.5
	SV	39.1	38.3	24.5^{y}	23.9^{x}	4.4^{y}	4.0^{x}
	Pooled SE	0.23	0.23	0.09	0.09	0.10	0.10
P-values	Transport (T)	0.206		0.157		**0.010**	
	Lairage (L)	**0.024**		**0.061**		0.110	
	Stress (S)	**0.001**		**0.002**		0.303	
	Muscle (M)	**0.001**		**0.001**		**0.001**	
	T × M	0.509		0.102		0.589	
	L × M	**0.009**		**0.001**		**0.016**	
	S × M	**0.001**		**0.001**		**0.001**	
	T × L	**0.020**		0.547		0.491	
	T × S	0.411		**0.003**		0.426	
	L × S	0.524		0.736		0.127	

[xy]Least squares means within muscle, 2-way interaction and colour attribute lacking a common superscript letter differ ($P < 0.05$).

SENSORY AND INSTRUMENTAL ANALYSIS OF LONGITUDINAL AND TRANSVERSE TEXTURAL VARIATION IN PORK *LONGISSIMUS DORSI*

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Background

Longitudinal and transverse textural variation in pork *longissimus dorsi* has been widely reported (e.g. Onate and Carlin, 1963; Rust et al., 1972; Alsmeyer et al., 1965; Weir, 1953; Møller and Vestergaard, 1986). It is essential to understand this variation when measuring different aspects of meat quality such that what is measured is the actual difference between treatments and not differences within muscles.

Objectives

The objective of the present study was to investigate the texture variation in pork *musculus longissimus dorsi* (LD) through sensory and instrumental analysis. Specifically, the aims were to determine how the transverse and longitudinal texture varied within and between left and right muscles. A further objective was to determine the dynamic nature of texture variation occurring in and between left and right muscles of pigs and as a function of ageing. Overall, in the present case the predictive and causal association of these methodologies was considered paramount, such that confusion and misrepresentations as to what textural variation in pork meat means from a sensory perspective versus texture measurement from instrumental perspective.

Materials and methods

Twenty seven pigs were selected at random from an abattoir production line with regard to weight at slaughter (73-79 kg), lean meat percentage (58-62%), and ultimate pH (5.5-5.8). Both left and right *Longissimus dorsi* (LD) from the pigs, a total of 54 muscles, were excised. During excision subcutaneous fat and bones were removed. The LD muscles were divided into two halves at the 13th rib (cranial and caudal ends) and vacuum packed in oxygen impermeable bags. The muscles were aged for 0, 4, and 7 days respectively at 4˚C before they were frozen at -20°C.

Both the cranial and caudal ends of the loins were sliced into 20 mm thick chops from the 13th rib upwards and downwards (producing 9 chops in each direction). The pork chops were pan cooked in neutral oil (grape seed oil) at 155˚C for approximately 8 minutes until a core temperature of 65˚C was reached.

The transverse sample cores for the sensory descriptive texture profiling were excised by using a template (4×5 cm) and then divided into three equally sized pieces (approximately 17 mm wide, 20 mm thick, and 40 mm long). For instrumental analysis transverse sample cores were excised parallel to the muscle fibre orientation to the cut chop surface as recommended in AMSA (1995). The cores from each chop were denoted according to their position on the chop from inner to outer edge, a (nearest spinal column, dorsal), b (medial), and c (lateral).

The samples were measured using sensory descriptive texture profiling (ISO 11036, 1994; Meilgaard et al., 1999) and a Texture Analyser (Stabel Micro Systems, UK) mounted with a Volodkewich shear blade.

In the present study a special use of 'two block' Partial Least Squares Regression (PLSR) known as APLSR (the 'ANOVA like use of PLSR') was utilised. This form of PLSR projects the response variables onto the design variables in order to determine to which degree each of the design variables in **X** contribute to the variation in the response variables **Y** (Martens & Martens, 2001).

In relation to significance testing at the 5% level, i.e. $P \leq 0.05$, a re-sampling technique termed 'jack-knifing', which is part of cross-validation, was utilised. All multivariate analyses were performed using the Unscrambler Software, Version 8.0 (CAMO ASA, Norway).

Results and discussion

In the sensory determined transverse muscle variation a trend (non-significant) in decreasing tenderness from the dorsal position (nearest the spinal column) to the medial position was found. Instrumental analysis showed that hardness increased from the dorsal to the lateral position, both being significant. It should however be mentioned that the medial position could not be ascribed any significance level. The sensory and instrumental analysis agreed that the dorsal position was the most tender, but neither method was able to determine if the medial or the lateral position was the least tender of the three positions investigated (see Figure 1). The reason for the dorsal position being most tender can be attributed to the anatomic location. This position is supported by the spinal column, thus less work is performed in this muscle area.

Transverse variation was better described instrumentally than from a sensory perspective, in that significance levels could be ascribed. This was most likely because fibre orientation when assessed in sensory analysis was more inconsistent in its orientation when physically placed in the mouth by panellists.

In the sensory determined longitudinal muscle variation a significant decrease in tenderness was found from the anterior to the posterior part of the muscle. The reason for the cranial end being the most tender was most likely related to the anatomical location of the muscles. The cranial end is strongly supported by the ribcage and thus less work is performed in this muscle part. Instrumental analysis showed a trend (non-significant) in decreasing hardness when approaching the caudal end of the muscle.

Variation between individual chops was found when assessed by the sensory panel but not when measured instrumentally. Because the instrumental measurements lacked information about the longitudinal texture variation, specific examination of the texture was only carried out using data from the sensory analysis (see Figure 2).

When tenderness ratings were determined as a function of individual chops for each of the three ageing periods it appeared that the first 7 chops showed similarity in tenderness level for 0, 4, and 7 days respectively. A marked decrease in tenderness took place at the region near the end of the ribcage. Below the ribcage the tenderness was rated as being constant or tending to slightly increase. As tenderness decreased hardness was found to concurrently increase. The turn-over point in tenderness could be applied to a general textural turn-over point in that hardness and juiciness also showed marked changes in this specific region of the muscle. A markedly higher difference between individual chops was seen in the cranial versus the caudal end at 0 days of ageing. When the meat was aged, larger individual chop variation occurred in the caudal end. It seemed that the individual chop variation in the cranial end decreased, whereas the variation in the caudal end concurrently increased when the meat was aged.

Marked differences between left and right muscles were observed. Right muscles showed bigger internal variation throughout the loin, when compared to left muscles. Moreover, it appeared that the longitudinal variation was more distinct and interacting in the right loins compared to the left loins. Qualitatively left and right loins displayed the same trends but differed quantitatively in the levels of variation. A plausible explanation may be that the majority of the pigs in the present study were ‘right-footed’. If this was the case, right muscles would perform more work than left muscles which may explain why the right side muscles varied considerably more internally than the left side muscles.

Both sensory and instrumental analyses were able to differentiate significantly between 0, 4, and 7 days of ageing. As expected 0 days of ageing was positively correlated to hardness. Ageing for 4 and 7 days respectively resulted in equally tender meat. The sensory analysis revealed that 4 and 7 days of ageing were differentiated by the levels of crumbliness and cohesiveness, 7 days of ageing leading to more crumbly and less cohesive meat.

Sensory and instrumental data revealed significance differences between left and right muscles. The left muscles were found to be significantly more tender than the right muscles in both the sensory and instrumental analysis.

The textural variation lengthwise was better described when assessed by the panel compared to when measured instrumentally. Of considerable importance regarding this discrepancy the shear force apparatus only measured deformation in terms of force (Newton) used for compression of the sample, whereas the sensory profiling described what caused the differences in the longitudinal variation.

Sensory and instrumental measurements were found to be predictive indexes of each other (see Figure 1) however these methods did not measure the same textural properties in the muscles. This is due to the fact that texture testing instruments are calibrated to respond linearly to the intensity of the tested mechanical property, which does not apply in the human perception of texture (Szczesniak, 1987). According to Spadaro et al. (2002) linearity will only occur if the biological material is homogeneous.

Conclusions

A trend of decreasing tenderness from dorsal to medial transverse positions was found in sensory analysis. Instrumentally determined transverse variation was found to significantly decrease from dorsal to lateral position, though the medial position did not display significance. Overall, the dorsal position was the more tender of the three positions (dorsal, medial, and lateral).
It was clear that the cranial end was significantly more tender than the caudal end when evaluated from a sensory perspective. However, no significance could be applied to the instrumental measurements hereof.
The individual chops in the left and right sides generally showed a gradual change in tenderness from cranial to caudal end of loins. Significance was found for most sensory evaluated individual chops, which again could not be derived from the instrumental analysis. A marked change in texture was observed at the end of the rib cage. Instrumental analysis was found unable to predict lengthwise texture variation. Sensory analysis was found to consistently describe the longitudinal variation.
An effect of ageing on the transverse variation could not be assigned. A greater decrease in tenderness between individual chops was seen in the cranial than in the caudal end at 0 days of ageing. When the meat was aged more pronounced individual chop variation occurred in the caudal end. Ageing was found to be similar qualitatively in left and right LD
Transverse and longitudinal variation was found to be more distinct in right LD compared to left LD. Furthermore, the decrease in tenderness longitudinally, was markedly higher and more defined into stages in the right loins. The cranial ends were more similar in tenderness from both left and right side of the animals than the caudal ends.

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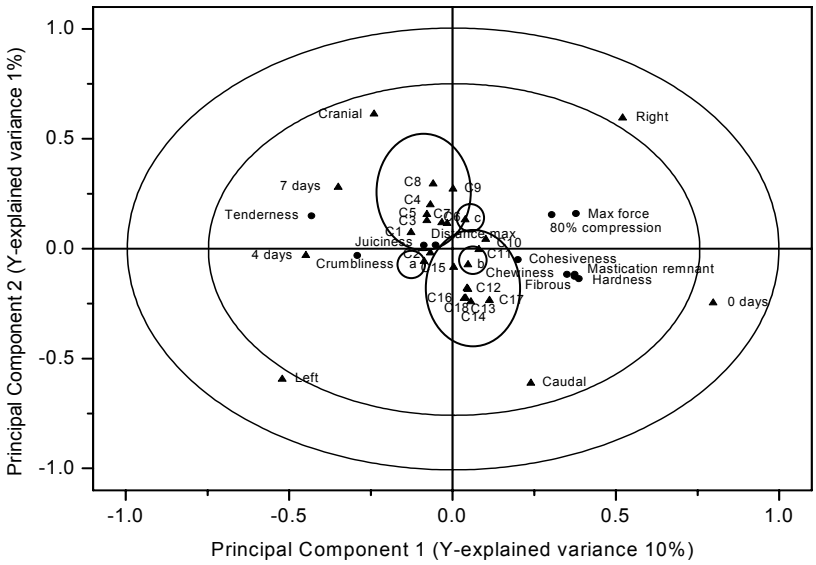

Figure 1. Instrumental and sensory predictive and causal analysis. ANOVA Partial Least Squares Regression (APLSR) correlation loading plot of the first two Principal Components (PCs). Total design (transverse positions and various chops, cranial/caudal, ageing, left/right) in the X-matrix and sensory and instrumental terms in the Y-matrix. Ellipses represent r^2=50 and 100%.

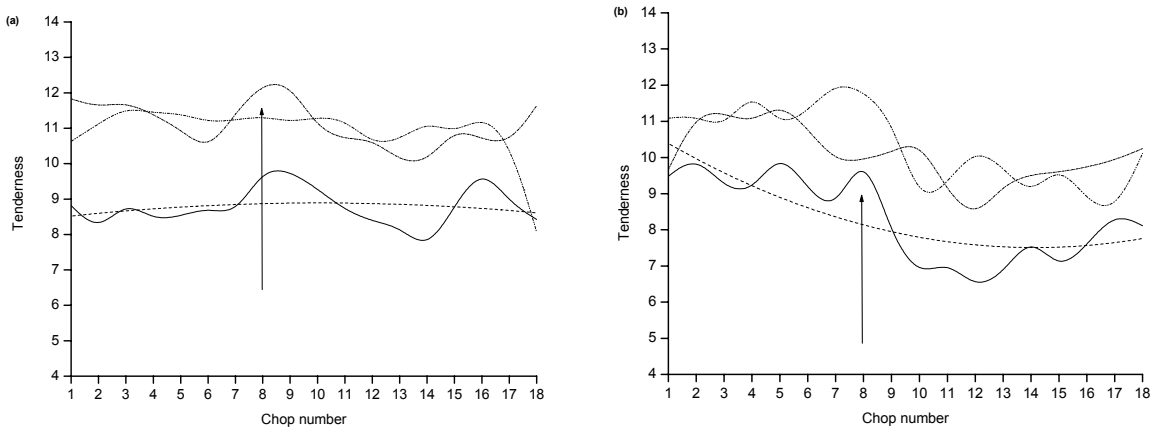

Figure 2. Longitudinal variation of tenderness in (a) left and (b) right loins within each ageing period. muscles respectively, at 0 The solid line represent 0 days of ageing. The dashed and dotted (–¨–) line represent 4 days of ageing. The dotted (¨) line represent 7 days of ageing. The dashed (---) lines represent the average of changes in left and right muscles respectively at 0 days of ageing. Data is averaged over transverse sample positions (a, b, and c) within each chop. Arrows indicate the change point at chop 8.

MODIFICATION OF VERY LONG CHAIN n-3 PUFA IN CHICKEN MEAT

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Background

The content of PUFA n-3 in chicken meat is of great importance for human nutrition. The ratio of n-6/n-3 PUFA in meat should be as narrower as possible (Okuyama and Ikemoto, 1999). This recommendation is based upon the fact that PUFA n-6 and PUFA n-3 affect differently the immunological functions and inflammatory processes in humans and animals (Caldor, 2001). PUFA n-6 acts pro-inflammatory, while PUFA n-3 has anti-inflammatory effects. Within n-3 PUFA group, EPA and DHA are more important than precursors of α-linolenic acid (Ollis et al., 1999). Recommended amounts of specific fatty acids for adults are the following: LA 4.4-20 g/day, LNA 1.35-2.2 g/day) and LC n-3 PUFA 0.16-1.6 g/day (Meyer et al., 2003). According to Ollis et al. (1999), only 15% of LNA is converted to VLC n-3 PUFA. Chicken meat is a valuable source of n-3 PUFA because of the possibility to change the profile of fatty acids (Kralik et al., 2003). The content of fatty acids and cholesterol level in chicken meat can be influenced by using the fats, which differ in their contents of fatty acids (Kralik et al., 1997). The content of EPA and DHA in chicken meat can be increased by addition of fish oil and fish flour to the diets (Chanmugam et al., 1992, Lopez-Ferrer et al., 1999). The content of linoleic and linolenic acids is increased by addition of soybean oil and rapeseed oil to the diets (Scaife et al., 1994).

Objectives

The aim of this research was to determine the effect of addition of PBE oil in different amounts to finishers and the content of n-3 PUFA, i.e. very long-chain fatty acid equivalent (VLCE) in white and dark meat.

Materials and methods

Chickens were divided into five groups. From the 1st to 21st day, they were fed with starter mixture (A), which contained 22.67% of crude proteins and 14.19 MJ/kg ME. From 22nd to 42nd day chickens were fed with finishers (B_1, B_2, B_3, B_4, and B_5), which were balanced at the level of 20.43% of crude proteins. The first control group was given B_1 mixture, while the 2nd, 3rd, 4th and 5th group were fed with B_2, B_3, B_4 and B_5 mixtures, which had the animal fat partially replaced by the Pronova Biocare Epax 3000 T6 preparation (PBE oil), in the amounts of 0.5%, 1%, 1.5% and 2%, respectively. This oil contains, besides other fatty acids, 15,36% eicosapentanoic acid (EPH) and 9.99% docosahexanoic acid (DHA). The content of fatty acids in the lipids of white and dark chicken meat was determined on 9 samples of each group by the Chrompack CP-9000 chromatograph with flame ionization detector. Each fatty acid was shown in % of the total fatty acids (Csapo et al., 1986). On the basis of the content of α-linolenic acid**,** eicosapentanoic and docosahexanoic acids in lipids, the content of stated fatty acids was calculated in white and dark meat. Veri long chain fatty acid equivalent was shown according to Komprda et al. (2003) as follows: VLCE = 0.15 LNA + (EPA + DHA).

Results and discussion

Table 1 shows the data, which refer to the content of fats and n-3 PUFA in white and dark chicken meat. Portions of fats in white meat fluctuated from 1.08 to 1.45%. Highly significant difference is found between 2nd and 3rd group, 2nd and 4th group, as well as between 3rd and 5th group. Chemical analysis of dark meat showed the portion of fats of 4.22-5.02%, but no statistically significant differences were found out between groups. Results that are similar to ours are stated also by Komprda et al. (1999), Škrtić (1999) and Crespo and Esteve-Garcia (2001). The contents of EPA and DHA in lipids of white meat are significantly higher in all experimental groups in comparison to the control groups. In comparison with the 1st group, the 5th group, which was fed with 2% of PBE preparation, contained 291.93% more EPA and 92.54% more DHA in the lipids of breast muscles. The results obtained in this research are similar to those of Hulan et al. (1988), but higher than those stated by Komprda et al. (1999a) and Zelenka et al. (2001). The feeding of chickens with

this preparation resulted in the increase of EPA and DHA in the thigh lipids. The highest content of unsaturated omega-3 fatty acids is found out in the lipids of dark meat in the 5th group, which was fed with mixture containing 2% of PBE preparation. Moreover, the content of EPA and DHA in the lipids of thigh muscles is increased from 0.48% and 1.45% (1st group) to 1.18% and 1,83% (2nd group); 1.30% and 2.83% (3rd group), 2.24% and 3.76% (4th group) and 2.76% and 4.91% (5th group). Statistical analysis showed highly significant differences ($P<0.01$) in the content of EPA and DHA between 1st and 2nd, 1st and 4th, then between 2nd and 5th, and 3rd and 4th, as well as between 3rd and 5th group. Table 2 presents the data of the n-3 PUFA content in g/100 g in white and dark meat. Graph 3 presents VLC n-3 PUFA equivalent in white and dark chicken meat. Komprda et. al. (2003) succeeded to increase the VLC n-3 PUFA equivalent in turkey meat by addition of linseed and fish oil to the diets. The addition of PBE preparation in the diets resulted in the increase of VLC n-3 PUFA equivalent from 48 mg/100 g and 93 mg/100 g to 59 and 143, 60 and 183, 73 and 258, and 119 and 390 mg/100 g, respectively, in white and dark chicken meat.
Comparison of the EPA and DHA contents in the lipids of white and dark chicken meat shows higher deposition of stated fatty acids in the lipids of white meat. Considering the fact that dark meat contains more fats than white meat, the contents of EPA and DHA in dark meat is thus considerably higher than in white chicken meat. Addition of the PBE preparation to diets resulted in the higher deposition of stated fatty acids in both white and dark meat (Table 2).

Conclusions

Based on the obtained results, it can be concluded that the addition of PBE preparation to the diets in the amounts of 0.0%, 0.5%, 1.0%, 1.5% and 2.0% resulted in the statistically significant increase of EPA and DHA in white and dark chicken meat. Very long-chain fatty acid equivalent (VLCE) was also increased in both meat kinds. Related to the increase of concentration of PBE oil in the diets, VLC equivalents were also increased from 48 to 119 in white meat, and from 93 to 90 mg/100 g in dark meat.

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Table 1. Content of fats and PUFA n-3 in lipids of white and dark chicken meat

	Groups					F-value
	1st	2nd	3rd	4th	5th	
			White meat			
Fat, %	1.32	1.38	1.13	1.08	1.45	**
α-Linolenic acid[1]	0.52	0.82	0.61	0.75	0.66	*
EPH[1]	0.62	1.11	1.49	2.21	2.43	*
DHA[1]	2.95	3.03	3.79	4.47	5.68	**
			Dark meat			
Fat, %	4,51	4.51	4.42	4.22	5.02	n.s.
α-Linolenic acid[1]	0.82	0.98	0.91	0.74	0.70	*
EPH[1]	0.48	1.18	1.30	2.24	2.76	**
DHA[1]	1.45	1.83	1.82	3.76	4.91	**

[1]g in 100 g of total fatty acids; n.s. = non significant; * P<0.05; **P<0.01

Table 2. Content of PUFA n-3 (g/100 g) in white and dark chicken meat

	Groups					F-value
	1st	2nd	3rd	4th	5th	
			White meat			
α-Linolenic acid*	0.007	0.011	0.007	0.008	0.010	*
EPH*	0.008	0.015	0.017	0.024	0.035	**
DHA*	0.039	0.042	0.042	0.048	0.082	**
			Dark meat			
α-Linolenic acid*	0.037	0.044	0.040	0.031	0.035	n.s.
EPH*	0.021	0.053	0.058	0,095	0.138	**
DHA*	0.066	0.083	0.125	0.159	0.246	**

n.s. = non significant; * P<0.05; **P<0.01

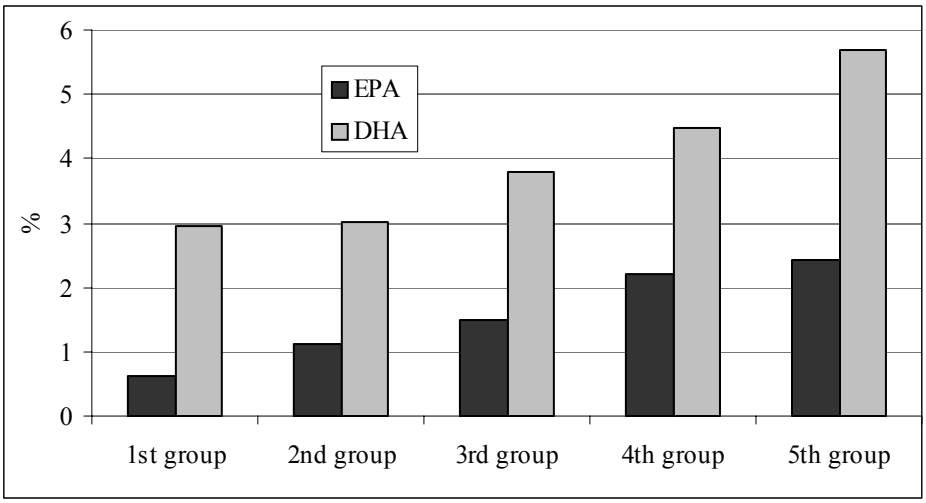

Figure 1. Content of EPA and DHA (%) in total fatty acids of white meat

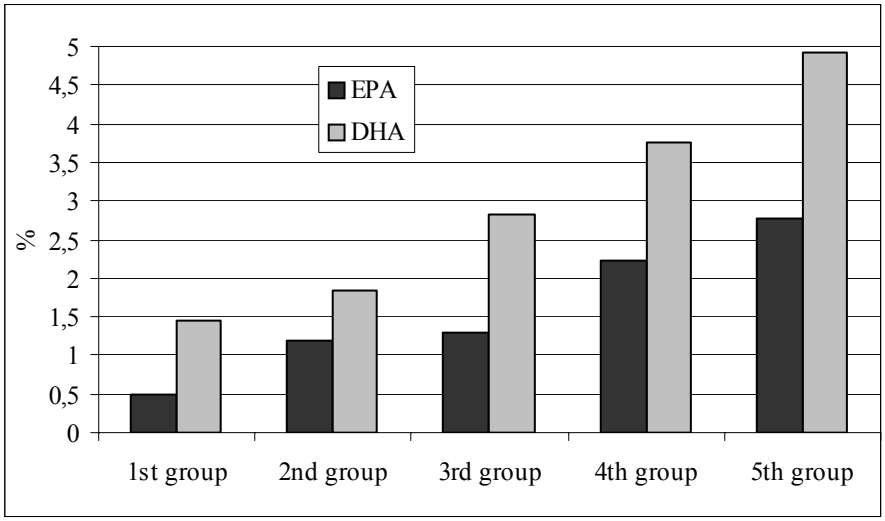

Figure 2. Content of EPA and DHA (%) in total fatty acids of dark meat

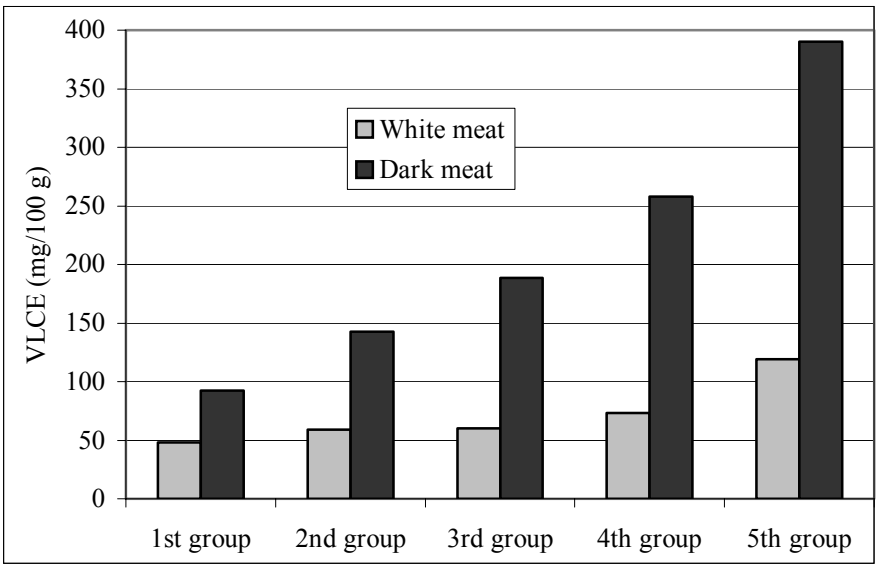

Figure 3. Equivalent of very long chain n-3 polyunsaturated fatty acids (VLCE) in white and dark meat

FACTORS AFFECTING CARCASS AND MEAT QUALITY OF THE HANWOO

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Background

Studies have shown that the fat embedded in the muscle or muscular tissue makes the meat juicy, tender, and tasty. These findings were reported by Parrish (1974) and Donald and Merkel (1993). Estimating quality of meat varies from country to country. Factors such as intramuscular fat content and maturity are important, since they directly affect the meat's tenderness, juiciness, and flavor. Parrish (1974) reported that intramuscular fat explained approximately 12-14% of tenderness. However, according to Gregory, Cundiff and Koch (1995), tenderness was reduced for highly marbled meat, while May, Dolezal, Gill, Ray and Buchanan (1992) reported that high WB-shear force reduced juiciness, flavor, and tenderness. The study also showed that tenderness had a correlation efficient of 0.19 and 0.73 for intramuscular fat content and juiciness, respectively. In addition, Miller, Carr, Ramsey, Crockett and Hoover (2001) reported that WB-shear force lower than 3.0 was tender, while that higher than 5.7 was tougher. Laila (1996) reported that correlation coefficients in relation to objective meat color and intramuscular fat content were 0.07 for L*, 0.06 for a*, and 0.12 for b* values.
However, Shackelford, Wheller, Meade, Reagan, Byrnes, and Koohmaraie (2001) reported that consumer preferences were significantly affected by doneness. As mentioned earlier, the intramuscular fat content was the prime factor affecting the meat quality in the U.S (Donald and Merker, 1993). Thus, according to Talamantes, Long, Smith, Jenkins, Ellis, and Cartwright (1986), beef production system for the effective fat deposit in muscles is important because carcass fat deposit is significantly correlated with intramuscular fat content. Considering sex as a variable, Hardt, Greene & Lunt (1995) observed that heifers had a higher intramuscular fat, with lower maturity and received a higher carcass quality grade.

Objectives

This study determined the meat quality of the Hanwoo (Korean native cattle) as a result of the diversity in the production system for sex and market weight. The physico-chemical traits and palatability of 773 *M. longisimus dorsi* muscles obtained from Hanwoo cattle were evaluated.

Materials and methods

Animals: A total of 773 cattle were sampled from 24 farms across the country and slaughtered in the National Livestock Research Institute (NLRI) slaughterhouse. Longissimus samples (2.5 kg) were taken from the 13th rib of each animal.
Analysis of objective and subjective meat quality:WB-shear force was determined by cooking steaks of 3-cm thickness of *m. longissimus dorsi* (LD) in a 70 ˚C water bath for 60 min. These were cooled in running water (ca. 18 ˚C) for 30 min so that core temperature reached below 30 ˚C. Eight cores were made, each measuring 1.27 cm in diameter. The shear force was measured by using a V-shaped shear blade at 400 mm/min speed (Wheeler et al., 2000). Cooking loss was calculated as percent of weight changes during cooking for WB-shear force measurement. Objective meat color was determined by using a Minolta Chromameter (CR301, Minolta, Japan) on a freshly cut surface of the WB-shear force block after a 30-min blooming at 1°C. Water-holding capacity was determined according to the filter paper method described by Ryoichi, Degychi, and Nagata (1993). Briefly, 0.5g of muscle tissue was placed on glass, and filter paper was pressed against the meat sample at 35-50 kg/cm^2 for 2 min. Water-holding capacity was calculated by using a planimeter. Sensory characteristics were determined by 10 semi-trained panelists who were randomly selected from a total of 15 recruits. They estimated the values of tenderness, flavor intensity, juiciness by using a six-point scale.
Statistical analysis:ANOVA, Duncan-test, and correlation coefficients were determined by using SAS package (1996). The following analyses of variations were performed to estimate the effects of sex and live weight on carcass quality. Yijk = μ + SEXi + WTCLASSj + (SEXxWTCLASS)ij + eijk, where Yijk =

carcass quality,μ = mean, SEXi = sex effect of ith carcass (i = 1, 2, 3), WTCLASSj = carcass weight effect of jth carcass (j = 1,2,3,4,5,6), (SEX x WTCLASS)ij = interaction between ith sex and jth carcass weight, eijk = error.

Results and discussion

Intramuscular fat content increased with increasing live weights for cows (3.07-4.00), bulls (1.00-1.96), and steers (2.75-4.22). Cows and steers contained higher intramuscular fat than bulls when live weight was lighter than 500 kg ($P < 0.05$). Maturity ranged from 1.00 to 2.02, with higher scores for heavier weights. Bulls matured faster than steers and cows ($P < 0.05$). The results showed a faster calcification for cows compared with that of other groups. A lighter meat color for cows and steers than that of bulls was thought to be related to the higher intramuscular fat content for these groups. Cows had a whiter fat color than that of other groups within the same weight group. Frequency of carcass quality grade "1" increased with a corresponding increase in live weights, and cows and steers had a higher frequency than that of bulls ($P < 0.05$). Sex significantly affected the intramuscular fat content, maturity, meat color, fat color, and texture ($P < 0.01$), while live weights (Table 3) had significant effects on intramuscular fat and maturity ($P < 0.01$). Carcass quality grade had a higher correlation with intramuscular fat content (r=0.87), meat color (r=0.32), and texture (r=-0.54); but had a relatively weaker correlation with fat color (r=0.07) and maturity (r=-0.04). These results were similar with those of the previous studies conducted by Griffin (1992) and Dikeman, Cundiff, Gregory, Kemp and Koch (1998). They all reported that high slaughter weights resulted in higher carcass quality grade. In addition, Mckenna et al. (2002) reported that higher slaughter weights resulted in higher intramuscular fat, but did not affect maturity. For meat color, that of bulls was darker than that of cows and steers. This result was consistent with that of Seideman, Cross, Oltjen and Schanbacher (1982). In terms of sex as a variable, Park et al. (2002) reported that the difference in sex for Hanwoo cattle significantly affected carcass quality factors, where intramuscular fat content was highly correlated with carcass quality grade (r=0.81). This finding is also consistent with that of the previous study.

Protein content decreased with increasing marbling scores from 22.4% to 16.6% ($P < 0.01$), while intramuscular fat content increased from 5.16 to 19.46% ($P < 0.01$). Similarly, moisture content decreased from 71.24% to 61.04% ($P < 0.01$) and ash content decreased from 0.95% to 0.67% ($P < 0.01$). Average protein content of Hanwoo meat (21.28%) was similar with that of the European breed (21.43%) as reported by Browning, Huffman, Egbert & Jungst, 1990 and that of the Japanese black cattle (20.4%) according to Konishi, Nade, Maeda & Uchiyama (1995). On the other hand, intramuscular fat content was lower than 11.9% in the Japanese black cattle (Konishi, Nade, Maeda & Uchiyama, 1995), and higher than 5.91% in the cross breeds (Miller, Ramsey, Claborn & Wu, 1995). In addition, the current result was consistent with that of the previous study (Jones, Savell & Cross, 1990) who reported that intramuscular fat content increased with increasing marbling score and carcass quality grade. Meanwhile, data on moisture obtained in this study (69.34%) was lower than 72.9-75% as reported by Van Koevering, Gill, Owens, Dolezal, and Strasia (1995) and Corah, Tatum, Morgan, Mortimer, and Smith (1995). For ash content, it is generally known that it is approximately 1% in beef muscle, and the ash content obtained from this study was similar with that obtained from the previous report (Miller, Cross, Baker, Byers & Recio, 1988) which showed that ash content was similar between breeds.

The results showed that higher fat contents significantly increased tenderness (from 3.35 to 4.46), flavor (from 4.22 to 4.76), and juiciness (from 3.99 to 4.70). These findings support the observation that intramuscular fat is the most significant variant that explains approximately 7-15% of meat tenderness (Parrish, 1974). These findings also agree with those of the previous report (Shackelford, Wheeler & Koohmarmie, 1995) which showed significant correlation between marbling score and meat tenderness. Likewise, the results agreed with those of Lorenzen et al.(2003) who reported that meat flavor and juiciness were improved by 0.3 and 0.4 between low select and top choice. Mckenna et al. (2004) similarly reported that increase in marbling score improved overall sensory characteristics.

The results showed that WB-shear force and cooking loss decreased as marbling score increased, with averages of 5.2 kg and 29.4% ($P < 0.01$), respectively. On the other hand, water-holding capacity significantly increased ($P < 0.01$) at an average of 47.39%. The result was similar to that in the Japanese black cattle (5.6 kg) as reported by Konishi, Nade, Maeda & Uchiyama (1995), and for and in the Angus and Hereford (5.06-5.38 kg) as reported by May, Dolezal, Gill, Ray & Buchanan (1992). Meanwhile, studies by Cross, Savell and Francis (1986) showed water-holding capacity results as higher than 39.0-43%. But the significant increase in water-holding capacity with increasing marbling score tended to be similar with that

of Kim and Lee's study (2003). Their study showed that the water-holding capacity increased from 51.3% to 55.6% from a carcass quality grade of 3 to 1. Van Koevering, Gill, Owens, Dolezal and Strasia (1995) reported 21.7-31.7% of cooking loss and Konishi, Nade, Maeda and Uchiyama (1995) reported 29.2% for American-Japanese black cattle, the current result was similar with the previous results.

Sex significantly ($P < 0.01$) affected the chemical composition and sensory characteristics. Sex also had significant ($P < 0.01$) effects on the objective color measurements (Table 6), and lightness had a low correlation with intramuscular fat content (r=0.17, Table 7). As far as slaughter weight is concerned, it significantly affected fat, moisture, and protein contents ($P < 0.01$), but this did not influence sensory characteristics. The significant differences in fat content between the sex groups and between slaughter weights indicated that these factors influenced fat deposition within the examined *longissimus* muscle. This result was consistent with that obtained by Miller, Cross, Baker, Byers and Recio (1988), where sex significantly affected the intramuscular fat content. The significant correlation between intramuscular fat content and juiciness (r=0.35), tenderness (r=0.23), and flavor (r=0.34); and between marbling score and juiciness (r=0.39), tenderness (r=0.26), and flavor (r=0.42) demonstrated that the amount of fat played a significant role in sensory characteristics. This result agreed with that of the previous studies (James, Mcbble & Jack, 1967; Shackelford, Wheeler & Koohmarmie, 1995) in that juiciness and tenderness received significantly higher scores from highly marbled muscles. Table 7 shows the effect of sex and slaughter weight on the objective meat quality traits. The result shows that sex had a significant effect on WB-shear force, but slaughter weight had no such effect. Sex also significantly affected water-holding capacity, and this result was similar with that of Waggoner, Dikeman, Brethour and Kemp (1990). WB-shear force significantly correlated with ($P < 0.01$) with juiciness (r=-0.29), tenderness (r=-0.34), flavor (r=-0.29), and intramuscular fat content (r=-0.32). The significant relationship between WB-shear force and intramuscular fat was similar with the result obtained by Gregory, Cundiff and Koch (1995). In addition, negligible effect of sex, slaughter weight, and intramuscular fat content on cooking loss was similar to that observed by Jones, Savell and Cross (1990). The high correlation between intramuscular fat content and moisture (r=-0.92) was similar to the previous result of Parrish (1974). On the other hand, the objective meat tenderness for carcass quality grades 2 and 3 was higher than 4.32 kg for Angus (Tatum, Smith & Carpenter, 1982) and 4.54 kg for Hereford (Patil, Goetsch, Lewis & Geird, 1993) for Angus. This suggests that the chiller aging treatment is required for the quality grade. Objective meat color of CIE L, a and b dimensions significantly ($P < 0.01$) increased as marbling scores increased from 1 to 7. Sex had significant ($P < 0.01$) effects on the objective color measurements (Table 6), and lightness had a low correlation with intramuscular fat content (r=0.17, Table 7). Subjective meat and fat color assessed by a 7-point scale indicated that higher intramuscular fat content reduced the subjective color scores of 4.54-4.80 for meat color and 2.58-3.04 for fat color. The result of objective color dimensions (i.e., CIE L, a and b) indicated that lightness and yellowness were similar with the previous results (Shackelford, Wheeler & Koohmarmie, 1995; Laila, 1996), but redness was stronger for Hanwoo beef.

Conclusions

As marbling degree increased, Warner-Bratzler shear force and cooking loss correspondingly decreased, while the water-holding capacity increased ($P < 0.01$). In the panel tests, the scores of tenderness, flavor, and juiciness increased ($P < 0.01$) with increasing marbling degrees. The shear force values had a negative correlation with juiciness, tenderness, and flavor. Sex of Hanwoo had significant effects on the carcass quality grade factors. The results of this study suggest a need for comparative studies on the range of marbling scores based on intramuscular fat content, and on objective and subjective meat quality for increasing sub-class of carcass grading which is included in the currently used four classes of carcass quality grading in Korea.

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CHANGES IN PURGE LOSS, WATER HOLDING CAPACITY AND TENDERNESS DURING AGING OF DIFFERENT MUSCLES FROM PIG CARCASSES

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Background

Pork shoulders and hams are traditionally used as raw material for further processing of meat products and often are considered to be lower value than other cuts such as the pork loin. Recently, the pork industry has been investigating the potential for adding value to the pork carcass by identifying individual muscles or muscle groups within the shoulder and ham that are well-suited for new uses in processed products or for new product development. However, while the general properties of primal cuts as a whole are understood, the characteristics of individual muscles making up the primal cuts are not well known. Therefore, there is a need for research on meat quality characteristics of individual muscles from pork shoulders and hams. Further, it is important to know the effects of aging on the specific muscles in order to make the best use of individual muscles in processed products.

Objectives

The objectives of this experiment were to determine the changes in purge loss, water holding capacity, and tenderness during aging for ten selected muscles dissected from pig carcasses following harvest.

Materials and methods

Animals, treatments and sample preparation: Seven barrows or gilts (Hampshire sire × crossbred dam) were humanely harvested at the Iowa State University Meat laboratory. Carcasses were chilled in a forced-air cooler at -5 °C for 24h. Live weights, carcass weights, 45-minute pH and 45-muscle temperatures were recorded and are shown in Table 1. At 1 day postmortem, the *biceps femoris* (BF), *gluteus medius* (GM), *gracilis* (G), *infraspinatus* (I), *longissimus dorsi* (LD), *pectoralis profundi* (Fan, PP), *rectus femoris* (RF), *semitendinosus* (ST), *spinalis* (S), *triceps brachii* (TB) were dissected from both sides of each carcass. Muscles were cut into 2.54 cm thick portions, vacuum-packaged and aged at 4 °C for 7days.

Purge loss, water holding capacity (WHC) and WB-shear force: Purge loss was determined by weighing the muscle portions before vacuum-packaging and after 1, 3 or 7 days of aging to calculate percentage weight losses. WHC was determined using the method described by Honikel and Hamm (1994). WB-shear force measurements were performed using cooked muscle portions (internal temperature at 71 °C) by a texture analyser (TA-XT2i texture analyser, USA). Preparation of the samples for the tenderness measurement was done in accordance to AMSA Guidelines (American Meat Science Association, 1995). The conditions for the texture analyser was a pre-test speed of 3.0mm/s, test speed of 3.3mm/s, post-test speed of 10.0mm/s, distance of travel at 80.0 %, and trigger force of 0.15kg.

Statistical analysis: Data were analyzed using PROC GLM (SAS, 1998).

Results and discussion

Purge loss (%) was significantly increased ($P < 0.05$) in all muscles during aging (Table 2). The GM had the highest ($P < 0.05$) purge loss, while the TB was significantly lower ($P < 0.05$) than the other muscles. After 7 days of aging, the range for purge loss values from all muscles was 3.46-8.93%.

The WHC value for GM and LD muscles was significantly increased by aging, but the G and S were decreased ($P < 0.05$) as the aging period increased (Table 3). The S had the highest WHC ($P < 0.05$), while the BF was lower in WHC than the other muscle after 7 days of aging. The range of WHC values in all muscles was 87.36-93.49% after 7 days of aging.

It is generally accepted that the source of drip from pork is intracellular water which is lost from the muscle fibre post-mortem, driven by a pH and calcium-induced shrinkage of myofibrils during rigor development (Offer et al., 1989). Moeseke and Smet (1999) reported an increase in WHC of fresh meat during aging using a traditional drip measurement where samples were taken several days after slaughter for comparison with samples taken at 1 day post-mortem. However, in our study, WHC of the G and S muscles decreased, and the I, RF, ST and TB muscles showed a tendency to decrease during aging. Honikel and Hamm (1994) reported that conventional drip losses during storage of pork were in the range of 1-10%.

The WB-shear force for the TB was significantly decreased ($P < 0.05$) during aging, but the other muscles were not affected during aging. After 7 days of aging, the WB-shear force value for the S was the lowest ($P < 0.05$) among the muscles studied, but the BF was higher than the other muscles. The range for WB-shear force in the 10 muscles studied was 3.29-7.10 kg after 7 days of aging.

It has been reported that pork was completed of aging-related changes within 4-6 days post slaughter (Rees et al, 2002). Also, Wheeler et al. (2000) reported that tenderness ratings from trained sensory evaluation were highest for *semitendinosus* (7.2) and *triceps brachii* (7.1), followed by *longissimus lumborum* (6.4) and *semimembranosus* (5.7) and were lowest for *biceps femoris* (4.0). In the current study, WB-shear force values were greatest for the BF and lowest for the S. The TB showed significant tenderization during aging but the 9 other muscles were not significantly affected during aging.

The simple correlations between WB-shear force and water holding were 0.26 (purge loss) and –0.41 (water holding capacity) ($P < 0.0001$).

Conclusions

Purge loss of the TB was significantly less than for the other muscles in this study, while the GM had higher purge loss than the other muscles during aging. The WHC of GM and LD was significantly increased, but that of the G and S was decreased as the aging period increased. The S had the greatest WHC, while the BF had lower WHC than the other muscles after 7 days of aging. The purge loss and WHC values suggest that individual muscles will perform quite differently in processes utilizing differential amounts of added water and will affect expected yields depending on how individual muscles may be used Tenderness of the TB was significantly increased by aging, but the other muscles were not affected during the aging period. After 7 days of aging, the S remained the most tender, while the BF remained tougher than the other muscles studied. Thus, tenderness differences in pork muscles appear to largely retain the differences inherent to individual muscles and do not show a great deal of change as a result of aging.

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Table 1. Information of pork carcasses used in the experiment (n=7).

Item	Live weight (kg)	Hot Carcass weight (kg)	pH $_{45\ min}$	Temp.(℃) $_{45\ min}$
Mean±SE	111.59±15.47	85.34±10.90	6.43±0.26	39.01±1.62

Table 2. Changes of purge loss (%) for 10 muscles from pork carcasses during aging.

Muscle***	Ageing time (day)		
	1	3	7
BF	4.00 ± 0.39^{BCc}	5.51 ± 0.51^{BCb}	7.12 ± 0.62^{BCa}
GM	5.42 ± 0.45^{Ab}	7.99 ± 0.49^{Aa}	8.93 ± 0.57^{Aa}
G	1.44 ± 0.20^{Dc}	2.55 ± 0.34^{Eb}	3.88 ± 0.49^{EFa}
I	3.03 ± 0.36^{Cb}	4.39 ± 0.45^{CDa}	5.43 ± 0.57^{CDEa}
LD	3.70 ± 0.60^{BCb}	4.99 ± 0.68^{BCab}	6.97 ± 0.88^{BCDa}
PP	3.07 ± 0.43^{Cc}	4.95 ± 0.51^{BCb}	6.59 ± 0.58^{BCDa}
RF	3.20 ± 0.52^{BCb}	4.98 ± 0.68^{BCab}	5.14 ± 0.65^{DEFb}
ST	4.46 ± 0.61^{ABb}	6.12 ± 0.42^{Bab}	7.66 ± 0.83^{ABa}
S	1.58 ± 0.21^{Db}	2.43 ± 0.36^{Eb}	3.56 ± 0.36^{EFa}
TB	1.51 ± 0.19^{Db}	3.08 ± 0.35^{DEa}	3.46 ± 0.31^{Fa}

* A-F : Means±SE in a column with different letters are significantly different ($p<0.05$).
** a-c : Means±SE in a row with different letters are significantly different ($p<0.05$).
*** Muscles are biceps femoris (BF), gluteus medius (GM), gracilis (G), infraspinatus (I), longissimus dorsi (LD), pectoralis profundi (Fan, PP), rectus femoris (RF), semitendinosus (ST), spinalis (S) and triceps brachii (TB)

Table 3. Changes of water holding capacity (%) for 10 muscles from pork carcasses during aging.

Muscle	Ageing time (day)		
	1	3	7
BF	87.06 ± 1.07^{CD}	87.76 ± 0.80^{C}	87.36 ± 0.57^{E}
GM	85.83 ± 0.67^{Db}	87.45 ± 0.80^{Cab}	88.72 ± 0.59^{DEa}
G	92.79 ± 0.45^{Ba}	92.71 ± 0.86^{Ba}	89.33 ± 0.73^{CDb}
I	92.18 ± 0.72^{B}	91.93 ± 0.82^{B}	91.38 ± 0.72^{B}
LD	85.03 ± 0.69^{Db}	87.79 ± 0.42^{Ca}	87.92 ± 0.41^{DEa}
PP	90.59 ± 0.71^{B}	90.79 ± 0.59^{B}	91.25 ± 0.43^{B}
RF	91.65 ± 0.97^{B}	91.83 ± 0.89^{B}	89.55 ± 0.44^{CD}
ST	88.50 ± 0.73^{C}	88.58 ± 0.84^{C}	88.35 ± 0.68^{DE}
S	96.35 ± 0.44^{Aa}	96.16 ± 0.40^{Aa}	93.49 ± 0.62^{Ab}
TB	92.10 ± 0.73^{B}	92.07 ± 0.63^{B}	90.46 ± 0.34^{BC}

* A-E : Means±SE in a column with different letters are significantly different ($p<0.05$).
** a-b : Means±SE in a row with different letters are significantly different ($p<0.05$).

Table 4. Change of WB-shear force (kg) for 10 muscles from pork carcasses during aging.

Muscle	Ageing day (day)		
	1	3	7
BF	7.32±0.20^{A}	7.31±0.21^{A}	7.10±0.19^{A}
GM	5.95±0.18^{B}	5.80±0.15^{B}	5.75±0.17^{B}
G	3.91±0.12^{D}	3.97±0.10^{E}	3.93±0.13^{D}
I	4.27±0.12^{D}	4.30±0.10^{DE}	4.00±0.12^{D}
LD	5.95±0.15^{B}	5.59±0.18^{BC}	5.55±0.18^{B}
PP	5.66±0.11^{B}	5.70±0.12^{B}	5.67±0.10^{B}
RF	5.22±0.22^{C}	5.20±0.16^{C}	5.35±0.20^{B}
ST	4.82±0.12^{C}	4.61±0.15^{D}	4.80±0.11^{C}
S	3.34±0.13^{E}	3.25±0.13^{F}	3.29±0.15^{E}
TB	5.75±0.14^{Ba}	5.76±0.09^{Ba}	5.41±0.12^{Bb}

* A-F : Means±SE in a column with different letters are significantly different ($p<0.05$).
** a-b : Means±SE in a row with different letters are significantly different ($p<0.05$).

EFFECTS OF HOUSING SYSTEMS ON CARCASS CHARACTERISTICS OF FINISHING PIGS

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Background

Production of quality pig meat in conditions that positively affect welfare and health of growing pigs is lately becoming more important. Growing of pigs on straw bedding meets the requirements that are set to producers as far as the pigs' health and welfare is concerned. In that sense, the scientific investigations of such housing system are being intensified. The most obtained results show many advantages of such housing system however, there are also some disadvantages of deep litter housing system in relation to the conventional housing systems. When compared to the conventional housing system, the majority of scientists agree that there is a cost benefit of deep litter housing system (Gentry et al., 2002a, Morrison et al., 2003a), as well as benefits for animal welfare and environment protection (Lyons et al., 1995, De Yong et al., 1998, Beattie et al., 2000, Kelly et al., 2000, Klont et al., 2001, Guy et al., 2002, Morrison et al., 2003b). Investigations of productivity and slaughtering characteristics of pigs point out not only advantages of deep litter housing system (Beattie, 1996; Morgan et al., 1998; Beattie et al., 2000; Spolder et al., 2000; Turner et al., 2000; Klont et al., 2001; Maw et al., 2001; Lombooij et al., 2004), but also some negative aspects that are caused by this way of pig housing (Gentry et al., 2002b; Honeyman and Harmon, 2003; Morrison et al., 2003a, 2003b).

Objectives

Having in mind the opposite results of our previous researches, the aim of this research was to compare productive, slaughtering and economic characteristics of conventional and deep litter housing systems.

Materials and methods

The crossbreeds (GLxLW) x GL, divided into two groups, were used for this research. The first group of pigs was kept on deep litter, while the second group was kept in a conventional way, on cross-barred floor, without straw bed. Pigs in both groups were fed equally. In the first fattening phase (27-60 kg), the pigs were fed with mixture that contained 17.68% of crude proteins and 12.98 MJ/kg ME; in the second phase of fattening (60-110 kg) that mixture contained 14.71% of crude proteins and 13.10 MJ/kg. Throughout the fattening process, the food consumption was controlled, and the average daily weight gain, food conversion and costs of live weight gain were calculated. Throughout slaughtering, the meat portion (M%) in carcasses was obtained by the "two points" method (Rule Book, 1999, 2001), using the following formula:

$$M\% = 47.978 + 26.0429\frac{F}{M} + 4.5154\sqrt{M} - 2.50181\log_{10} F - 8.4212\sqrt{F}$$

F = the minimum thickness of visible fat (including rind) on the midline of the split carcases in millimeters, covering the lumbar muscle (*M. glutaeus medius*), M = the visible thickness of the lumbar muscle on the midline of the split carcases in millimeters, measured at the shortest connection between the front (cranial) end of the lumbar muscle and the upper (dorsal) edge of the vertebral canal.

According to the meat portions, the carcasses were divided into the (S)EUROP commercial classes. Costs of housing, feeding, health protection, as well as other costs that are in relation to specific conditions were taken into consideration in order to determine the economic factors.

Results and discussion

At the beginning of fattening process, live weight of growing pigs in both groups was almost the same (27.50 kg and 26.90 kg, respectively). Fattening of pigs on straw-bedded floor lasted for 117 days, and on cross-barred floor for 114 days. Pigs of both groups had equal average daily weight gains (0.67 kg and 0.66 kg, respectively), but the food consumption per kg of live weight gain was better in pigs that were kept on deep

litter than in pigs of the second group that were kept conventionally (3.15 kg and 3.31 kg, respectively). Food conversion was also better in the first group, kept on deep litter, than in the second group. Data obtained for mortality and waste of pigs did not show results that can differentiate between the two housing systems (Table 1). Throughout the fattening process, it was noticed that the pigs kept on deep litter spent more time moving around and were less aggressive than the pigs on cross-barred floor. Similar conclusions were stated by Lyons et al. (1995) and Morrison et al. (2003a). Positive effects of deep litter on the welfare and behavior of growing pigs was also pointed out by De Jong et al. (1998), Kelly et al. (2000), Turner et al. (2000), as well as Day et al. (2002).

Productive and slaughtering characteristics significantly affect the effectiveness and cost benefits of pig production. Beatie et al. (2000) state that pigs kept on deep litter in their finishing growth phase had better food consumption, less food usage per kg of live weight gain, better weight gain, and thicker back fat when compared to pigs that were kept in a conventional way. Lambooij et al. (2004) found out that the pigs kept on deep litter had significantly higher weight of carcasses and better water holding capacity. Honeyman and Harmon (2003) found out that, in comparison to the pigs kept on half-cross-barred floor, pigs kept on deep litter had higher average weight gain in the summer months, while in the winter months, they had equal average daily gain, but weaker conversion.

Data on the slaughtering traits of carcasses (Table 2) show that pigs kept on deep litter had higher weight of warm carcasses, thinner back fat and higher portion of muscular tissue in carcasses. Therefore, the classification of carcasses proved the advantages of deep litter housing system. The S and E commercial classes had the portion of 77.14% and 66.67%, respectively. Carcasses of the R class were not present in pigs kept on deep litter (Graph 1). The deep litter housing system resulted in better average meatiness of carcasses than the conventional system (58.25% and 57.07% of muscular tissue). Classification of carcasses into commercial classes was also in favor of the deep litter housing system. However, Klont et al. (2001) and Gentry et al. (2002a) did not find differences in meatiness between pigs of two different housing systems.

Analysis of economic aspects of housing systems (Table 3) shows that the deep litter housing system has better financial results. This is in relation to the lowering the costs per finishing pig, and gaining the better market price of the product as well as of the produced fertilizer. Similar conclusions were made by Gentry et al. (2002a) and Morrison et al. (2003a). Lowered costs per finishing pig and satisfactory quality of pork are the main preconditions of productivity and profitability of pig production.

Conclusions

Based on the obtained results, it can be concluded that pigs, which were kept on deep litter had better food consumption than pigs kept conventionally. Analysis of average daily gain values did not show differences related to housing systems of finishing pigs. Slaughtering characteristics show that pigs kept on deep litter had heavier warm carcasses, thinner back fat and greater portion of muscular tissue in carcasses. Moreover, classification of warm carcasses was in favor of deep litter housing system. Lowered costs and higher market price of such finishing pigs proved that deep litter housing system is also financially better than the conventional housing systems.

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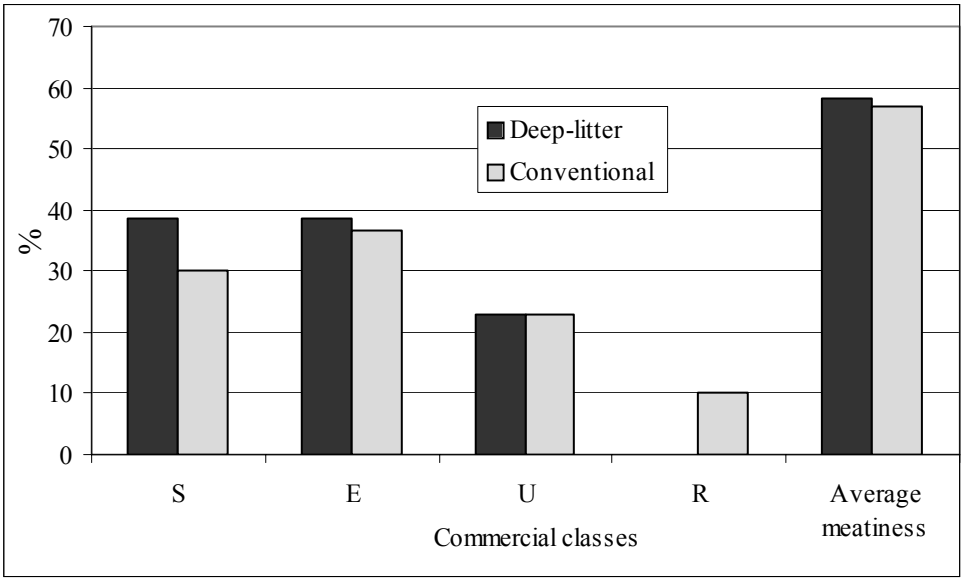
Table 1. Fattening productivity data

Indicators	Housing system	
	Deep litter	Conventional housing
Beginning of fattening, no. of pigs	106	117
End of fattening, no. of pigs	100	110
Fattening period, days	117	114
Starting weight, kg	27.50	26.90
End weight, kg	106.60	104.50
Total weight gain, kg	79.10	77.60
Average weight gain, kg	0.67	0.66
Food/HD, kg	2.10	2.17
Food/kg of gain	3.15	3.31
Food usage, %	47.62	46.08
Mortality, %	3.77	3.42
Waste, %	1.89	2.56

Table 2. Slaughtering traits of carcasses

Commercial classes	%	Weight, kg	Average F (mm)	Average M (mm)	Meatiness %
Deep litter housing system					
S	38.57	79.33	8.63	71.07	62.36
E	38.57	82.00	13.56	72.37	57.44
U	22.86	82.69	18.36	67.06	52.69
Total	100.00	81.13	12.76	70.66	58.25
Conventional housing system					
S	30.00	78.94	8.67	81.94	62.49
E	36.67	80.41	10.18	69.32	57.30
U	23.03	81.36	18.93	71.14	53.02
R	10.00	83.17	24.50	69.17	49.38
Total	100.00	80.47	14.30	70.52	57.07



Graph 1. Division of carcasses according to (S)EUROP classification

Table 3. Cost benefit analysis of two different housing systems

Structure of incomes and expenses	Value of pig (in EUR)	
	Deep litter housing	Conventional housing (without deep litter)
Expenses		
Growing pig	35.85	36.85
Food	41.00	42.30
Veterinary costs	6.00	8.00
Other costs	6.65	6.65
Straw	1.85	-
1. Total expenses	91.35	93.80
Incomes		
Fattened pig	155.20	147.40
Fertilizer	10.50	-
2. Total incomes	165.70	147.40
Profit (2– 1)	**74.35**	**53.60**

EFFECTS OF DIETARY *RHUS VERNICIFLUA* STOKES SUPPLEMENTATION ON MEAT QUALITY CHARACTERISTICS OF HANWOO (KOREAN CATTLE) STEERS DURING REFRIGERATED STORAGE

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Background

In recent years, there has been a global trend toward the use of natural substances present in fruits, vegetables, and herbs as antioxidants and functional foods (Farr, 1997). Greene (1969) was one of the first investigators to report on the potential color-preserving effect of antioxidants in meat. *Rhus verniciflua* Stokes (RVS) belongs to Anacardiaceae and has been used traditionally for medicinal purposes and for the protection of antiquities in Korea for a long time (Kim, 1996). Recently, it was shown that RVS has an antioixdant function. Antioxidant activity of *Rhus verniciflua* Stokes has been reported to correspond to well known enzymatic and non-enzymatic antioxidants in model linoleic emulsion experiments (Lim and Shim., 1997). The stem bark of *Rhus verniciflua* contains a high level of urushiols, which are polymerized formation of a lacquer film by the radical-chain reaction (Hirota et al., 1998). The exudate was previously found to have anti-AIDS, a strong antioxidant and immune-enhancing activities (Miller et al., 1996). However, urushiol was the main irritating component of exudate constituents of *Rhus verniciflua.* The heartwood of *Rhus verniciflua* dose not cause this type of allergenic action, which implies that it does not contain urushiols (Park et al., 2004). However, information on the application of *Rhus verniciflua* Stokes is still limited.

Objectives

The objective of this study was to determine the effects of different levels (0%, 2%, 4% and 6%/feed) of dietary *Rhus verniciflua* Stokes supplementation on meat color, water-holding capacity, volatile basal nitrogen, lipid oxidation and fatty acid composition in *M. longissimus* from Hanwoo (Korean cattle) steers during refrigerated storage at 3 ˚C.

Materials and methods

Animals, diets and treatments. Sample of *Rhus verniciflua* Stokes was collected from nature at Gapyeong, Gyeonggi province, Korea in 2003. The bark and heartwood of *Rhus verniciflua* Stokes were reduced to sawdust by means of an electrical saw. Hanwoo (Korean cattle) steers were divided into four groups. Control group (n=3) was fed a common basal diet (Control group) for 26 months. The other groups (n=5/group) were fed a supplemented concentrate diet with a *Rhus verniciflua* Stokes supplement of 2%, 4% and 6%/feed for 4 months before slaughter. The *Longissimus* muscle was removed about 48 hr after slaughter. Muscles were sliced (1.2 cm thickness), then overwrapped in polyethylene wrap film (oxygen transmission rate 35,273 $cc/m^2/24hr/tm$, thickness 0.01 mm). Samples were then held 7 days at 3˚C.

Analytical procedures. CIE L^* (lightness), a^* (redness), and b^* (yellowness) values for Illuminant C were measured by a color difference meter (CR-310, Minolta Co., Tokyo, Japan). Also, chroma (C^*) and hue-angle (h^o) values were calculated as $C^* = (a^{*2}+b^{*2})^{1/2}$, and $h^o = \tan^{-1}(b^*/a^*)$, respectively. The relative content of metmyoglobin at the meat surface was calculated by the method of Kryzwicki (1979) using reflectance at 473, 525, 572, and 730 nm. Reflectance readings were converted to absorbance[2-log(%reflectance)] and used in the equation (Demos et al., 1996). The pH value was determined by homogenizing 10 g sample with 100 ml distilled water for 1 min. A press technique reported by Grau and Hamm (1953) was used to determine water-holding capacity (WHC). Thiobarbituric acid reactive substances (TBARS) was measured according to the modified method of Sinnhuber & Yu (1977). VBN (volatile basic nitrogen) value was measured by Conway's method (Kohsaka, 1975). Total lipids for fatty acid analysis were extracted from muscle using the method of Folch et al (1957). Fatty acid methyl esters were prepared according to the procedure of Sukhija and Palmquist (1988). Data were analyzed as a 4 (diet condition) by 4 (storage day) factorial design using the General Linear Model procedure of SAS.

Results and discussion

As shown in Table 1, The CIE L^*, a^*, b^* and C^* values of 4% group were significantly ($p<0.05$) higher than those of the other groups over time. The CIE a^*, b^* and C^* values except L^* value were significantly ($p<0.05$) decreased during refrigerated storage in all of the groups. In particular, control and 6% groups were more accelerated compared to the other groups. The a^* value of day 7 was significantly ($p<0.05$) higher in 2% and 4% groups than in the other groups. The trend for C^* value was similar to that for a^* value. Hue angle increased ($p<0.05$) as storage time increased. And control group at day 7 had higher ($p<0.05$) hue angle than the other groups. As shown in Figure 1, the metmyoglobin (%) of day 0 was significantly ($p<0.05$) higher in 4% and 6% groups than in the other groups. It was significantly ($p<0.05$) increased during storage time in all of the groups, but 4% group had a lower rate of metmyoglobin accumulation during storage. As a whole, water-holding capacity (Fig. 2) was significantly ($p<0.05$) lower in control group than in the other groups. TBARS value (Fig. 3) which represent fat rancidity tended to increase as storage time increased in all of the groups. The TBARS value of day 0 was not significantly ($p>0.05$) different among 4 diet conditions, but the TBARS value after 5 days of storage was significantly ($p<0.05$) higher in control group than in other groups. The volatile basic nitrogen (Fig. 4) of 0 day was significantly ($p<0.05$) higher in control group than in the other groups. VBN increased gradually during storage, the VBN of day 7 was significantly ($p<0.05$) lower in 4% groups. As shown in Table 2, the proportions of C18:1, MUFA, UFA and MUFA/SFA ratio were significantly ($p<0.05$) higher in the meat from *Rhus verniciflua* Stokes-supplemented Hanwoo (Korean cattle) than in the control group. But, the proportions of SFA was significantly ($p<0.05$) highest in control group.

Conclusions

The meat from *Rhus verniciflua* Stokes-supplemented Hanwoo (Korean cattle) was effective in increasing color stability, water-holding capacity, unsaturated fatty acids and retarding lipid oxidation than was the control meat. In particular, dietary *Rhus verniciflua* Stokes supplementation with 4% of Hanwoo beef extended color display life compared to the other groups.

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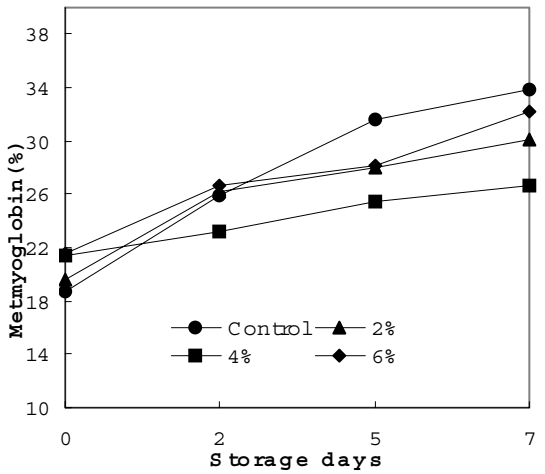

Fig. 1. Effect of dietary *Rhus verniciflua* Stokes supplementation on metmyoglobin in Hanwoo (Korean cattle) steers beef during refrigerated storage at 3℃.

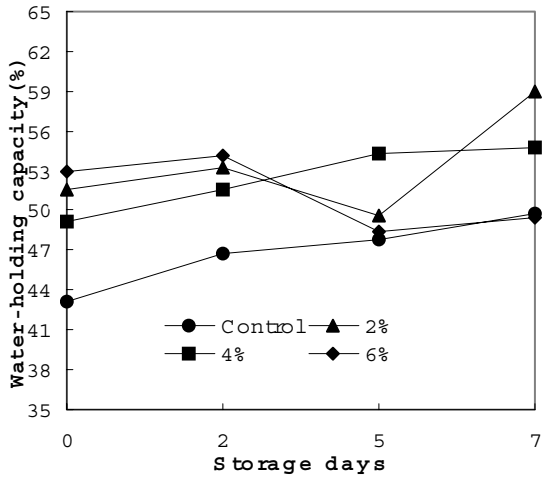

Fig. 2. Effect of dietary *Rhus verniciflua* Stokes supplementation on water-holding capacity in Hanwoo (Korean cattle) steers beef during refrigerated storage at 3℃.

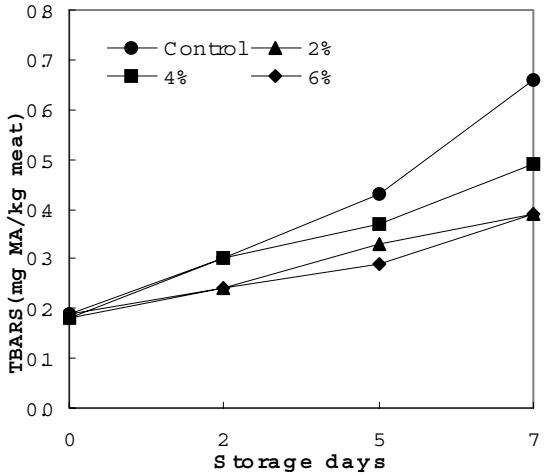

Fig. 3. Effect of dietary *Rhus verniciflua* Stokes supplementation on TBARS (thiobarbituric acid reactive substances) value in Hanwoo (Korean cattle) steers beef during refrigerated storage at 3℃.

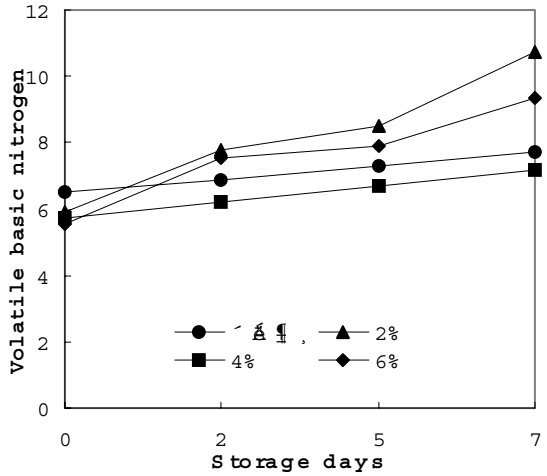

Fig. 4. Effect of dietary *Rhus verniciflua* Stokes supplementation on VBN (volatile basic nitrogen) in Hanwoo (Korean cattle) steers beef during refrigerated storage at 3℃.

Table 1. Effects of dietary *Rhus verniciflua* Stokes supplementation on pH and meat color in Hanwoo (Korean cattle) steers beef during refrigerated storage at 3˚C.

Items	Storage days	Diet conditions			
		Control	2%	4%	6%
pH	0	5.44$^{a\ A}$	5.42$^{a\ A}$	5.44$^{a\ A}$	5.40$^{a\ A}$
	2	5.36$^{b\ B}$	5.44$^{a\ A}$	5.43$^{ab\ A}$	5.44$^{a\ A}$
	5	5.38$^{ab\ A}$	5.43$^{a\ A}$	5.43$^{a\ A}$	5.41$^{a\ A}$
	7	5.38$^{ab\ A}$	5.46$^{a\ A}$	5.38$^{b\ A}$	5.39$^{a\ A}$
L^*	0	40.56$^{a\ B}$	38.98$^{a\ C}$	43.34$^{a\ A}$	40.39$^{a\ B}$
	2	41.16$^{a\ B}$	39.42$^{a\ B}$	43.32$^{a\ A}$	40.65$^{a\ B}$
	5	40.72$^{a\ B}$	39.49$^{a\ C}$	43.05$^{a\ A}$	40.46$^{a\ BC}$
	7	42.23$^{a\ B}$	39.57$^{a\ C}$	44.52$^{a\ A}$	40.89$^{a\ BC}$
a^*	0	21.88$^{a\ B}$	21.97$^{a\ B}$	24.60$^{a\ A}$	22.39$^{a\ B}$
	2	20.13$^{b\ C}$	21.41$^{ab\ B}$	23.69^{aA}	21.94$^{a\ B}$
	5	18.78$^{b\ C}$	20.92$^{b\ B}$	22.39$^{b\ A}$	20.34$^{b\ B}$
	7	16.91$^{c\ B}$	19.95$^{c\ A}$	20.67$^{c\ A}$	16.91$^{c\ B}$
b^*	0	11.11$^{a\ B}$	10.47$^{a\ B}$	12.87$^{a\ A}$	10.84$^{a\ B}$
	2	10.91$^{a\ B}$	10.15$^{a\ B}$	11.88$^{ab\ A}$	11.03$^{a\ B}$
	5	10.59$^{a\ B}$	9.89$^{a\ B}$	11.76$^{b\ A}$	10.15$^{b\ B}$
	7	10.03$^{a\ B}$	9.70$^{a\ BC}$	11.24$^{b\ A}$	9.23$^{c\ C}$
C^*	0	24.49$^{a\ B}$	24.34$^{a\ B}$	27.76$^{a\ A}$	25.16$^{a\ B}$
	2	22.90$^{ab\ C}$	23.70$^{ab\ BC}$	26.51$^{ab\ A}$	24.55$^{a\ B}$
	5	21.56$^{bc\ C}$	23.15$^{bc\ B}$	25.29$^{b\ A}$	22.73$^{b\ BC}$
	7	19.66$^{c\ C}$	22.19$^{c\ B}$	23.53$^{c\ A}$	19.29$^{c\ C}$
h^0	0	26.93$^{d\ A}$	25.32$^{a\ B}$	27.52$^{ab\ A}$	25.40$^{b\ B}$
	2	28.34$^{c\ A}$	25.25$^{a\ C}$	26.48$^{b\ B}$	26.61$^{b\ B}$
	5	29.33$^{b\ A}$	25.16$^{a\ D}$	27.61$^{ab\ B}$	26.45$^{b\ C}$
	7	30.61$^{a\ A}$	25.81$^{a\ C}$	28.44$^{a\ B}$	29.02$^{a\ AB}$

$^{a-d}$Means in the same column with different superscripts are significantly different ($p<0.05$).
$^{A-D}$Means in the same row with different superscripts are significantly different ($p<0.05$).

Table 2. Effect of dietary *Rhus verniciflua* Stokes supplementation on fatty acid composition in Hanwoo (Korean cattle) steers beef

Fatty acids	Diet conditions			
	Control	2%	4%	6%
C14:0	3.38^{AB}	2.80^{B}	3.45^{A}	3.39^{AB}
C16:0	27.25^{A}	21.21^{B}	24.13^{AB}	23.06^{B}
C16:1	5.63^{A}	6.02^{A}	6.34^{A}	6.62^{A}
C18:0	12.52^{AB}	14.96^{A}	10.67^{B}	11.43^{B}
C18:1	45.37^{B}	50.58^{A}	50.76^{A}	51.45^{A}
C18:2	3.71^{A}	3.54^{A}	3.27^{A}	3.55^{A}
C18:3	0.37^{A}	0.31^{AB}	0.33^{AB}	0.19^{B}
C20:1	0.42^{A}	0.41^{A}	0.26^{A}	0.29^{A}
C20:4	1.19^{A}	0.68^{B}	0.71^{B}	0.74^{B}
C22:4	0.16^{A}	0.00^{B}	0.08^{AB}	0.04^{B}
SFA[1]	43.15^{A}	38.78^{B}	38.25^{B}	37.58^{B}
MUFA[2]	51.41^{B}	56.71^{A}	57.36^{A}	57.93^{A}
PUFA[3]	5.44^{A}	4.51^{A}	4.39^{A}	4.48^{A}
UFA[4]	56.85^{B}	61.22^{A}	61.75^{A}	62.42^{A}
MUFA/SFA	1.19^{B}	1.47^{A}	1.50^{A}	1.54^{A}

$^{A-B}$Means in the same row with different superscripts are significantly different ($p<0.05$).
[1]Saturated fatty acids, [2]Monounsaturated fatty acids, [3]Polyunsaturated fatty acids,
[4]Unsaturated fatty acids.

DYNAMICS OF MYOGLOBIN LAYER CHANGE DURING DISPLAY OF COLOR-STABLE AND COLOR-LABILE BEEF MUSCLES

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Background

The ability of myoglobin (Mb) to oxygenate or "bloom" to a bright cherry red color of oxymyoglobin (OMb) and to retain its stability during retail display differs among different muscles (Hood, 1980; O'Keeffe and Hood, 1982; Renerre and Labas, 1987). Muscle cell respiration is temperature dependent (Urbin and Wilson, 1958; Bendall and Taylor, 1972) and was found to deteriorate with post mortem age (Bendall and Taylor, 1972; O'Keeffe and Hood, 1982). Lower temperatures enhance oxygen solubility in water, increase Mb oxygenation (Urbin and Wilson, 1958), and reduce enzyme activity (Urbin and Wilson, 1958; Bendall and Taylor, 1972). Bloom color on a muscle surface is influenced by oxygen consumption of muscle cells, oxygen partial pressure (pO_2) on the muscle surface, and the depth of Mb oxygenation (Brooks, 1929; O'Keeffe and Hood, 1982). Research has determined the depth of oxygen penetration into muscles directly by determining pO_2 (Morley, 1971; Feldhusen et al., 1995) or indirectly by observing pigment oxygenation (Brooks, 1929; O'Keeffe and Hood, 1982). We investigated both OMb and metmyoglobin (MMb) layer depths, and their relationship to surface color using novel open-topped clear Plexiglas® containers for continuous observation of pigment changes in muscle cubes and digital image analysis to quantitate the dynamics of pigment layer change.

Objectives

We investigated the combined effects of post mortem time (PT; 3, 10, or 14 d), storage temperature (ST; 0° or 4.4°C), and display temperature (DT; 0° or 3.3°C) on depths of oxymyoglobin (OMb) and MMb layers and instrumental color of beef *Longissimus lumborum* (LL) and *Psoas major* (PM) muscle cubes at 3 h and 1, 3, and 5 d of display.

Materials and methods

For each of 4 replications, sixteen paired muscles of LL and PM from the right side of USDA Select (n=48) and Choice (n=16) carcasses were obtained 48 h post slaughter from a commercial processing facility. Eight paired LL or PM were assigned randomly to 0ºC ST, while another eight pairs were assigned to 4.4ºC ST. At 3, 10, and 14 d PT, tissue from the anterior end of LL or posterior end of PM (same carcass for each ST) was cut into a cube and placed into an open-top, clear Plexiglas container (3.8-cm^3 for LL, 3.2-cm^3 for PM). Immediately after cutting, two adjacent sides of each muscle cube, with muscle fibers running perpendicular to the container base, were placed tightly in one corner of the Plexiglas container to maintain the deoxygenated form of Mb on muscle surfaces next to the Plexiglas. The extra portion of the muscle cube which extended above the top of the Plexiglas container was cut off at the open-top edge by cutting across the muscle fibers, to allow oxygen diffusion along the muscle fibers. The top muscle surface was covered immediately with PVC film (23,250 cc O_2/m^2/24 h at 23ºC and 0%RH) and exposed to ambient air. The LL or PM muscle cubes from each ST were placed in open-top display cases at 0º or 3.3ºC. The display luminance of 1614 lux was provided by the Ultralume™ 30 continuous fluorescent lighting (34 watts, 3000 K, Phillips, Sommerset, NJ, USA).

Due to time demands for muscle cube preparation, surface instrumental color measurement on top surface for each muscle cube was first done at 3 h bloom time. CIE L*, a*, b* (Illuminant A / 10 º observer; 1.27-cm diameter aperture) and reflectance spectral data (400-700 nm, 10-nm increments), were obtained using a HunterLab LabScan 6000 spectrophotometer (Hunter Associates Laboratory, Inc.; Reston, VA, USA). Values for %R 630 nm - %R 580 nm were calculated. The percentages of OMb and MMb on the muscle surfaces were estimated using K/S spectral data (AMSA, 1991). Surface instrumental color measurements and digital image photography of the pigment layers were recorded during 3 h, and 1, 3, and 5 d of display.

Following instrumental color measurement, digital image photography was performed on each muscle cube using a Sony Digital Mavica Still Camera model MVC-FD91 (Sony Corporation, Japan). Extreme care was taken to standardize digital photography. A scale in 1-mm increments, was included in each photograph for unit calibration for pigment layer measurement. Analysis of images was performed on an IBM ThinkPad T20 personal laptop computer model 2647-41U 700 MHz 128.0 MB RAM 12 GB storage capacity (IBM Corp., Armonk, NY, USA). Images were displayed on a high resolution (1024 x 768 pixels) TFT IBM ThinkPad LCD on S3 Inc. Savage/IX with true color (32- bit per pixel) screen setting. Digital images were processed using Adobe Photoshop 5.0 software (Adobe Systems Inc., San Jose, CA, USA). OMb and MMb layer depths were analyzed using the National Institutes of Health (NIH, Bethesda, MD, USA) *Scion Image* software (ScnImage release Beta 3b).

Color processing of images for the OMb layer depth measurement was performed by using the RGB mode TIFF image in Adobe Photoshop, converting it to CYMK color mode (Ringkob, 1997), and adjusting the color balance. The adjustments resulted in a bright orange yellow color of the OMb layer contrasting with a greenish brown color of the deoxymyoglobin layer. To separate the three Mb layers, the CYMK images were adjusted for color balance, hue, and saturation. Image processing and analysis for MMb layer depth measurement was performed on images recorded during 1, 3, and 5 d display. The image analysis for pigment layer depth determination was performed on ScnImage Software by acquiring the color processed RGB image. Stacks of an originally adjusted 24-bit color image were generated from a slice of the red channel (grayscale) with a "Stacks" drop down menu. The analysis of an image could be performed on this converted 24–bit color TIFF image. To calibrate the measurement unit, for each acquired image, a line selection tool was used to drag a 10-mm straight line on the scale presented underneath the muscle cube Plexiglas container. On the "Analyze" and "set scale" menu, the unit was set to mm. The measured distance in pixels was then calibrated with a known 10-mm scale. Five locations were measured for each pigment layer depth and averaged. The experiment was in a strip-split-split plot design. Analysis of Variance was performed utilizing the MIXED procedure of SAS (2000). Least-squares means for all variables and interactions were generated and separated using the DIFF option.

Results and discussion

As expected, muscle type and PT had the major effects on the depths of OMb and MMb layers and surface color. Their interactions also occurred in the data, especially on d 5 of display (Table 1). The effects of DT and ST on the OMb and MMb layers and surface color are presented in Table 2 and 3, respectively. Influences of main effects (muscle, PT, DT, and ST) and interactions will be discussed by each day of display.

At 3 h of bloom, the color-stable LL had a deeper ($P<.05$) OMb layer (3.45 mm) than the color-labile PM (2.02 mm). LL developed greater ($P<.05$) surface OMb, was ($P<.05$) lighter (higher L*), more ($P<.05$) yellow (higher b*), and had higher ($P<.05$) %R630 - %R580 values than PM, but it was not ($P>.05$) redder (a*). Feldhusen et al. (1995) reported no clear relationship between Mb oxygenation and a* value on muscle surface during 5 h of air exposure. PT affected ($P<.05$) surface MMb and bloom color attributes where muscles stored longer had a better bloom color. Surface OMb for 3, 10, and 14 d PT was similar (95.1, 95.1, and 96.4%, respectively). However, muscles 10 and 14 d PT had less ($P<.05$) surface MMb than 3 d PT and were brighter red and more yellow than 3 d PT. The greater surface MMb on muscles 3 d PT was likely due to lower pO_2 on muscle surface (Feldhusen et al., 1995), which likely resulted from higher oxygen consumption in muscles with less PT (O'Keeffe and Hood, 1982). Feldhusen et al. (1995) found that the oxygen penetration measured at 5 h after cutting increased with storage time. However, we found no effect ($P>.05$) of PT on the OMb layer at 3 h of bloom. Compared to 3.3ºC DT, muscles displayed at 0ºC were ($P<.05$) lighter, more yellow, and had higher %R630 - %R580 values, but were similar ($P>.05$) in OMb layer, surface OMb and MMb, and a* values (Table 2). There was no ($P>.05$) influence of ST (Table 3) on 3 h bloom color attributes.

At 1 d of display, the OMb layer of LL increased to 4.55 mm, while that of PM decreased to 1.73 mm. Feldhusen et al. (1995) reported a 4.5-mm deep OMb layer in *Longissimus dorsi* at 5 h of air exposure. They indicated a clear increase in oxygen penetration with time of air exposure during 5 h. The MMb layer of LL was ($P<.05$) thinner (1.18 mm) than PM (1.67 mm-MMb), which resulted in a brighter red and more yellow surface color (Table 1) in LL than PM. As expected, muscles 10 d PT had greater ($P<.05$) surface OMb, were redder and were more yellow than those at 3 d PT (Table 1), but 10 d did not ($P>.05$) differ from 14 d PT. We did not find an influence ($P>.05$) of PT on OMb and MMb layer depths. Colder DT (0°C) promoted a

deeper (P<.05) OMb layer formation (Table 1), likely due to better oxygen solubility in the intracellular water and less enzyme respiration (Urbin and Wilson, 1958). Surface color of muscles displayed at 0°C was better than those displayed at 3.3°C, however, the differences were not significant (P>.05).

At 3 d display, OMb layer (4.60 mm) in LL increased slightly from d 1, while that in PM continued to decrease (1.15 mm). In contrast, MMb layers of both muscles increased (2.24 mm for LL and 2.88 mm for PM). The OMb layer of LL was thicker (P<.05) and MMb layer was thinner (P<.05) than those in PM. As a result, LL had (P<.05) greater surface OMb, less surface MMb, was brighter red and more yellow than PM (Table 1). O'Keeffe and Hood (1982), however, found no differences in depth of OMb layer between these 2 muscles at 2 d PT. Muscles stored 14 d PT developed a thicker (P<.05) MMb layer than 3 d PT, but did not differ (P>.05) from 10 d PT. Storage at 0°C resulted in a thinner (2.34 mm, P<.05) MMb layer than at 4.4°C (2.78 mm, Table 3). Muscles stored and displayed at higher temperatures (4.4°C ST, 3.3°C DT) provided (P<.05) the least surface OMb and lowest value of %R630 - %R580 (data not shown).

After 5 d display, more interactions occurred between muscle and PT (Table 1). The a*, b*, %R630 - %R580, surface OMb and MMb, and OMb layer were influenced (P<.05) by muscle x PT. Among LL from 3 different PT, LL stored 14 d had (P<.05) the lowest values of a*, b*, %R630 - %R580, surface OMb, and OMb layer, but the most surface MMb. The OMb layer of 14 d PT LL, however, was similar (P>.05) to those of PM stored 3 or 10 d. PM from 3 different PT had similar (P>.05) a*, b*, and %R630 - %R580, which were (P<.05) lower than those in all the LL. Although not always significant, PM stored 14 d had the worse overall color, least surface OMb, most surface MMb, and thinnest OMb layer. More degradation of substrates and coenzymes may occur during longer PT of muscles, which likely causes less MMb to be reduced (O'Keeffe and Hood, 1982). Interestingly, no differences (P>.05) in MMb layer were observed. L* was affected by muscle main effect where LL was (P<.05) lighter than PM. ST at 0°C had (P<.05) greater a*, %R630 - %R580, and less surface MMb (Table 3).

Conclusions

This study suggests that longer PT (10 or 14 d) provided better color bloom, but as PT increased, color stability decreased as shown by faster OMb layer thinning and faster MMb layer thickening. The more color stable LL allowed a deeper O_2 penetration during bloom, a thicker OMb layer, and slower developing MMb layer during display. While MMb layer of the 2 muscle types reached similar thickness at 5 d display, the deeper OMb layer had a greater influence on surface color stability. The dynamics of myoglobin layer during bloom, which may be explained by their inherent biochemical traits of oxygen consumption and reducing capacity, were related to surface color bloom attributes and stability during display of the LL and PM muscles.

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Table 1. Effects of muscle and postmortem time (PT) on color attributes, oxymyoglobin (OMb) layer, and metmyoglobin (MMb) layer depths at 3 h, 1, 3, and 5 d of refrigerated display

Attributes	Muscle	3 h display			1 d display			3 d display			5 d display		
		3 d PT	10 d PT	14 d PT	3 d PT	10 d PT	14 d PT	3 d PT	10 d PT	14 d PT	3 d PT	10 d PT	14 d PT
L*	LL	**37.7ay**	**40.5ax**	**41.7ax**	**38.6a**	**41.1a**	**41.2a**	**39.7a**	**40.6a**	**40.9a**	**39.7a**	**39.3a**	**38.4a**
	PM	**36.9by**	**37.5bx**	**39.5bx**	**35.5b**	**35.8b**	**36.9b**	**35.9b**	**35.3b**	**36.1b**	**36.2b**	**35.1b**	**36.2b**
a*	LL	**29.2y**	**32.9x**	**32.8x**	**29.2ay**	**30.8ax**	**30.1axy**	**27.8a**	**29.4a**	**27.6a**	**26.0p**	**25.6p**	**21.7q**
	PM	**28.5y**	**32.7x**	**32.6x**	**22.7by**	**26.1bx**	**23.6bxy**	**18.7b**	**20.8b**	**18.6b**	**17.1r**	**18.1r**	**15.3r**
b*	LL	**24.9ay**	**27.3ax**	**26.9ax**	**24.5ay**	**26.1ax**	**25.4axy**	**23.6ay**	**25.0ax**	**23.4ay**	**22.4p**	**23.0p**	**21.2p**
	PM	**23.7by**	**26.4bx**	**26.9bx**	**20.8by**	**23.0bx**	**21.9bxy**	**19.0by**	**20.1bx**	**19.5by**	**18.5q**	**19.0q**	**18.3q**
%R630-%R580	LL	**23.2ay**	**27.3ax**	**28.3ax**	**21.8ay**	**25.6ax**	**25.1ax**	**21.0a**	**23.1a**	**20.8a**	**19.3p**	**19.0p**	**14.4q**
	PM	**22.3by**	**24.8bx**	**26.2bx**	**12.6by**	**16.6bx**	**14.5bx**	**8.6b**	**10.3b**	**7.9b**	**7.0r**	**8.1r**	**5.8r**
%OMb	LL	**97.1a**	**99.3a**	**98.4a**	**88.2aq**	**92.0ap**	**90.7aq**	**83.4a**	**87.7a**	**82.1a**	**77.5p**	**76.7p**	**62.9q**
	PM	**93.0b**	**91.0b**	**94.4b**	**55.9bq**	**70.5bp**	**60.7bq**	**37.7b**	**47.3b**	**37.7b**	**30.7rs**	**34.7r**	**18.2s**
%MMb	LL	**2.9bp**	**0.7bq**	**1.5bpq**	**11.4s**	**8.1s**	**8.6s**	**16.1b**	**11.2b**	**16.0b**	**21.5s**	**23.0s**	**37.2r**
	PM	**7.0ap**	**2.0aq**	**5.0apq**	**40.3p**	**28.6r**	**39.3pq**	**58.3a**	**49.7a**	**60.8b**	**67.7pq**	**59.7q**	**75.7p**
OMb layer (mm)	LL	**3.2a**	**3.4a**	**3.5a**	**4.1a**	**4.9a**	**4.4a**	**5.2a**	**4.6a**	**3.5a**	**4.8p**	**3.1q**	**1.8r**
	PM	**2.1b**	**2.2b**	**1.9b**	**1.5b**	**2.1b**	**1.6b**	**1.5b**	**1.4b**	**0.8b**	**0.9rs**	**0.7rs**	**0.2s**
MMb layer (mm)	LL	**N/A**	**N/A**	**N/A**	**1.0b**	**1.4b**	**1.2b**	**2.0by**	**2.1bxy**	**2.8bx**	**3.1**	**3.4**	**3.9**
	PM	**N/A**	**N/A**	**N/A**	**1.5a**	**1.6a**	**2.0a**	**2.3ay**	**3.2axy**	**3.3ax**	**3.4**	**3.8**	**3.5**

a,b **LSmeans for each attribute with a different letter within a column on the same display time differ (P<.05)**
p,q,r,s **LSmeans for each attribute with a different letter on the same display date differ (P<.05)**
x,y,z **LSmeans for each attribute with a different letter in the same row on the same display time differ (P<.05)**

Table 2. Effects of display temperature (DT) on color attributes, oxymyoglobin (OMb) layer, and metmyoglobin (MMb) layer depths at 3 h, 1, 3, and 5 d of display

Attributes	3 h display		1 d display		3 d display		5 d display	
	0°C DT	3.3°C DT	0°C DT	3.3°C DT	0°C DT	3.3°C DT	0°C DT	3.3°C DT
L*	**39.4x**	**37.9y**	**38.9**	**37.1**	**38.7**	**37.0**	**38.3**	**36.7**
a*	**31.4**	**31.1**	**27.8**	**26.6**	**24.5**	**23.0**	**22.0**	**20.0**
b*	**26.0x**	**25.4y**	**23.9**	**22.9**	**21.9**	**21.2**	**21.0**	**19.9**
%R630-%R580	**25.5x**	**24.0y**	**20.2**	**17.8**	**16.5**	**13.9**	**13.9**	**10.9**
%OMb	**94.3**	**94.1**	**78.0**	**73.9**	**64.9**	**59.8**	**55.1**	**47.2**
%MMb	**4.0**	**3.4**	**20.8**	**23.8**	**32.9**	**38.3**	**44.0**	**49.7**
OMb layer (mm)	**2.9**	**2.6**	**3.4x**	**2.9y**	**3.1**	**2.7**	**2.3**	**2.0**
MMb layer (mm)	**N/A**	**N/A**	**1.5**	**1.4**	**2.7**	**2.4**	**3.6**	**3.1**

x,y **LSmeans for each attribute with a different letter in the same row on the same display time differ (P<.05)**

Table 3. Effects of storage temperature (ST) on color attributes, oxymyoglobin (OMb) layer, and metmyoglobin (MMb) layer depths at 3 h, 1, 3, and 5 d of refrigerated display

Attributes	3 h display		1 d display		3 d display		5 d display	
	0°C ST	4.4°C ST	0°C ST	4.4°C ST	0°C ST	4.4°C ST	0°C ST	4.4°C ST
L*	**38.2**	**39.1**	**37.7**	**38.3**	**37.6**	**38.1**	**37.1**	**37.9**
A*	**31.3**	**31.2**	**27.1**	**27.2**	**24.1**	**23.4**	**22.0x**	**19.9y**
B*	**25.8**	**25.6**	**23.4**	**23.5**	**21.7**	**21.5**	**20.8**	**20.1**
%R630-%R580	**24.4**	**25.0**	**18.9**	**19.1**	**15.5**	**14.9**	**13.5x**	**11.4y**
%OMb	**93.6**	**94.8**	**76.6**	**75.5**	**64.4**	**60.3**	**55.7**	**46.6**
%MMb	**3.7**	**3.7**	**22.1**	**22.5**	**33.6**	**37.5**	**42.2y**	**51.4x**
OMb layer (mm)	**2.7**	**2.8**	**3.2**	**3.1**	**3.1**	**2.8**	**2.4**	**1.8**
MMb layer (mm)	**N/A**	**N/A**	**1.5**	**1.3**	**2.3y**	**2.8x**	**3.1**	**3.6**

x,y **LSmeans for each attribute with a different letter in the same row on the same display time differ (P<.05)**

EFFECT OF BREED AND STRATEGIC FEEDING ON PORK TENDERNESS EVALUATED WITH INSTRUMENTAL AND SENSORY ANALYSES

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Background

Consumers have high expectations to outdoor produced pig meat, especially from organic outdoor pigs. However, these expectations are seldom achieved. Recent studies have shown that strategic feeding of pigs can change many pork quality characteristics in a consumer-wise positive direction. Accelerated growth rates occur in pigs with free access to feed following a period of restricted feeding (Oksbjerg *et al*., 2002), and feeding intensity is directly related to muscle growth rate and in turn to meat tenderness (Kristensen *et al*., 2002). Muscle growth rate is principally determined by the ratio between the rates of protein synthesis and protein degradation. A high rate of protein degradation at slaughter accelerates meat tenderness development post mortem. Pigs produced outdoors on a restrictive feeding regime and with free access to roughage, have reduced muscle growth rate and lower tenderness score (Danielsen *et al*., 2000). Contradictory results concerning the influence of the intramuscular fat content (IMF) on pork tenderness are reported (Göransson *et al*., 1992; Fernandez *et al*., 1999; van Oeckel *et al*., 1999).

Objectives

The aim of the present study was to evaluate the effect of various rearing systems including strategic feeding on pork tenderness using both instrumental and sensory analyses, as well as to evaluate the relationship between (IMF) and pork tenderness.

Materials and methods

During two years, 280 growing/finishing pigs were raised to approximately 107 kg live weight in four different production systems. Pigs were equally distributed within litter and breed to housing systems (outdoors/indoors), crossbreed (Duroc*LargeWhite/Landrace*LW) and gender (castrates/females). Outdoor pigs were fed *ad libitum*, with either an organic diet diluted with 20% alfalfa roughage throughout (org.dil; decreased energy density) or with first this diet and thereafter the undiluted organic diet (org.dil./org.; strategic feeding for compensatory growth). In two indoor treatments, pigs were fed restrictively with either an undiluted organic diet or a conventional diet. A random part of the material, 135 pigs (67 D*LW and 68 L*LW) was used in the present study.

Sensory profiling was performed on *M. longissimus dorsi* (LD) using a trained sensory panel of 9 assessors. The loins were sliced in 20 mm thick chops and fried on a frying plate at 155°C to a core temperature of 65°C. No salt or spices were used. Sensory attributes related to meat structure, ie., tenderness, hardness at first bite, stringiness and crumbliness, are reported in this paper. The intensity of each attribute was evaluated on an unstructured scale from 0 to 15 (0 for low intensity and 15 for high intensity).

300 g pieces of LD were cooked in a water bath at 70°C for 90 min for determination of Warner-Bratzler (WB) shear force. Maximal WB peak force (WBPF) and total WB-work (WBwork) were measured on 8 strips (10x10x50 mm), sheared across the fibre direction (speed: 55 mm/min, TA-HDI texture Analyser; Stable Micro Systems, Surrey, UK). The intramuscular fat content (IMF) was analysed from some samples (48 D*LW loins and 50 L*LW loins) by the SBR-method after hydrolysis with HCl using diethyl ether and petroleum ether for extraction.

Statistical analyses of meat quality traits were performed with the MIXED procedure in SAS (SAS Institute Inc., Cary, N.C., USA, version 8.2). The model included treatment, crossbreed (D*LW or L*LW) and gender (female or castrate) as fixed effects. Two-way interactions between the fixed factors were included in the

model, when significant ($p \leq 0.05$). Sire within crossbreed, as well as dam within crossbreed and sire, were treated as random. Each assessor, as well as the interaction between assessor and sensory session, was also included as random in the model of sensory traits. In the tables, data are expressed as least square means with pooled standard errors. The Unscrambler version 8.0 (Camo Process AS, Oslo, Norway) was used for partial least squares regression (PLS) using standardised variables and full cross validation. Means of the sensory scores from all the assessors were used in the PLS. Martens' uncertainty test was used for significance testing of variables in PLS.

Results and discussion

The rearing system, crossbreed and rearing year affected the sensory attributes of tenderness, hardness at first bite, stringiness and crumbliness (Table 1). Organic outdoor rearing resulted in more tender pork than conventional indoor rearing for both crossbreeds (year 2). Organic outdoor D*LW pork was also more tender than indoor organic D*LW pork (year 2). Pork from strategic organically fed outdoor L*LW pigs was more tender than pork from indoor organic L*LW pigs both years. Strategic outdoor organic feeding improved tenderness of L*LW pork (year 2), whereas no such effect was found in D*LW pork. Strategic outdoor feeding increased hardness at first bite and stringiness and decreased crumbliness in D*LW pork, whereas the opposite was seen in L*LW pork (year 2). Indoor rearing of the pigs produced pork with the highest hardness at first bite, especially with conventional feeding. The most stringy and least crumbly D*LW pork was produced by organic indoor rearing (year 2).

Contrary to sensory tenderness, no interaction between crossbreed and rearing was found on WB shear force. Rearing effect was only seen on WBPF (year 2). WBPF was highest in pork from indoor organic pigs and lowest in pork from strategic fed organic outdoor pigs. The improving effect of strategic outdoor feeding (compensatory growth) on pork sensory tenderness was not seen on WB shear force. However both tenderness and WB shear force were improved when compared to indoor organic restricted feeding. The effect of strategic feeding was not as pronounced in the present study as in the study of Kristensen *et al*., 2002. The reason might be that the pigs were not slaughtered at an optimal compensatory growth period in the present study.

There were significant relationships between most of the measured traits (Table 2). WBPF and WBwork were, as expected, highly correlated, and both were correlated to sensory tenderness at almost the same level within both crossbreeds. The correlations were generally higher for D*LW pork except for crumbliness with higher correlations for L*LW pork. The most marked breed difference was for the correlations with IMF. Significant correlations were found for all traits, except WBwork, within D*LW pork, whereas IMF only was significantly correlated to stringiness within L*LW pork. Van Oeckel *et al*. (1999) found correlations between WBPF and sensory tenderness of pork loin between -0.39 and -0.69 depending on different modifications of the WB-method, which is in agreement with the present study.

All sensory traits were to some degree correlated to WB shear force (Table 2) and thus it was interesting to evaluate the total influence of the investigated sensory traits on shear force. It has been discussed whether IMF has an impact on pork tenderness or not. Göransson *et al*. (1992) found some negative influence of IMF on tenderness in pork of Hampshire crossbreeds, whereas van Laak *et al*. (2001) found a linear relationship accounting for 47% of the variation in WB shear force in Duroc pork loin, but no relationship in Hampshire or Berkshire pork loins. Fernandez *et al*. (1999) found a tendency to improved tenderness with higher IMF in one experiment, but no effect in another experiment. Therefore IMF was included in the PLS in the present study. The rearing system did not affect IMF (Table 1), but IMF was higher in D*LW compared with L*LW loin (2.0% vs 1.4%, P=0.029). IMF varied between 1.1-3.8% in D*LW loin and between 0.7-3.1% in L*LW loin. PLS on WBPF showed significance for IMF only in D*LW loin, not in L*LW loin (Figures 1 and 2), which was in agreement with the single correlation coefficients (Table 2). Tenderness, hardness at first bite and stringiness were significant for loin of both crossbreeds, but crumbliness only for L*LW loin.

Conclusions

Outdoor strategic feeding for compensatory growth improved the sensory attributes of tenderness and hardness at first bite on L*LW pork loin, but this effect was not seen in D*LW pork loin. The outdoor rearing systems resulted in more tender pork, evaluated both with instrumental and sensory analyses,

compared with the indoor restricted rearing systems. IMF influenced WBPF in D*LW pork loin, but not in L*LW pork loin.

Acknowledgements

The study is part of the EU-project "Sustainability in the production of pork with improved nutritional and eating quality using strategic feeding in out-door production". QLK5-2000-00162.

Table 1. Intramuscular fat, sensory scores and Warner-Bratzler shear force on *M. longissimus dorsi*[1]

Trait	Breed (B)	Year	Rearing (R)				SE	P-value	
			Outdoor		Indoor				
			Org. dil.	Org.dil./Org.	Org.	Conv.		R	B*R
IMF, %		1	1.9	1.9	1.9	–	0.2	0.900	NS
		2	1.6	1.7	1.6	1.6	0.1	0.579	NS
Sensory quality									
Tenderness	D*LW	1	8.4	8.4	8.8	–	0.7	0.222	0.004
	D*LW	2	10.4^{a}	9.7^{a}	8.7^{b}	8.6^{b}	0.7	0.001	0.015
	L*LW	1	8.3^{ab}	9.0^{a}	7.6^{b}	–	0.7	0.222	0.004
	L*LW	2	9.1^{a}	9.9^{b}	9.1^{a}	8.2^{c}	0.7	0.001	0.015
Hardness at 1st bite	D*LW	1	5.4	5.3	5.1	–	0.6	0.098	0.006
	D*LW	2	4.8^{a}	5.3^{b}	5.9^{bc}	6.2^{c}	0.6	0.001	0.044
	L*LW	1	5.3^{a}	5.1^{a}	6.3^{b}	–	0.6	0.098	0.006
	L*LW	2	5.6^{a}	4.8^{b}	6.0^{ac}	6.6^{c}	0.6	0.001	0.044
Stringiness		1	3.9^{a}	3.1^{b}	4.0^{a}	–	0.8	0.005	NS
	D*LW	2	3.5^{a}	4.4^{b}	5.3^{c}	4.8^{bc}	0.8	0.001	0.008
	L*LW	2	4.5^{a}	3.3^{b}	4.9^{a}	5.0^{a}	0.7	0.001	0.008
Crumbliness		1	2.3^{a}	3.2^{b}	2.3^{a}	–	0.8	0.001	NS
	D*LW	2	6.2^{a}	5.3^{b}	5.0^{b}	5.5^{b}	0.9	0.001	0.017
	L*LW	2	6.0^{a}	6.9^{b}	5.3^{a}	6.0^{a}	0.9	0.001	0.017
Warner Bratzler									
WBPF, N		1	30.5	28.9	30.6	–	1.4	0.506	NS
		2	28.1^{ac}	27.6^{a}	33.8^{b}	31.7^{bc}	1.8	0.007	NS
Wbwork, Nmm		1	175	162	168	–	5.6	0.243	NS
		2	151	145	163	156	6.0	0.143	NS

[1] Significant differences between LSM with different letters in the same row, P≤0.05; NS = not significant.

Table 2. Pearson's correlation coefficients for relationships between the measured traits within crossbreed[1] D*LW correlations are shown in the upper diagonal and L*LW correlations in the lower diagonal.

L*LW \ D*LW	WBPF	WBwork	Tenderness	Hardness 1st bite	Crumbl.	String.	IMF
WBPF		0.850	-0.635	0.641	-0.236	0.647	-0.488
Wbwork	0.845		-0.622	0.557	-0.404	0.536	-0.257
Tenderness	-0.564	-0.492		-0.917	0.423	-0.729	0.333
Hardness at 1st bite	0.552	0.363	-0.919		-0.189	0.818	-0.474
Crumbliness	-0.337	-0.532	0.614	-0.422		-0.200	-0.286
Stringiness	0.523	0.355	-0.810	0.853	-0.528		-0.531
IMF	-0.193	-0.135	0.145	-0.178	0.017	-0.302	

[1] Bold coefficients are significant (P≤0.05).

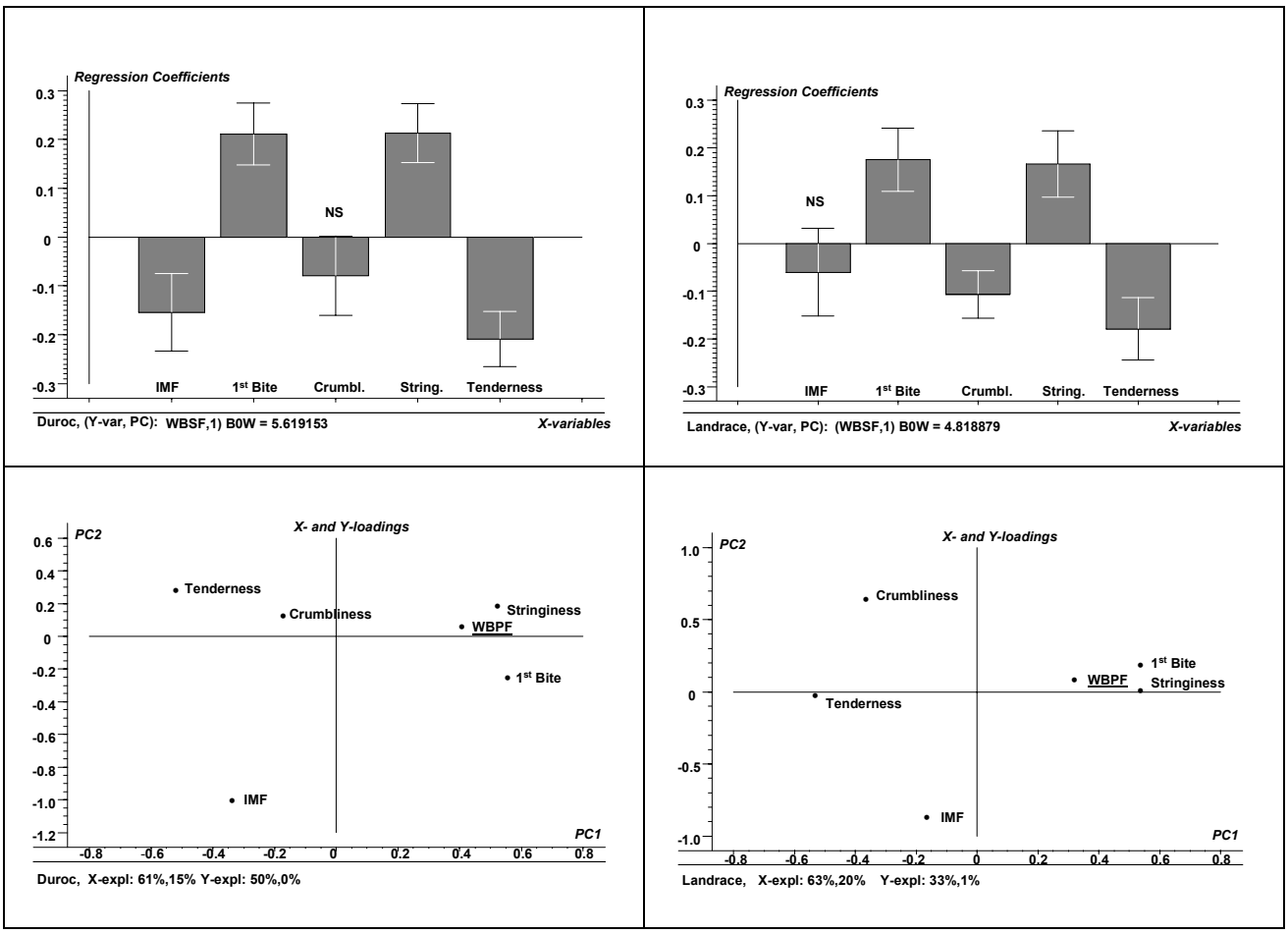

Figure 1. PLS regression coefficients and loadings plot on WBPF related to sensory traits and IMF on D*LW loins. NS = not significant

Figure 2. PLS regression coefficients and loadings plot on WBPF related to sensory traits and IMF on L*LW loins. NS = not significant

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FACTORS AFFECTING BEEF LUMBAR VERTEBRAE DISCOLORATION

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Background

Marrow discoloration is a factor that influences the appearance of bone-in meat products. This is particularly true in beef steaks containing lumbar vertebrae. However, to date, little research has focused on beef bone marrow discoloration, regardless of its affect on product display life.

Bone discoloration appears to be promoted by high-oxygen modified atmosphere packaging (MAP), which likely favors hemoglobin oxidation within exposed bone marrow (Lanari *et al.* 1995; Sorheim *et al.* 1999). In contrast, removing oxygen from modified atmosphere packages should encourage the deoxygenated form of hemoglobin; therefore, stabilizing vertebrae marrow color during storage and display. In addition, low levels of carbon monoxide in MAP should maintain a bright-red marrow color due to the formation of carboxyhemoglobin. Sorheim *et al.* (1999) speculated that carbon monoxide and oxygen exclusion might limit bone blackening. Although packaging technology has the potential to influence bone color, no published reports have evaluated the effects of package atmosphere on bone discoloration.

The oxidative state of hemoglobin released from red blood cells on the surface of cut bones likely determines marrow color (Lanari *et al.* 1995; Gill 1996). Thus, antioxidants that limit heme-protein oxidation within marrow should prove useful for preserving vertebrae surface color. Although ascorbic acid's ability to reduce methemoglobin in purified solutions has been reported, no published work has assessed the effects of ascorbic acid on bone marrow. The ability of ascorbic acid to delay myoglobin oxidation has made it a useful tool for minimizing surface discoloration in muscle (Lee *et al.* 1999; Shivas *et al.* 1984).

Objectives

Because bone discoloration is (1) an undesirable result of prolonged storage and (2) a process for which there is no established preventative measure (Warren *et al.* 1992; Gill 1996), our objectives were to:

1.) Investigate ultra low-oxygen and carbon monoxide modified atmosphere packaging (CO MAP) as a means of limiting lumbar vertebrae discoloration during storage.
2.) Investigate the ability of ascorbic acid, ascorbate-6-palmitate, and sodium erythorbate to minimize beef lumbar vertebrae discoloration during display.

Materials and methods

Experiment 1

Ten beef short loins (6 days postmortem) were cut perpendicular to the vertebral column into 2.54 cm-thick steaks. All lean, fat, and ribs were removed, yielding 2.54 cm-thick sections of only lumbar vertebrae. From each loin, 4 vertebrae were packaged separately in either ultra low-oxygen (80% N_2 and 20% CO_2) or carbon monoxide MAP (0.4% CO, 30% CO_2, and 69.6%N_2). One vertebra from each loin was packaged in high-oxygen (80%O_2 and 20% CO_2). Before packaging, initial instrumental color on the surface of each vertebrae section was evaluated (CIE L*a*b* and reflectance from 400 to 700nm; Illuminant A, 0.64 cm aperture, 10° observer). Packages were stored at 4°C.

From each loin, 1 vertebra per packaging treatment was evaluated after 1 day in storage. Because of the rapid discoloration resulting from high-oxygen MAP, vertebrae packaged in 80%O_2 were no longer evaluated. Instrumental color variables at 2, 4, and 6 weeks after packaging were then used to evaluate atmosphere-induced stability during storage (low-oxygen and 0.4% carbon monoxide packaging). The experimental design was a completely randomized block with repeated measures. Vertebral columns served as blocks (n = 10) to which packaging was assigned to vertebrae sections within each loin. Time of color evaluation was a

repeated measure assigned to units within a column. Data for all experiments was analyzed using the mixed procedure of SAS. Significance represents probabilities < 0.05.

Experiment 2:
Eight vertebral columns containing lumbar vertebrae were obtained at 6 days postmortem. Each was cut perpendicular to the length of the column into 1.91 cm-thick sections. From each column, two vertebral sections (n = 2 per treatment per column) with at least 2.54-cm² of freshly exposed lumbar marrow were randomly assigned to 1 of 7 treatments: Treatment 1 = No topical antioxidant; Treatment 2 = Distilled water; Treatment 3 = 1.5% Ascorbic acid; Treatment 4 = 2.5% Ascorbic acid; Treatment 5 = Ethanol; Treatment 6 = 1.5% Ascorbate-6-Palmitate; and Treatment 7 = 2.5% Ascorbate-6-Palmitate. Ascorbic acid and ascorbate-6-palmitate were made on a wt/wt basis using distilled water and ethanol, respectively. Vertebrae were topically treated with 1 ml of an assigned treatment, which thoroughly covered the entire fresh cut vertebrae.

To minimize package-to-package variation, 7 vertebrae sections from each column (1 per treatment) were packaged together in 80% O_2 and 20% CO_2. Packages were displayed at 1°C for 5 days in open-top display cases under continuous fluorescent light (1614 lux; 3000 K).

The color of marrow from each vertebrae section was evaluated initially and on days 1, 3, and 5 of display by 8 trained visual-color panelists using a scale of 1 = Bright reddish-pink to red (typical fresh cut bone), 2 = Dull pinkish-red, 3 = Slightly grayish-pink or grayish-red, 4 = Grayish-pink or grayish-red, 5 = Moderately gray, 6 = All gray or grayish-black, 7 = Black discoloration (0.5 point intervals). Instrumental color (CIE L*a*b*; Illuminant A, 0.64 cm aperture, 10° observer) was evaluated at 0, 1, and 5 days of display. Initial color (day 0) was evaluated prior to topical antioxidant treatment and packaging. The experimental design and analysis were similar to experiemnt 1.

Experiment 3
Ten bone-in short loins containing *longissimus* muscle and lumbar vertebrae (6 days postmortem) were fabricated into 1.9 cm-thick steaks. From each loin, 6 steaks with at least 2.54-cm² of freshly exposed lumbar marrow were assigned to one of the following treatments: Treatment 1 = Untreated control (0% Erythorbate); Treatment 2 = 0.05% Erythorbate; Treatment 3 = 0.1% Erythorbate; Treatment 4 = 0.5% Erythorbate; Treatment 5 = 1.0% Erythorbate; and Treatment 6 = 1.5% Erythorbate. All treatments were made on a wt/wt basis using distilled water. Experiment 2 indicated that there was little advantage to using 2.5% ascorbic acid; thus, 1.5% erythorbate was selected as the maximum concentration. Five milliliters of an assigned treatment was applied to the entire fresh cut surface of each steak, including the bone surface. To maximize distribution, treatments were spread over the entire steak surface using sterile cell spreaders.

Before treatment, the initial color (0 hours) on the fresh cut surface of lumbar vertebrae and *longissimus* muscles was measured instrumentally (Illuminant A, 1.27 cm aperture, 10° observer). Following treatment, steaks were packaged in 80% O_2 (20% CO_2) and displayed for 24 hours at 1ºC before instrumental color again was evaluated (24 hours post packaging) on both the *longissimus* muscle and the porous bone marrow of lumbar vertebrae for each steak. The experimental design and analysis were similar to experiment 1.

Results and discussion

Experiment 1
Initial vertebrae color prior to packaging was bright-red, typical of freshly-cut, oxygenated marrow (Table 1). High-oxygen packaging resulted in a rapid and significant loss of both redness ($\Delta a^* = 7.7$) and red color intensity (Δchroma = 6.8) within 24 hours after packaging. In addition, high-oxygen MAP decreased reflectance at 630 nm and increased reflectance between 540 and 580 nm, both of which indicate the conversion of oxy- to met- pigments. Thus, discoloration on the surface of lumbar vertebrae was likely due to methemoglobin. Our results support those with pork (Lanari *et al.* 1995), indicating that high-oxygen is detrimental to beef bone marrow color stability. Our reflectance data supports the role of hemoglobin's oxidative state (redox stability) in bone darkening (Lanari *et al.* 1995; Gill, 1996).

The benefits of 0.4% CO in MAP were immediately noted, and this packaging system increased surface redness within as little as 24 hours of storage (Table 1). In addition, vertebrae packaged in 0.4% CO MAP

remained bright-red for up to 6 weeks while those packaged in ultra low-oxygen MAP discolored after 2 weeks of storage. Sorheim *et al.* (1999) hypothesized that low levels of CO might limit bone discoloration. Our results support this, suggesting that formation of carboxyhemoglobin maintained a bright-red marrow color on the surface of lumbar vertebrae for up to 6 weeks in storage at 4°C. We agree that bone discoloration is increased by storage time (Warren *et al.* 1992; Gill 1996), but also recognize that beef vertebrae stored in high-oxygen MAP will darken within as little as 24 hours after packaging.

Experiment 2

Initial vertebrae color prior to treatment was bright-red. Untreated vertebrae and vertebrae treated with water or ethanol significantly discolored within 1 day after packaging in high-oxygen, resulting in no significant difference between negative and positive controls (Table 2). Applying ascorbic acid to vertebrae marrow significantly limited the rapid discoloration observed in control samples. Concentration of antioxidant had no significant affect on discoloration (1.5% was comparable to 2.5%). Overall, surface color during the 5-day display was preserved by topical antioxidant treatment in the following order: ascorbic acid > ascorbate-6-palmitate > controls; $P < 0.05$). During the 5-day display, ascorbic acid treated marrow had the least surface discoloration, the ascorbate-6-palmitate treatments were intermediate, and applying only water or ethanol had no benefits compared to untreated samples.

Experiment 3

Prior to display, all vertebrae were bright-red with no surface discoloration. Sodium erythorbate at 0.5, 1.0, or 1.5% prevented vertebrae discoloration within the first 24 hours of high-oxygen MAP display whereas untreated vertebrae and vertebrae treated with 0.05 or 0.1% erythorbate significantly discolored (Table 2). The *longissimus* from all treatments was bright-red with no signs of discoloration; therefore, erythorbate had no detrimental affects on muscle color (data not shown).

When bone-in beef steaks containing lumbar vertebrae were packaged in high-oxygen MAP, the marrow discolored rapidly while the *longissimus* muscle remained bright-red early in display. In experiments 2 and 3, both ascorbic acid and its isomer (erythorbate) proved useful for minimizing lumbar vertebrae discoloration. The reducing activity of ascorbic acid plays a role in muscle color stability via metmyoglobin reduction (Lee *et al.* 1999). We speculate that ascorbic acid maximized color stability on the surface of lumbar vertebrae by reducing methemoglobin. However, it is also possible that ascorbic acid limited methemoglobin accumulation by protecting hemoglobin from prooxidants derived from lipid oxidation.

Conclusions

We conclude that the detrimental effects of storage on beef lumbar vertebrae can be minimized through package atmosphere. Removal of oxygen from packages will limit vertebrae marrow discoloration during storage (2 weeks at 4 °C). To maximize the stability of hemoglobin on the surface of bone, combining 0.4% carbon monoxide with oxygen exclusion from modified atmosphere packages will maintain bright-red lumbar vertebrae for up to 6 weeks after packaging.

Although storage tends to promote discoloration, we conclude that high-oxygen MAP will induce rapid bone marrow discoloration. However, this rapid color deterioration on the surface of lumbar vertebrae can be prevented by ascorbic acid and sodium erythorbate. To maximize color stability through a 5-day display period at 1ºC, 2.5% ascorbic acid may be required. While ascorbate-6-palmitate minimizes vertebrae discoloration compared to untreated bone-in product, it does not perform as well as its water-soluble counterparts, ascorbic acid and sodium erythorbate.

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Table 1: Effects of packaging atmosphere[a] and storage time at 4°C on the instrumental color of marrow from beef lumbar vertebrae

Variable	Package atmosphere	Storage time				
		Initial color	Day 1	Week 2	Week 4	Week 6
a*	High-O_2	25.7c	18.0d	---[b]	---[b]	---[b]
	Ultra-low O_2	25.7c	24.9c	24.4c	17.6d	18.5d
	CO MAP	25.7c	32.1e	34.4f	31.0de	29.3d
Chroma	High-O_2	31.9c	25.1d			
	Ultra-low O_2	31.9d	30.2d	29.5d	22.5c	23.8c
	CO MAP	31.9c	38.9de	41.1e	36.9d	34.9d

[a]High-O_2 = 80%O_2 & 20% CO_2; Ultra low = 80% N_2 & 20% CO_2; CO = 0.4% CO, 30% CO_2, & 69.6%N_2.
[b]Vertebrae packaged in high-oxygen were bright red prior to packaging and moderately gray within 24 hours of storage. Thus, these vertebrae significantly discolored and were no longer evaluated.
[cdef]Least square means within a row with a different letter differ ($P < 0.05$).

Table 2: Effects of ascorbic acid and ascorbate-6-palmitate on the visual[a] color and a* values of beef lumbar vertebrae packaged in 80% O_2 and displayed at 1°C

Visual color[a]	Controls			Ascorbic acid		Ascorbate-6-palmitate	
Display (days)	Untreated	Water	Ethanol	1.5%	2.5%	1.5%	2.5%
0[b]	Prior to treatment, all vertebrae were bright-red (typical fresh cut bone)						
1	5.4c	5.4c	5.9c	1.8e	2.0e	4.2d	4.6d
3	5.8c	5.8c	6.2c	2.9e	3.0e	4.4d	4.7d
5	6.0c	6.0c	6.4c	4.2e	4.2e	5.1d	5.5d
a* values	Prior to treatment[b], all vertebrae had an average a* value of 30.8						
1	14.0c	14.0c	16.3c	33.2e	32.7e	25.4d	23.6d
5	15.7c	15.7c	14.8c	19.4e	18.3e	20.9d	22.8d

[a]Color scale of 1 = Typical fresh cut bone, 2 = Dull pinkish-red, 3 = Slightly grayish-pink or grayish-red, 4 = Grayish-pink or grayish-red, 5 = Moderately gray, 6 = All gray or grayish-black, 7 = Black discoloration.
[b]Day 0 initial color for all vertebrae prior to antioxidant treatment had an average color score of 1.0.
[cde]Least square means within a row with a different letter differ ($P < 0.05$).

Table 3: Effects of sodium erythorbate on the a* values of beef lumbar vertebrae packaged in 80% O_2 and displayed for 24 hours at 1°C

Display	Sodium erythorbate					
	Control[a]	0.05%	0.1%	0.5%	1.0%	1.5%
Initial[b]	Prior to treatment, all vertebrae were bright-red with an average a* value of 24.5					
24 hours	17.9c	17.1c	16.4c	26.9d	32.7de	32.5de

[a]Control samples recieved no topical antioxidant treatment (untreated control samples).
[b]Initial color was measured on all vertebrae prior to antioxidant treatment.
[cde]Least square means within a row with a different letter differ ($P < 0.05$).

DIET AND AGEING EFFECT ON INSTRUMENTAL AND SENSORY CHARACTERISTICS OF MEAT FROM PODOLIAN YOUNG BULLS

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Background

Meat quality largely depends on organoleptic properties such as colour, texture, flavour and juiciness which are related to production (breed, age, sex, diet) and technological (refrigereration or ageing time) factors.
Some studies (Bidner et al. 1985; Fortin et al. 1985; O'Sullivan et al. 2003) found no effect of different forage to concentrate ratio supplementations on meat quality. Ageing produces changes in meat characteristics (Zamora et al., 1996, Ruiz de Huidobro et al. 2003), influencing the final perception of the product. Tenderness has been considered the most important meat quality characteristic for consumers (Risvick 1994). It is well known that throughout ageing muscles undergo a series of physical and biochemical changes which are responsible of their conversion to meat. Particularly these modifications concern the Z-disk weakening and the myofibrilis degradation which are highly related to meat tenderness (Koohmaraie, 1994). Both instrumental and sensorial trials have been commonly used for assessing it.

Objectives

The present study aims to assess the effect of different ageing periods and diet supplementation on physicochemical and sensory properties of meat from Podolian young bulls. Such information is at present deficient in literature and has the potential to improve meat quality, primarily tenderness, and so to satisfy consumer desires.

Materials and methods

Experimental design

The experimental animals were twenty organically farmed Podolian young bulls, reared at pasture and divided into two groups of 10 each according to hay to concentrate ratio. In the high concentrate (HC) group the forage to concentrate ratio was 60 to 40 while in the low concentrate (LC) group was 70 to 30. The dry matter chemical composition of supplementation was determined by standard procedures (AOAC, 1990). On average, oats hay used as forage contained 6-7% crude protein, 27-34 % crude fibre, 62-63% NDF, and 0.5 MFU/kg, while the d.m. chemical composition of durum wheat flour shorts as concentrate was 17% crude protein, 7% crude fibre, 27% NDF and 0.95 MFU/kg. Animals were slaughtered at 16-18 months of age. Mean slaughter weight was 378±14.8 kg. The carcasses were assessed for conformation and fatness according to the SEUROP-system (UE n.1208/1981 and UE n.1026/1991). Dressed carcasses were weighed and split into two sides within 1 h *post mortem*, chilled for 48 h at 1-3°C. Dressing percentage (DP) was calculated as carcass weight to slaughter weight ratio x 100. After 48 h post mortem, each side was divided in hind and fore quarters and each quarter was dissected into different anatomical regions. The pH was measured at 1 and 24 h *post mortem* on *Longissimus dorsi* (LD), using a portable pH-meter (Hanna, HI 9025) and a combined glass electrode. The LD muscle was removed from the left and right carcass side and aged in vacuum-packaging at 4°C until 15 and 21 days *post mortem* respectively.

Meat quality

Colour parameters (lightness-L*, redness-a* and yellowness-b*) were measured according to the CIE system (CIE, 1986) using a Minolta Chromameter CR 200. Chroma (C) and hue-angle (H) were calculated according to Liu et al. (1996).
Two 1 cm wide cores were removed from each muscle parallel to the muscle fibre and placed as raw samples in the Warner-Bratzler Shear attachment, which was attached to the model 1140 Instron texture machine. The sensory analysis (flavour and tenderness) was performed by a trained eight-member panel on steaks grilled to an internal temperatue of 75°C. The values were normalised standardising each assessor by his standard deviation according to Cifuni et al., (2004).

Data were subjected to an analysis of variance, using the GLM procedure of the SAS statistical software (1999). Individual animal variation within different supplementation and muscle was used as the error term. When significant effects were found the Student t-test was used to locate significant differences between means.

Results and discussion

Podolian young bulls produced carcass of 198±9.55 kg with an average carcass yield of 52.4±0.78%. Carcass conformation of both groups was classified as good, R according to the SEUROP system, while fattening condition received a score of 2+ and 2- for the HC and the LC groups, respectively.There was a significant decrease in pH during the 24 hours post mortem. Values vary from 6.5±0.07 at 1 hours post mortem to 5.5±0.06 at 24 hours. Meat with high ultimate pH values (dark-cutting or Dark, Firm, Dry-DFD) is a persistent quality defect that shortens shelf life, especially for vacuum-packaged meat and affects meat colour, texture and water holding properties (Gill & Newton, 1981). No DFD carcasses were identified in this study and, in general, the rate of pH fall was similar for all animals.
Table 1 shows colour parameters evolution in *Longissimus dorsi* of Podolian meat as affected by ageing and different supplementation. Colour parameters were unaffected by different supplementation. Lightness (L*) was not affected by ageing while red index and chroma (color saturation) decreased as ageing time increased and yellow index and hue angle (proportions of redness and yellowness) were found higher ($P < 0.001$) at 21 days post mortem. Meat colour changed during ageing period. The colour of the muscle surface is determined mainly by both the amount and the redox state of myoglobin (Fox, 1987). In the present trial, we found that red index decreased during ageing, in agreement with the results obtained by Feldhusen et al. (1995) that found no obvious connections between oxygenation and measurements of red index. These authors claimed that higher percentages of oxymyoglobin did not lead to an increase in a* in the muscle surface of the meat that has been ripening for time periods longer than 5 days.
In Figure 1 tenderness parameters of meat from Podolian young bulls are reported. Warner-Bratzler shear force (WBS) was affected by ageing and different supplementation. After 21 days meat from HC group was significantly more tender ($P < 0.001$) than after 15 days. Meat from the HC group showed, after 15 days, higher WBS values than LC ($P < 0.01$). Extending ageing period produced an improving in tenderness, intended as sensory attribute, in both group. Tenderness was scored significantly higher ($P< 0.05$) in the LC group than HC after 15 days of ageing.

Both instrumental and sensorial analyses gave the same results: 1) the meat from LC group was more tender than that from the HC group after 15 days of ageing; 2) during the ageing period the meat became more tender even if only in the HC group the increase in tenderness was significant. Comparison between sensory and objective measures of meat tenderness in previous researches gave very variable results. Some authors have found a good correlation (e.g. Touraille, 1982) whereas others found very poor coefficients (e.g. Shackelford et al. 1995). We found a positive relation between WBS and sensorial tenderness using raw meat. Indeed, shear force on raw meat reflects the background of collagen toughness, collagen being the main component of muscle connective tissue and the major determinant of the texture of meat, whereas shear force on cooked meat may be considered a measure of myofibrillar toughness (De Smet et al. 1998). Our WBS values are in agreement with values found by Torrescano et al.(2003) in raw samples of 14 bovine muscles. Forage finishing of beef has produced mixed results on tenderness and palatability attributes. Our findings showed that major forage fed animals produced a more tender meat than major concentrate fed animals after 15 days of ageing along with French et al.(2001), who found that supplementing grass with low levels of concentrates produced the most tender and acceptable meat two days post mortem, and in disagreement with Mitchell et al. (1991), who reported a negative effect of forage finishing on meat tenderness. After 21 days the different forage to concentrate ratio didn't affect meat tenderness.

Figure 2 shows flavour evolution of Podolian meat as affected by ageing and different supplementation.

The flavour was unaffected by different supplementation and ageing time. In both experimental group the ageing time improved flavour and HC and LC groups showed very similar values at 15 and 21 days of ageing. Flavour intensity increased with ageing time. This result is due to post-mortem processes such as proteolysis and lipolysis which result in the development of flavour precours. We found no effect of different forage to concentrate ratio supplementation of meat flavour. Muir et al. (1998) claimed that the differences in flavour and acceptability due to feed accounted for differences in carcass fatness.

Conclusions

The results of this study showed that a longer ageing time improved the tenderness of meat as determined through an instrumental and sensorial approach. A higher forage to concentrate ratio can improve tenderness but a longer ageing eliminates all diet effects on eating quality of beef. A longer ageing time didn't affect the colour parameters regarded by consumers as appreciating features.
We can conclude that ageing time is one of the main technological factors affecting beef quality and that the choice of a proper ageing time is critical for optimising the organoleptic properties of meat.

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Table 1 Colour characteristics of Podolian meat as affected by ageing (days) and different supplementations during the finishing period (means ± SE).

		Ageing					P		
	diet	**15 d**		**21 d**		**SEM**	**days**	**diet**	**days x diet**
L*	HC	35.06		35.88		0.67	NS	NS	NS
	LC	36.22		35.90					
a*	HC	19.81	a	13.74	b	0.55	***	NS	NS
	LC	18.94	a	13.86	b				
b*	HC	4.25	b	5.58	a	0.29	***	NS	NS
	LC	4.04	b	5.07	a				
C	HC	20.29	a	14.82	b	0.54	***	NS	NS
	LC	19.37	a	14.78	b				
H	HC	12.24	b	22.24	a	1.06	***	NS	NS
	LC	11.96	b	20.20	a				

NS = not significant; ***=P<0.001. Means followed by different letters differ significantly at P < 0.05.

Fig.1 Tenderness parameters (WBS and tenderness as sensory attribute) of Podolian meat as affected by ageing (days) and different supplementations (means ± SE).

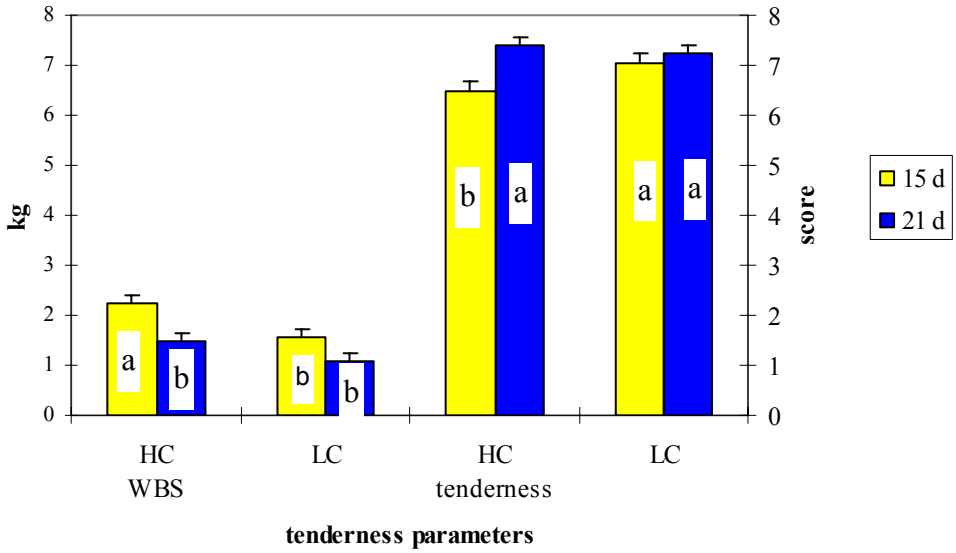
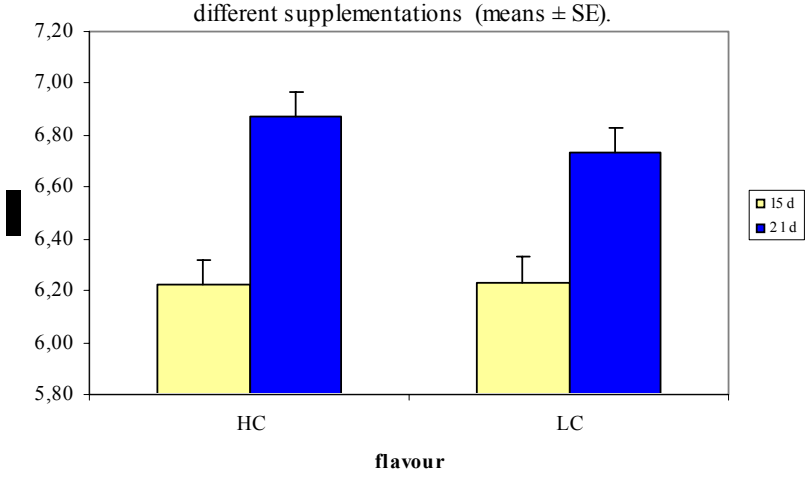


Fig. 2 Flavour of Podolian meat as affected by ageing (days) and different supplementations (means ± SE).



EFFECT OF CLASSING THE BULL CARCASSES WITHIN QUALITATIVE CLASSES (EUROP) ON CHEMICAL AND PHYSICAL-TECHNOLOGICAL PROPERTIES OF MEAT

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Background

Classification of the slaughtered bodies of cattle according to the EUROP system gives the processor and farmer good survey on the quality in fattening cattle. It makes it easier for the meat processor to plan the production and meat distribution more effectively, and the farmer can take rational breeding measures that increase the portion of muscular substance in the carcass of cattle, and at the same time enable better economic valorization of the animal at purchase and realization. The task of a meat processor should be to supply the network of shops not only with valuable parts of the carcass but also with meat of high nutritional and sensorial quality. Is it possible to predict at least partially the quality of muscular substance by the assessment of the quality class of the carcass (meatiness and fattiness)? Which are then the relations between the carcass quality and quantitative parameters of meat? A number of authors have tried to answer this question (Temisan and Augustini, 1987; Shemeis *et al*., 1994; Fiems *et al*., 2000; Saňudo *et al*., 2000; Maher *et al*., 2001; Kim and Lee, 2003). Results of the mentioned authors are quite variable and they show that the classes of fattiness have better expression ability to predict the quality of meat (Bach *et al*., 1986).

Objectives

To evaluate the relationship between the quality class of the carcass of bulls assessed after the EUROP system and the meat quality (*m. longissimus thoracis*).

Materials and methods

We used total 340 adult slaughter bulls of different breeds (Slovak Spotted, Slovak Pinzgau, Holstein, Belgian Blue, Braunvieh, Limousine and Blond d'Aquitaine) in the experiment. Their average weight before slaughter was 515 kg. After slaughtering the carcass sides were examined and classified by a classifier after the official EU scheme (EC 1208/1981) and classed within the classes of meatiness (E, U, R, O P) and classes of fattiness (1, 2, 3, 4, 5). A muscle sample was taken from *m. longissimus thoracis* from the right side of carcass 24 hrs. post mortem. The sample was stored in a refrigerator at the temperature between +1^{o}C and 3^{o}C for further qualitative analyses. Content of total water, protein and intramuscular fat was determined in the apparatus Infratec 1265 48 hrs. post mortem. Furthermore the pH value (firm Radelkis), meat colour (Spekol) and water holding capacity (Hašek and Palanská, 1976) were measured. In the remaining sample the weight loss by cooking and shear force in the cooked meat (Warner-Bratzler) were determined on the 7th day after the slaughter. Values $\overline{x} \pm s$ of the studied qualitative parameters of meat were calculated and the differences between the quality classes of the carcasses (t-test) were tested by means of the STATGRAPHIC programme.

Results and discussion

In table 1 there are the average values of qualitative parameters of meat according to the qualitative classes of the carcasses considering the degree of meatiness (E, U, R, O, P). The results show that there are no significant differences among the classes of quality, except for small exceptions. The values are variable, there are no visible marked trends towards increasing or decreasing the values of qualitative parameters with the improvement or deterioration of carcass quality. The evaluation of shear force in cooked meat is worth mentioning; the most tender meat was in the least meaty carcass sides. The value of shear force in E class was 4.93 and in P class 3.44 kg ($P<0.05$). We noticed increasing content of intramuscular fat with deterioration of the meatiness in the slaughtered body (E = 1.99 and P = 2.49 g . 100 g^{-1}). Maher *et al*. (2001) found no constant influence of the effect of classification on the values of qualitative parameters in *m. longissimus dorsi* and *m. semimembranosus* in bullocks and heifers, using the most frequent classes

according to the statistics in Ireland ($R4^+$, $R4^-$, O^{4+} and O^{4-}). If we compare the meat quality taking into consideration the classes of fattiness in the slaughtered body (1, 2, 3, 4, 5) we obtain results given in tab. 2. The content of intramuscular fat increased linearly with increasing fattiness of the carcass (class 1 = 1.19; class 5 = 2.52 g .100 g^{-1}). The differences between classes were statistically significant $P<0.05$). On the contrary, water holding capacity decreased (class 1 = 35.69; class 5 = 32.45 g . 100 g^{-1}). Results with other parameters of meat quality are quite variable. Bach *et al*. (1986) observed in bulls and Shemeis *et al*. (1994) in cows that with increasing fattiness of the carcass also the content of intramuscular fat increased, the values of shear force decreased, and the smell and flavour of meat were evaluated more favourably. Fiems *et al*. (2000) calculated the correlation coefficient between the degree of fattiness in carcass and content of intramuscular fat r = 0.704, values of shear force r = -0.244 and colour of meat r = -0.159 in bulls of Belgian Blue breed. The correlations, we calculated, were weak (0.104; -0.170 and –0.132). We also found out that the degree of marbling in meat correlated strongly with the content of intramuscular fat (r = -0.781).

Conclusions

Our results confirmed hitherto knowledge that the estimation of beef quality assessment is more precise if we use the classes of fattiness in the slaughtered body. We found most linear changes with the intramuscular fat using the meat classes as well as the classes of fattiness. Practical utilization of the results considering separately the classes of meatiness or classes of fattiness is questionable as the resulting quality class is in the EUROP system always the combination of the class of meatiness and the class of fattiness.

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Tab.1. Mean values ($\bar{x}$, s) of qualitative parameters in *m. longissimus thoracis* considering the classes of meatiness

Parameter	E	U	R	O	P	t – test
Water g.100g^{-1}	75,63±098	75,16±0,95	75,21±1,00	74,95±0,92	75,12±1,07	E:O*
Proteins g.100g^{-1}	21,43±0,63	21,63±0,83	21,83±0,75	21,88±0,74	21,89±0,71	E:O*
Fat g.100g^{-1}	1,99±0,78	2,19±0,95	1,98±0,83	2,32±0,84	2,46±0,82	R:O*
pH_{48}	5,75±0,18	5,71±0,21	5,74±0,24	5,71±0,20	5,71±0,17	
Colour %	8,86±1,64	9,70±2,34	9,43±2,53	9,30±2,23	9,62±2,50	
WHC g.100g^{-1}	34,15±3,22	34,50±3,59	33,56±3,62	33,88±3,42	33,92±1,30	
Cooking losses g.100g^{-1}	43,44±4,30	43,85±3,33	43,61±4,33	43,74±4,35	45,20±2,17	
Shear force kg	4,93±2,98	4,55±2,05	3,91±1,85	3,73±1,47	3,44±1,19	U:R,O*

* $P < 0.05$

Tab. 2. Mean values ($\bar{x}$, s) of qualitative parameters in *m. longissimus thoracis* considering the classes of fattines

Parameter	1	2	3	4	5	t – test
Water g.100g^{-1}	76,34±0,25	75,50±0,97	75,01±0,96	74,89±0,88	74,88±0,86	1:2*,3,4**
Proteins g.100g^{-1}	21,41±0,64	21,51±0,75	21,85±0,78	22,03±0,68	21,43±0,70	
Fat g.100g^{-1}	1,19±0,35	2,01±0,84	2,21±0,92	2,19±0,76	2,52±0,71	1:2,3,4*
pH_{48}	5,63±0,12	5,70±0,20	5,73±0,23	5,74±0,21	5,60±0,20	
Colour %	7,86±1,14	10,21±2,78	9,20±2,15	8,93±2,00	10,00±2,65	2:3,4*
WHC g.100g^{-1}	35,69±1,65	34,56±3,42	33,84±3,53	33,03±3,48	32,45±3,19	2:3*,4**
Cooking losses g.100g^{-1}	45,96±0,25	43,85±3,20	43,58±4,34	43,87±4,59	46,01±3,93	1:3**
Shear force kg	3,53±0,61	4,71±2,44	3,68±1,55	4,06±1,55	4,68±1,47	2:3**,4*

*$P < 0.05$, **$P < 0.01$

CHEMICAL AND PHYSICAL CHARACTERISTICS OF FRESH SUBCUTANEOUS FAT FROM ALENTEJANO PIG BREED

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Background

The Alentejano pig differs from the modern highly selected breeds regarding growth rate and body composition. Comparatively with others, this pig shows a slow rate of growth and a high lipogenesis activity at the early stages of development. The lipids are deposited mainly in subcutaneous, renal and pelvic regions. The percentages of fatty cuts can attain more than 50% of the carcass weight and the backfat thickness at the last rib level can grow to 60 mm at 120 kg live weight (LW) (Almeida et al., 1993; Neves et al., 2001). This kind of carcass is considered ideal for the manufacture of dry cured products, and was for decades the main source of meat in the diet of the people living in Alentejo region. Nowadays, the production fulfills a double function: it provides meat for the manufacture of cured products and for fresh consumption. The manufacture industry (cured hams, forelegs and sausages) requires pigs reared in traditional extensive systems, slaughtered at 140-160 kg BW and with 18-24 months of age (De Pedro and Olmo, 2000). The emerging market of the fresh meat requires animals with 90-100 kg LW, obtained at 10-12 months of age. The adipose tissue plays an important role on the characteristics of cured or cooked products and fresh meat. The animal growth implies chemical, biochemical and physical changes in the adipose tissue, mainly due to an increase on the lipids content. These changes affect the gross chemical composition or the fatty acid composition, which could determine its global quality (firmness or softness, color or oxidation sensibility) (Lebret and Mourot, 1998).

Objectives

The aim of this work was to investigate the evolution of the chemical composition of fatty tissues and its effect on chemical and physical traits.

Materials and methods

Thirty Alentejano pigs were weaned at 28 days and castrated at 60 days old. After weaning, they were transferred to open-air individual pens with a protecting roof and fed a commercial diet (15 % CP; 3100 kcal DE) at 85 % of *ad libitum*. This diet had 3 % crude fat, distributed by the following major fatty acids: C16:0 (17,7 %), C18:1 (28,9 %) and C18:2 (31,2 %). The animals were weekly weighted and after a 24 h fasting period, 5 pigs were initially slaughtered at 40 kg LW. The remaining animals were fasted and slaughtered (5 animals) at 70, 80, 90, 100 and 110 kg LW. After slaughter, the left side of each carcass was submitted to a 24 h chilling, followed by commercial cuts and sample collection of backfat at last rib level. Adipose subcutaneous backfat at the 12^{th}-13^{th} rib level and ham were also sampled. All the samples were vacuum packaged and stored (-20 ºC) until analysis. Analyses included moisture (Portuguese norm - 1614), total protein (Portuguese Norm - 1612) and lipids (calculated as 100 - [protein + water]). Color CIE L* a* b* (Minolta CR-200) and pH (Portuguese Norm - 3441) were also determined. Lipids for fatty acid determination were extracted at 50 ºC, and prepared to obtain the methyl esters to be analyzed by GC/FID. An ANOVA was carried out and the means comparison was made by SNK test. The correlations between the variables studied were determined by the Pearson coefficient. SPSS statistical software was used.

Results and discussion

Table 1 presents the results of gross chemical composition and some physical traits. The slaughter weight affected significantly the gross chemical composition and the subcutaneous color values. As the slaughter weight increased, the water and protein amount decreased and the lipids concentration increased, mainly between 40 and 70 kg LW. From 70 kg to 110 kg LW, we observed the same significant differences between the two slaughter groups, but the differences among the tree major chemical compounds were less pronounced. The evolution of the gross chemical composition was paralleled by the increase of backfat depth and the increase of this adipose depot on the Alentejano pig carcass. This evolution of the chemical

composition seems to agree with the three phases of development of the adipose tissue: hyperplasia between 7 and 20 kg, hyperplasia and hypertrophy between 20 and 70 kg, and finally an exclusive hypertrophy after 70 kg LW (Anderson and Kauffman, 1973). The higher increase between 40 and 70 kg LW could be explained by a predominant hypertrophy of the adipocytes, accompanied by a decrease of the amount in protein and water in the tissue, whether the amount of lipids increased (Camara *et al*., 1994).
Between 40 and 70 kg LW the backfat became less colored and mainly less red. The a* (red) value decreased (7,4 to 3,7), the b* (yellow) increased (1,39 to 4,2), the hue angle increased (from 10,45 º to 49,00 º) and the cromatocity decreased (7,6 to 5,6). These results could be explained by a dilution effect of the connective network and capillary infiltration, as a result of the lipids synthesis and deposition. The pH values showed large variations among the six groups studied, and no trend was identified. The results from the correlation matrix showed a high correlation between lipids and pH (0,88; P>0,05). As known, the lipids have a neutral pH, and an increase of their concentration implies an increase of the pH value. The deposition of lipids in adipose tissue (adipocytes) seemed to produce a great change in color backfat evidentiated by the high correlation between lipids and the chromatic coordinates a* and b* (-0,85 and 0,82; P>0,05); and in hue angle (0,94; P>0,05) and croma (-0,59).

Table 2 presents the results of fatty acid composition. The slaughter weight affected greatly the amount of the major fatty acids between 40 and 70 kg LW, showing a slight difference between 70 and 100 kg LW. At 40 kg LW, subcutaneous tissue presented less C16:0, C18:0 and total saturated, and C18:1 and total monounsaturated fatty acids, but more C18:2 and total polyunsaturated fatty acids. After 70 kg LW the slaughter weight affected the amount of C16:1, C18:1, C18:2, total monounsaturated and total polyunsaturated fatty acids. This heavier group registered a significantly great amount of C16:1, C18:1 and total monounsaturated fatty acids, and a lower content of C18:2 and total polyunsaturated fatty acids. However, quantitatively, the differences between the upper and the lower values were rather small. In general, the adiposity increase during the growth process induced a reduction of the unsaturation degree of the fat depots (Nürnberg, 1995). This could be explained by the greater participation of the metabolic pathway synthesis *de novo,* mainly saturated. In our experiment, we didn't observe a reduction in the unsaturation degree, but a change in the proportions of poly and monounsaturated fractions was detected (increase of monounsaturated and decrease of polyunsaturated fractions). Since the diet used in our trial had only a 3% crude fat, the increase of the saturated fatty acids through a *de novo* synthesis is suggested. In another study with Alentejano pigs slaughtered at 95 kg LW, Neves (1998) found less amounts of C16:0 (23,50%) and C18:1 (44,45%) and a greater amount of C18:2 (12,06%) than the ones obtained in this study. However, although the diet used had a similar fatty acid composition, it was richer in crude fat (6%) which could have stimulated the exogenous synthesis (Mourot *et al*., 1994) and a great deposition of C18:2.

Conclusions

- The Alentejano pig revealed a great adipogenic capacity at early developmental stages. Lipid concentration in subcutaneous fat showed a high increase between 40 and 70 kg LW and only a slight increase between 70 and 100 kg LW.

- The high adipogenic activity in the backfat at early development stages was accompanied by a large change in the fatty acid profile, with the increase of total saturated and monounsaturated fatty acids, and a decrease of total polyunsaturated fatty acids. After 70 kg LW, the differences found in fatty acid composition didn't seem to be important enough to change the global quality of the Alentejano pig subcutaneous fat.

- The evolution of gross chemical composition had a great effect on the backfat color attributes.

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Acknowledgements

We thank to the AGRO program

Table 1. Chemical and physical characteristics of the backfat of Alentejano pigs at a various live weights

Slaughter weight groups	40	70	80	90	100
Water	18,76± 3,7 [a]	5,29 ± 0,3 [b]	5,37 ± 0,5 [b]	5,14 ± 0,6 [b]	5,05 ± 0,5 [b]
Protein	3,80 ± 1,3 [a]	1,19 ± 0,3 [b]	0,92 ± 0,1 [b]	0,94 ± 0,4 [b]	0,86 ± 0,2 [b]
Lipids	77,44 ± 5,0 [a]	93,53 ± 0,5 [b]	93,72 ± 0,6 [b]	93,92 ± 0,9 [b]	94,09 ± 0,7 [b]
pH	6,32 ± 0,2 [a]	7,00c ± 0,1	6,76 ± 0,1 [b]	6,91 ± 0,1 [bc]	6,84 ± 0,04 [bc]
L*	80,46 ± 0,1 [a]	79,38 ± 1,0 [b]	80,09 ± 1,0 [b]	80,06 ± 1,5 [b]	79,24 ± 1,2 [b]
a*	7,43 ± 0,3 [a]	3,70 ± 0,9 [b]	4,16 ± 0,6 [b]	3,94 ± 1,2 [b]	3,66 ± 0,8 [b]
b*	1,39 ± 0,6 [a]	4,20 ± 0,8 [b]	4,98± 0,5 [b]	4,21 ± 0,5 [b]	4,23 ± 0,4 [b]
Hue	10,50 ± 4,2 [a]	49,00 ± 3,7 [b]	50,20 ± 1,8 [b]	47,99 ± 8,0 [b]	49,57 ± 3,6 [b]
Croma	7,58 ± 0,3 [a]	5,61 ± 1,19 [b]	6,49 ± 0,8 [ab]	5,80 ± 1,1 [b]	5,60 ± 0,8 [b]
Saturation	0,09 ± 0,004 [a]	0,07 ± 0,002 [b]	0,08 ± 0,01 [ab]	0,07 ± 0,01 [b]	0,07 ± 0,01 [b]
Backfat depth (cm)	1,3 ± 0,4 [a]	3,0 ± 0,6 [b]	4,1 ± 0,5 [c]	5,1 ± 0,3 [d]	5,6 ± 0,4 [d]

Means within the same line with same letter were not significantly different (P>0,05)

Table 2. Fatty acids composition of the backfat of Alentejano pigs at a various live weights

Slaughter weight groups	40	70	80	90	100
C14:0	1,46 ± 0,1	1,33 ± 0,1	1,43 ± 0,1	1,39 ± 0,1	1,51 ± 0,1
C16:0	22,07 ± 1,1^{c}	24,34 ± 0,5^{ab}	24,46 ± 1,0^{ab}	24,19 ± 0,4^{ab}	24,92 ± 0,5^{ab}
C16:1	1,77 ± 0,1^{c}	2,18 ± 0,2^{a}	2,12 ± 0,1^{a}	2,31 ± 0,3^{ab}	2,57 ± 0,3^{b}
C18:0	11,41 ± 0,7^{b}	12,57 ± 0,7^{a}	12,75 ± 0,6^{a}	12,52 ± 1,4^{a}	12,29 ± 0,4^{a}
C18:1	41,75 ± 1,0^{c}	46,61 ± 0,6^{ab}	45,90 ± 0,5^{a}	46,38 ± 0,6^{ab}	47,11 ± 0,7^{b}
C18:2	13,60 ± 0,7^{c}	9,60 ± 0,4^{b}	9,87 ± 0,7^{b}	10,13 ± 0,7^{b}	8,65 ± 0,6^{a}
C18:3	1,02 ± 0,04	0,84 ± 0,1	1,01 ± 0,3	0,88 ± 0,3	0,74 ± 0,1
Total Saturated	36,96 ± 1,7^{a}	38,67 ± 1,0^{b}	39,01 ± 1,6^{b}	38,50 ± 1,0^{b}	39,15 ± 0,8^{b}
Total monounsaturated	44,88 ± 0,9^{c}	50,19 ± 0,6^{ab}	49,34 ± 0,6^{b}	49,89 ± 0,8^{ab}	50,93 ± 0,8^{a}
Total Polyunsaturated	15,87 ± 0,8^{c}	11,05 ± 0,5^{a}	11,50 ± 1,0^{a}	11,63 ± 0,8^{a}	9,98 ± 0,8^{b}

Means within the same line with same letter were not significantly different (P>0,05)

PHYSICAL CHARACTERISTICS OF THE LONGISSIMUS TRAIT MUSCLE OF THE ITALIAN AND POLISH HORSE MEAT

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Background

Of the commonest horse-breeding in Italy the Italian Trotter ranks the first (11.260) heads of cattle). It is used as a sports-horse followed by rustic breeds which are currently utilised in country's resorts or for meat production such as the "Avelignese" (9.782), the "Cavallo Agricolo Italiano da Tiro Pesante Rapido" (heavy-cart horse) (6.377), the "Maremmano" (3.540), the "Murgese" ("2.722), the "Bardigiano" (2.200), the 'Sanfratellano" (1.469) and the "Tolfetano" (1.377) (FAO, 2002).

The Italian horse-meat sector is one of the most important all over the world. In 2002 the Italian production accounted for 77.34% of the world wide one and 35.92% of the European one (Fao 2002). In 2002 horse-breeding in Italy counted 285.000, and such a number kept unchanged with respect to the previous year. Also the number of slaughtered animals (278,283) was the same as the previous year (Istat, 2001) (National Poll Institute).

In 2001, according to the last ISTAT information (data) processed by "Assocarni" little less than 300,000 horses were slaughtered in Italy, with an increase by 19,9 related to 2000 of these 69,3% in privately owned slaughter houses. In detail, 278,283 horses and 2,228 including asses, mules and hynnies. To this end it is noteworthy to mention that horse-slaughtering since 1999 at the time of the bovine BSE onset has a positive trend Martuzzi et al., 2002) with significant influence on import-export, and increase in quality of imported meat.

The average yearly per capita meat consumption in Italy is about 13 kg which is 1.6% of total meat quantity eaten in one year (82 kg per capita) (Istat, 2001). Italy, then, ranks the first in Europe for horse-meat consumption which in the other E.U. Countries accounts for 0.4 kg per capita (Martin-Rosset, 2001). Horse-meat consumption greatly varies within the national territory, most horse-slaughter in Italy occurs in Apulia (33.4%), Lombardy (13.7%), Piedmont (10.5%), Veneto (8.5 %) and Latium (5.8%) (Istat, 2001). Home production, despite the limited consumption, cannot meet the market demand, hence the need of importing heads of cattle and meat from abroad mainly Eastern Europe Countries (Martuzzi et al.,2001); for, in 2001 23,156 tons of horse meat were imported with an increase by 25.5% compared to 2000 (Fao, 2002).

Objectives

The aim of this research is to study some quality indicators of carcasses and of horse meat belonging to two different genetic type on the Italian market.

Materials and methods

Twelve female crossbred foals, seven TPR x Abruzzese genetic type and five TPR x Polish genetic type were slaughtered at the age between 24 and 26 months.

After slaughtering all carcasses were weighed and measured pH value. This last was taken from shoulder, leg and Longissimus dorsi (Ld) muscles by means of pH meter (Hanna Instruments HI 9023) equipped with an immersion glass electrode. Then, a sample of the Ld muscle from the 8th and 9th rib was taken to the lab and stored at 4°C. This sample was measured pH values at 24,48, 96 and 168 h, and of colour at 0.75, 48 and 196 h from slaughter. Colour was measured by Microscan XE colour meter through Hunter L* a* b* tridimensional system. Also hue (arctg b/a) and chroma or colour saturation values were measured (= (a2+b2)1/2). Shortly after slaughter samples were taken and put in plastic bags and immediately frozen at –80°C. All samples were defrosted at the same time to carry out the shear force tests before and after cooking and to evaluate cooking loss. For the shear force each meat sample was tested to the core 3 times before and after cooking, and their shear force value was determined by means of WBS test (Warner Blatzer Shear Force) through Instron 9000 texurometer.

In order to evaluate losses due to cooking, samples were weighed before and after cooking, in a convector oven at an inside temperature of the sample of 60° C over 30 minutes, measured by a diving (sinking) probe thermometer. Cooking loss was considered as a percent of the initial weight. The data analyzed for variance werw evaluated by using Student's 't' test (SAS, 1996).

Results and discussion

Table 1 shows post mortem pH values of Italian and Polish mares meat. pH measurements 45 minutes after slaughter were considerably higher in Polish mares for both the fore-quarter and rear one muscles ($P < 0.01$). The meat pH of the Italian mares is higher ($P< 0.05$) than the Polish mares one only 48 hours after slaughter.
Ld colour values measured at different post mortem times are shown in table 2. Shortly after slaughter meat colorimetric parameters of the Polish mare were remarkably different from those of the Italian mares. Conversely, at 48 hours both a* and b* values appeared higher in the foreign meat ($P<0.05$). Moreover, always at 48 hours, the Polish mares meat showed a lesser hue ($P <0.05$) and a higher saturation ($P <0.05$), appearing more intensely coloured. After one week storage (168 h) the situation is alike, since Polish mares showed higher redness index and chroma ($P <0.05$), thus indicating a more vivid colour. Investigations by Robelin et al (1984) on other breeds confirm our results related to the effect of sex and genotype on the horse meat colour.
The consistency of meats (table 3) belonging to the two genotype under investigation is nearly the same both for raw and cooked. Ld shear force data show that force needed for cutting raw meat of mares was similar. An instrumental force significantly ($P<0.05$) higher was necessary to cut cooked meat samples of polish mares. Literature reads that the genotype as well as other factors (Boccardi et al., 1979;Robelin et al, 1984; McCormick, 1999) affect meat tenderness relating to three classes of muscular proteins: the connective tissue, (collagen, elastin, reticulin, mucopolysaccharide of the basal substance) the myofibris (actin, myosin and tropomyosin) and the sarcoplasm (sarcoplasmic proteins, sarcoplasmic reticulum) (Lawrie 1983).
Shrink or drop in weight results following to cooking loss evidence a water retention capacity being nearly the same between the Italian and Polish mares. Final pH, obtained after post-mortem glicolysis, directly affects denaturation and proteolysis degree of sarcoplasmic proteins. These phenomena, in turn, influence meat tenderness, water retention capacity and colour (Lawrie, 1985). A high pH indicates a lesser proteic denaturation and a lesser proteolysis during ripening (Raduco-Thomas, 1989) and explain the major capacity of proteins in retaining water (Cook, 1926; Empey, 1933).

Conclusions

The results of this research leads to the following considerations :

- pH was not influenced by the genotype effect ;
- when slaughtered meats of the two genetic types under study showed a nearly similar colour. During storage lapse Polish mares meat intensified its colour more than the Italian mares one.
- The Italian and Polish meat tenderness was the same both cooked and raw. Similarly this behaviour was also noted for cooking loss.

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Table 1 - pH values of different sections of the carcass and Ld, at different post mortem times, of the Italian and Polish mares.

			ITALIAN	POLISH
		Rear quarter	6,50 B	6,80 A
45 min.	pH_1	*Longissimus dorsi*	6,36	6,46
		Fore quarter	6,37 b	6,78 a
24 h	pH_2	*Longissimus dorsi*	5,77	5,71
48 h	pH_3	*Longissimus dorsi*	5,84 a	5,71 b
96 h	pH_4	*Longissimus dorsi*	5,79	5,76
168 h	pH_2	*Longissimus dorsi*	5,85	5,77

Table 2 - Colorimetric characteristics of Ld of Italian and Polish mares at different post mortem times.

TIME	COLOR	ITALIAN	POLISH
45 min	L*	30,79	31,03
	a*	11,32	12,93
	b*	12,29	13,31
	HUE	47,27	45,85
	CHROMA	16,72	18,57
48 h	L*	34,79	36,50
	a*	10,63 b	14,44 a
	b*	15,72 b	18,39 a
	HUE	56,33 a	52,00 b
	CHROMA	19,02 b	23,40 a
168 h	L*	34,02	34,87
	a*	10,15 b	11,58 a
	b*	14,71	15,96
	HUE	55,28	53,94
	CHROMA	17,91 b	19,71 a

Table 3 - Physical characteristics.

		ITALIAN	POLISH
WBS	Row	0,87	0,86
	Cooked	2,47 b	2,92 a
Cooking loss (%)		38,30	39,15

EFFECTS OF *POST MORTEM* DEBONING TIME ON PHYSICAL, CHEMICAL, MORPHOLOGICAL AND ORGANOLEPTIC PROPERTIES OF CHICKEN BREAST MEAT DURING *POST MORTEM* AGING

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Background

It is often said that chicken beast meat is not favourite for lack of its juiciness and tenderness in Japan, although chicken is most cheap in meat. The improvement of chicken beast meat qualities is now being required. It is well known that rigor mortis happens immediately after slaughter and the muscles in an animal become tough. It was reported that rigor mortis reached to the maximum at 2 hours after slaughter, which leads to make chicken muscle the toughest (Negishi and Yoshikawa, 1994). This phenomenon not only hardens meat but also reduces juiciness of meat, resulting in decline in meat quality even if meat is stored for *post mortem* aging at low temperature.
In Japan, the production of broiler meat is expanded. The deboning process in a factory in Japan is immediately conducted after slaughter in order to save time for production of broiler meat from a living body, because freshness of chicken meat is required from a market. In general, boiler breast meat in Japan is evaluated non-juiciness. This problem is caused by rigor mortis after slaughter. In order to prevent such a decline in meat quality, it is important to make influence of rigor mortis as small as possible. It is reported to pass a time zone of rigor mortis in the state on the bone as one of the methods of making influence of rigor mortis small (McKee *et al.*, 1997). However, it has not clarified how deboning time after slaughter influences rigor mortis and meat quality after *post mortem* aging.

Objectives

The objective of this work was to clarify the effects of *post mortem* deboning time on physical, chemical, morphological and organoleptic properties of chicken breast meat after *post mortem* aging in order to the improvement of juiciness and tenderness.

Materials and methods

Preparation of chicken breast: In analysis and sensory evaluation, chicken breast meat obtained from male broiler (White Rock×White Cornish) deboned at 1, 2, 4, 6 or 24 h (hours) *post mortem* was aseptically put into polyvinylidene chloride bags, vacuum-sealed, and stored at 0 ˚C for 4 days. In morphological observation, one obtained from female broiler deboned at 1, 8 or 16 h *post mortem*. Each sample deboned at 1 h *post mortem* was observed after aging until 8 or 16 h at 0 ˚C.

Sensory evaluation: Samples for sensory evaluation were skinned and grilled at 140˚C until internal temperature became 70 ˚C. Tenderness, juiciness and palatability were evaluated by well-trained panels using Kramer ranking tests (1963).

Cooling loss and cooking loss: Amounts of drips separated from samples after storing at 4 ˚C for 4 days were measured and the cooling loss was expressed with the rate of drip to sample weight. Their samples were skinned and vacuum-sealed. Amounts of drips separated from samples during heating at 70 ˚C for 70 minutes in a water bath were measured and the cooking loss was expressed with the rate of drip to sample weight before heating.

Shear force values: Pectoral muscles separated from chicken breast used for measurement of cooking loss were cut into 4×1×1 cm. Their shear force values were determined by Tensipresser™ (TTP-50BX, Taketomo) with a cylindrical plunger of 5.5 mm in diameter (Ozutsumi *et al.*, 1988).

Morphological observation: Pectoral muscles of chicken breast deboned at 1, 8 or 16 h *post mortem* were immediately fixed with 0.5 % paraformaldehyde in K-phosphate buffer (pH 7.3) by standard method. The samples for observation with optical microscope (DX-50, Olympus) were stained with hematoxylin and eosin. The pre-fixed samples were fixed with 2% osmium tetroxide and dehydrate by standard method. They were embedded in epoxi resin and the blocks were cut with ultracut ultramicrotome. Their sections were stained with uranyl acetate and lead citrate for observation with transmission electron microscope (1200EX, Joel). The distance between Z lines of each sample was measured as arbitrary average value of ten points.

Myofibrillar fragmentation index (MFI): Myofibrils prepared from chicken breast meat were suspended, observed at 1,000 magnification under a phase-contrast microscope, and photographed. MFI (%) was measured as the ratio of myofibrillar fragments composed of 1-4 sarcomeres.

Results and discussion

Sensory evaluation: Table 1 shows the sensory properties after post-deboning aging for 4 days at 0℃. Chicken breast deboned at 4 h *post mortem* was the most tender and juicy among those deboned at 1, 2, 4, 6 h *post mortem*. Chicken breast deboned at 6 h *post mortem* was more palatable than that deboned at 4 h *post mortem*. It was shown that chicken breast deboned at 4-6 h *post mortem* were the most tender, juicy and palatable those after post-deboning aging.

Cooling loss and cooking loss: Figure 1 shows cooling loss and cooking loss after post-deboning aging. Cooling loss of chicken breast deboned at 1 or 2 h *post mortem* was higher than those of ones deboned at 4, 6 and 24 h *post mortem*. On the other hand, the cooking loss of chicken breast deboned at 1 h *post mortem* was also lager than those of meats deboned at 4, 6 and 24 h *post mortem*.

Shear force values: Shear force value of chicken breast meat after post-deboning aging was shown in Figure 2. Shear force value of breast meat deboned at 1 h *post mortem* was lager than those of meats deboned at 2, 4, 6 and 24 h *post mortem*, and shear force value of breast meat deboned at 2 h *post mortem* was also lager than those of meats deboned at 6 and 24 h *post mortem*. These results were consistent with those of the sensory evaluation for juiciness and tenderness.

Morphological observation: Optical micrographs of pectoral muscle deboned at 1, 8 and 16 h *post mortem* were shown in Fig. 3. Some crooked portions were observed in the myofibrils of pectoral muscle deboned at 1 h *post mortem*, while they were not observed in breast muscle deboned at 8, 16 h. The observation by transmission electron microscope showed that the distance between Z lines in myofibrils of chicken pectoral muscle deboned at 8 or 16 h *post mortem* was longer than that of muscles deboned at 1 h *post mortem* (Table 2). It was suggested that the latter myofibrils were observed to be wounded partially, because the rigor mortis was accelerated after deboning.

Myofibrillar fragmentation index (MFI): Figure 4 shows MFI after post-deboning aging. The MFI of chicken breast meat deboned at 1 or 2 h *post mortem* was smaller than those of meat deboned at 4, 6 and 24 h. It was also shown that weakening of Z lines of chicken pectoral muscle deboned after 4 h *post mortem* was significantly increased, and its tenderization was also accelerated.

Conclusions

From these results of this work, it was concluded that deboning of chicken breast after 4 h *post mortem* improved meat qualities such as tenderness, juiciness, cooling loss, cooking loss and shear force value.

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Table 1. Effect of *post mortem* deboning time on the tenderness, juiciness and palatability of chicken breast meat aged at 0 ˚C for 4 days

1. Deboning time at 1, 2, 4 h postmortem

	Ranking		
	1 hour	2 hours	4 hours
Tenderness	3 **	2	1 **
Juiciness	3 *	2	1 **
Palatability	3 *	2	1 *

2. Deboning time at 1, 4, 6 h postmortem

	Ranking		
	1 hour	4 hours	6 hours
Tenderness	3 **	1 **	2
Juiciness	3 **	1 **	2 *
Palatability	3 **	2	1 **

****p<0.01 ; *p<0.05 (ranking test, Kramer method).**

Fig. 1. Effect of *post mortem* deboning time on the cooling loss and cooking loss of chicken breast meat aged at 0 ˚C for 4 days

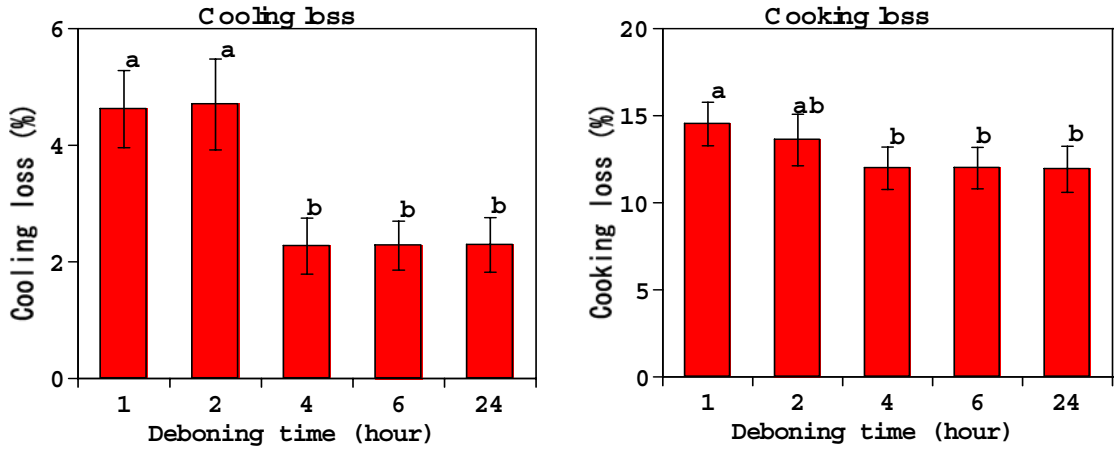


Fig. 2. Effect of *post mortem* deboning time on the shear force value of chicken breast meat aged at 0 ˚C for 4 days

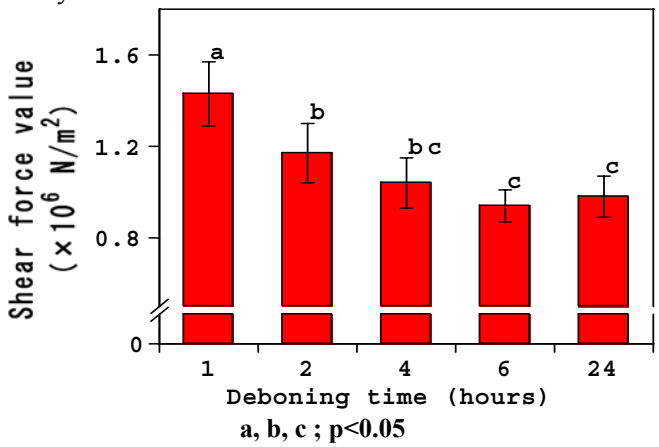


Fig. 3. Optical micrographs of pectoral muscle deboned at 1, 8 and 16h *post mortem*
(Vertical section ; ×800)

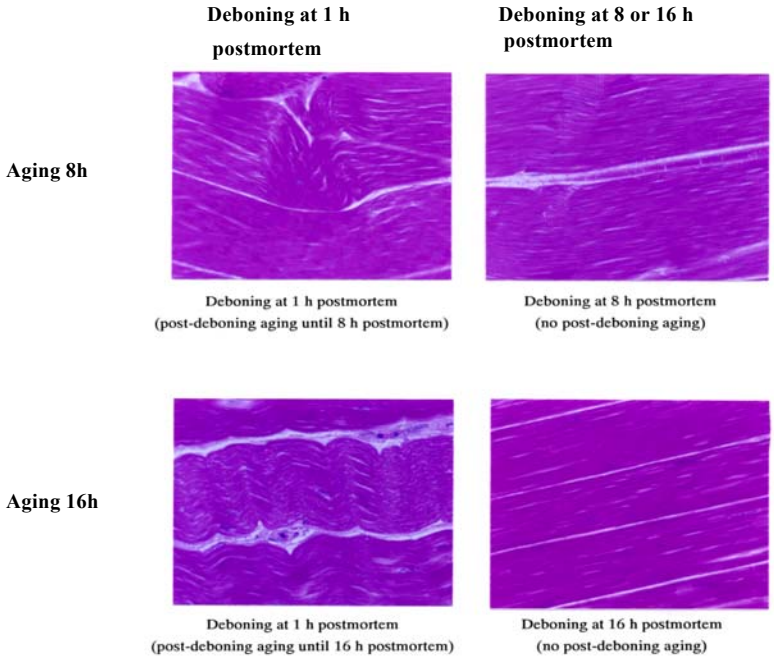
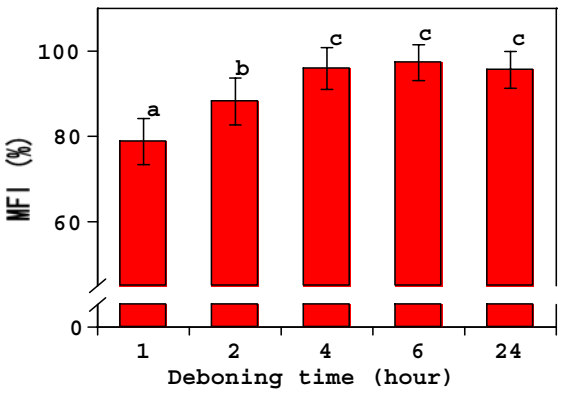


Table 2. Effect of *post mortem* deboning time on the distance between Z-lines in myofibrils of pectoral muscle of chicken breast meat

Postmortem time (hours)	Deboning time after postmortem (hours)			
	1		8	16
	ÿ ÿ ÿ ÿ ÿ ÿ ÿ ÿ ÿ	ê m	ÿ ÿ ÿ ÿ ÿ ÿ ÿ ÿ	
8	2.24 ß 0.12**		2.57 ß 0.07	
16	2.41 ß 0.07**			2.63 ß 0.08

** Values within the same horizontal line are significantly different (p<0.01).

Fig. 4. Effect of *post mortem* deboning time on the MFI of chicken breast meat aged for 4 days at 0 ˚C



a, b, c ; p<0.05

RELATIONSHIP BETWEEN ELECTRICAL IMPEDANCE SPECTROSCOPY AND SENSORY QUALITY OF BEEF

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Background

Prediction of the sensory quality of beef from on-line carcass measurements would be of great value for the beef industry. During the last years different optical probes have been developed for the on-line evaluation of meat. Fluorescence and reflectance measurements have been used to detect connective and adipose tissue in beef (Swatland & Findlay, 1997; Swatland, 2000) and spectrophotometric measurements have been correlated with important attributes of meat quality, such as water-holding capacity or intramuscular fat (Swatland, 1995). The electrical impedance spectroscopy (EIS) has been applied with success as a predictor of the intramuscular fat content in beef and pork (Madsen et al., 1999; Marchello et al., 1999), and can be used to select green hams on the basis of the pH and fatness (Oliver et al., 2001) and even to predict sensory quality attributes of dry cured hams (Guerrero et al., 2004).

Objectives

The objective of this study was to evaluate an EIS (Electrical Impedance Spectroscopy) on-line probe, based on electrical impedance measurements, as predictor of the sensory quality of beef.

Materials and methods

This study was carried out on a set of 30 carcasses of yearling bulls (500 kg live weight) of two local breeds from northern Spain: Asturiana de los Valles (AV) and Asturiana de la Montaña (AM). These carcasses were selected from 5 different biological types derived from different combinations of breed (AV and AM), physiological state (bull and steer) and presence of muscular hypertrophy (*mh*/*mh*, *mh*/+, +/+), in order to obtain a sample set that included a wide range of carcass and meat traits.

At 24 h *post-mortem* carcasses were quartered between the 5th and 6th ribs and several measurements were performed on the *Longissimus* muscle of the left carcass: pH (pH24) was recorded on the loin of the 5th rib and electrical measurements were performed with an EIS on-line probe, supplied by the company NTE (Riu et al., 2001), inserted perpendicularly to muscle between the 4th and 5th lumbar vertebrae (*Longissimus lumborum*, LL) and between the 10th and 11th ribs (*Longissimus thoracis*, LT). The probe has two needles (length 50 mm) separated by 8 mm. Each needle is composed of 2 electrodes having the device a total of 4 electrodes. A temperature sensor was included in the probe to record the inner temperature of the sample. This device measures two parameters: R_∞ and R_0, that record the resistence at the higher (112 kHz) and the lower (5 kHz) frequencies, respectively, expressed in ohms (Ω). A third parameter, called K-value, is calculated, corresponding to the ratio R_∞ over R_0.

The loin comprised between the 6th and the 11th ribs was extracted and transported to the laboratory. The 6th rib joint was kept on a poliexpan tray covered with plastic permeable to O_2 at 4 ºC during 7 days, for subsequent determination of moisture (ISO 1442), intramuscular fat (IMF, ISO 1443) and water holding capacity measured as expressible juice of raw meat (EJ) according to a modification of the method of Grau and Hamm described by Sierra (1973). The rest of the loin was sliced, vacuum packed and kept at 4ºC for 7 days ageing, being later frozen at -18 ºC. The instrumental toughness (kg/cm^2) was evaluated on cooked meat in an Instron 1011 equipment with a Warner-Bratzler shearing device. Sensory analysis was performed by an eight member trained panel on steaks cooked to an internal temperature of 70º C. On a 100 mm scale, panellist assessed a profile composed of hardiness, juiciness and chewiness.

Analysis of variance was performed to study the effect of biological type on the physico-chemical, electrical and sensory traits. Differences between means were calculated with the LSD test. Multiple linear regressions were assessed between variables by the stepwise method. Principal component analysis was made to describe the relationships between meat quality and electrical parameters and discriminant analysis was applied to

study the ability of electrical measurements to classify the meat samples according to the water holding capacity (WHC), measured as expressible juice (EJ): high (H, EJ>21%) or low (L, EJ<21%). All the statistical analyses were performed using the SPSS programme 11.5.1 (2002).

Results and discussion

There was a significant effect of biological type on EJ ($p<0.001$) and IMF ($p<0.05$) of meat (Table 1). Meat of AV bulls with muscular hypertrophy (*mh*/*mh* or *mh*/+) showed higher juice losses and lower intramuscular fat than any other meat type, and there was a general tendency of EJ to increase as the intramuscular fat content of meat decreased.

Biological type affected also significantly to R_∞ ($p<0.05$) and K ($p<0.001$) when measured on the LT (10^{th}-11^{th} ribs). Values of R_0 were higher as the IMF of meat increased and K values decreased as the juice losses increased. There were not significant differences between meat types on toughness or hardiness, but there was a tendency of meat with lower fat content and higher juice losses (AV bulls) of showing lower juiciness and higher chewiness than meat of AV steers or AM bulls.

The principal component analysis (Fig. 1) showed that the electrical variable K (ratio R_∞:R_0) was positively related with juice losses (EJ) and moisture, as it is a parameter proportional to the ratio of extracellular water to total water in meat (Lozano et al. 1995). However, R_∞ and R_0 were positively related with the IMF content of meat, because the resistance of the muscle at high frequencies depends directly on the geometry of the conductive medium under measurement, hence it reflects the amount and/or size of fat cells. Furthermore, because fat is an insulator, the greater the fat content the higher the impedance reading. The first principal component explained 33% of the total variance and contrasted meat of high juice losses (EJ), high K value and high chewiness, hardiness and toughness with meat of high IMF content and juiciness.

Multiple regression analysis of electrical variables confirmed that R_0-LT and R_0-LL were negatively related with EJ and contributed significantly to predict this variable ($r= -0.66$, r.s.d.= 2.39, $p<0.001$). These results agree with those presented by Swatland (1982), Whitman et al. (1996) and Gobantes et al. (2000) who showed that low frequency electrical conductivity (R_0) is related to water holding capacity of meat. When including in the stepwise regression analysis physico-chemical and electrical variables, the best prediction of EJ was obtained from the IMF, R_0-LT and R_∞-LT, that explained 67% of the variance ($r=0.82$, r.s.d.= 1.82, $p<0.001$). This confirms that the intramuscular fat content of meat has a great influence on the water holding capacity, as described by Oliván et al. (2003), who compared meat quality of different breeds (AV and AM) and/or genotypes (*mh*/*mh*, *mh*/+, +/+) and found that the higher the intramuscular fat content the lower are juice losses of raw meat. Therefore, electrical variables that estimate the amount of fat cells in the tissue (R_0-LT and R_∞-LT) may be used as predictors of the expressible juice losses of meat.

The electrical variable that contributed significantly to predict the IMF content of meat was R_0-LL ($r= 0.39$, r.s.d.= 0.96, $p<0.05$). However, when including all quality traits in the stepwise regression analysis, none of the electrical variables was selected but moisture and juiciness were significantly related with IMF as they explained together 81% of the variance of data ($r=0.90$, r.s.d.= 0.46, $p<0.001$).

Discriminant analysis was used in order to test the ability of on-line measurements (pH and electrical variables) to classify meat samples according to the amount of expressible juice (EJ): high (EJ>21%) or low (EJ<21%) (Table 2). When using pH24 and the electrical variables measured on the LL (4^{th}-5^{th} lumbar vertebrae), 60.0% of samples were well classified. However, pH24 and electrical measurements performed on the LT (10^{th}-11^{th} ribs) allowed to classify 73.3% of the meat samples correctly in high (H) or low (L) juice losses. When the chemical composition of meat (moisture + IMF) was also included, the proportion of well classified samples increased to 90.0 %, being 100% in the group of high EJ (H). This is not the first time that electrical impedance measurements allow the classification of meat by quality traits. Guerrero et al. (2004) showed that EIS measures detected correctly 69% of green hams with problems of pastiness, due to its relationship with PSE meat.

Conclusions

The results of the present study indicate that the electrical measurements performed with the EIS on-line probe on the carcass of yearling bulls at 24 h *post-mortem* are useful predictors of the sensory and technological quality of meat at 7 days *post-mortem* and could be used for on-line beef quality grading.

Acknowledgements

This study has been supported by the INIA (Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria) under project INIA OT-00-042 C61.

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Table 1. Effect of biological type on physico-chemical, electrical and sensory traits.

	Biological type					
Breed	**AV**				**AM**	
Muscular hypertrophy	**mh/mh**	**mh/+**		**+/+**	**+/+**	**Significance**
Physiological state	**Bull**	**Bull**	**Steer**	**Bull**	**Bull**	
N	4	7	5	7	7	
pH24	5.4	5.5	5.4	5.5	5.5	NS
EJ (%)	25.64 c	23.76 cd	17.47 a	21.93 bd	19.56 ab	***
Moisture (%)	73.89	73.28	73.26	73.06	73.06	NS
IMF (%)	2.02 a	3.12 ab	3.54 ab	3.34 ab	4.07 b	*
Toughness (kg/cm^2)	2.62	2.17	2.04	2.29	2.29	NS
Hardiness	3.53	2.74	2.16	2.80	2.80	NS
Juiciness	2.98	3.64	4.01	3.89	4.27	+
Chewiness	4.15	3.53	3.20	3.43	3.49	+
EIS on LL (4th-5th lumbar vertebrae):						
R_∞-LL (Ω)	12.0	10.5	12.3	12.1	12.5	NS
R_0-LL (Ω)	49.6	57.5	76.9	70.0	65.9	NS
K-LL	0.27	0.19	0.16	0.17	0.22	NS
EIS on LT (10th-11th ribs):						
R_∞-LT (Ω)	8.9	10.4	12.3	11.1	12.9	NS
R_0-LT (Ω)	20.5a	33.3ab	58.1b	43.7ab	51.1ab	*
K-LT	0.45b	0.32ab	0.23a	0.26a	0.26a	***

NS: no significant; +: p<0.1;*: p<0.05; **: p<0.01; ***: p<0.001
For each variable, data followed by different letters are significantly different (p<0.05).

Table 2. Classification of meat samples according to low (L) or high (H) expressible juice by discriminant analysis based on pH24, electrical variables and chemical composition.

		pH24 + impedances on LL			**pH24 + impedances on LT**			**pH24 + impedances on LT + moisture + IMF**		
		L	**H**	**Total**	**L**	**H**	**Total**	**L**	**H**	**Total**
L	**n**	8	5	13	10	3	13	13	0	13
	%	61.5	38.5	100.0	76.9	23.1	100.0	100.0	0.0	100.0
H	**n**	7	10	17	5	12	17	3	14	17
	%	41.2	58.8	100.0	29.4	70.6	100.0	17.6	82.4	100.0

H: high EJ (>21%), L: low EJ (<21%).

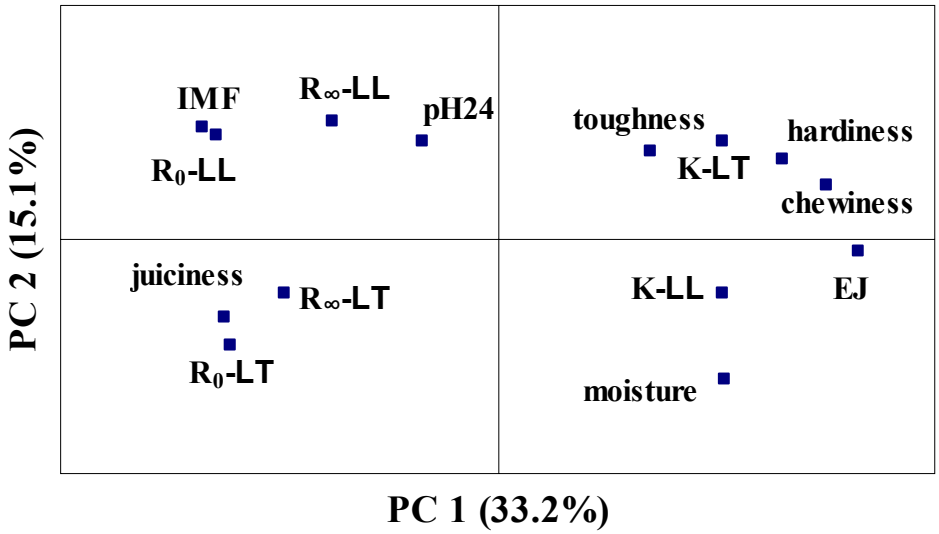

Figure 1. Principal component analysis of physico-chemical, electrical and sensory traits.

EFFECT OF PORK PIGMENT ADDITION ON THE COLOUR OF COOKED HAMS FROM EXUDATIVE MEAT

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Background

Pork quality refers to meat technological aspects such as colour, water-holding capacity, cooking losses or texture, as well as to sensorial properties. The genetic background of the pigs (Oliver et al., 1994; Gil et al., 2003) and the ante-mortem treatment constitute sources of variation in pork quality (Van der Wal, Engel & Hulsegge, 1997). Pale, soft and exudative meat (PSE meat) may appear as a result of both factors (Gispert et al., 2000) and affects the colour and quality of cooked ham. The pale colour of PSE meat is explained by the protein denaturation (Bendall & Wismer-Pedersen, 1962). PSE is a technological defect found on average in 6% of the hams in Spain, but this percentage can be higher depending on genetics and pre-slaughter treatment in the abattoir (Gispert et al., 2000). As a consequence, the hams become exudative and some muscles can be very pale or show bicolour with a decrease in the quality of the final product. Moreover, other factors such as the diet can influence meat colour as well. In this sense, Ramirez et al. (2002) found that supplementation with Fe in the pigs'diet s modified the lightness of the meat positively, producing a reddish pork meat. On the other hand, colour stability in cooked ham is affected by light and by oxigen content and probably by some components of the brine such as sodium ascorbate (Farkas et al., 1990).

Objectives

The aim of this study was to ascertain the effect on the colour and colour stability of the final product from exudative hams by the addition of porcine pigments to the brine used in the processing of cooked hams.

Materials and methods

Selection of the raw hams: Thirty-three hams were selected at 15 h *postmortem* (pm) in a local processing plant on the basis of pH and electrical conductivity (EC) in the *Semimembranosus* muscle (if pH< 5.6 or CE > 6 μS, PSE meat) in order to have three groups of 11 hams each: group 1 (control, with normal meat quality hams), group 2 (control, with exudative hams) and group 3 (APRORED -pork pigments- with exudative hams). The weight of the hams was 10,62 ± 0,74 kg.

Pork pigment: APRORED is a 100% water soluble food preparation based on pork pigments (stabilized hemoglobine) used as an ingredient in the meat processing industry (APC Europe).

Cooked hams process: The hams were weighed before and after muscles were boned-out. Subcutaneous and intermuscular fat, connective tissue, bone and rind were removed. Two hundred and ten grams of brine per kg of meat were injected into the pork legs. The brine - containing 0.35 % phosphate, 0.05 % ascorbate, 0.80 % dextrose, 0.05 % carragenate, 1.7 % NaCl, 0.01 % nitrite, and with 0.04 % of APRORED in the case of group 3 - was injected into the pork legs to increase their weight by 21 %. After injection, raw hams were placed in the vacuum tumbler and a vacuum was drawn to 200 mbar. The tumbling schedule was set for the hams to rotate a total of 2100 revolutions at 4 ºC (3 periods of 60-90 min of continuous tumbling). The hams were packed into aluminium moulds and then placed in an oven and cooked to an internal temperature of 65 ºC using a cabinet temperature of 70 ºC. The total cooking time was approximately 8 h.

Colour measurements: Colour measurements were carried out with a Minolta Chroma Meter CR-200 using the white tile provided by the manufacturer as the internal standard and set to illuminant C. Triplicate measures were taken on *Biceps femoris* (BF) and *Semimembranosus* (SM) muscles. The measurements were expressed as CIE L*a*b* (CIE, 1976). After this, a study of the stability of the colour was done on slices 15 mm thick on the SM and BF muscles of cooked hams. Measurements at time 0 were taken immediately after cutting the slices, and then at times 15, 30 and 120 minutes. The pigment content was determined in the fresh and cooked hams using the method of Hornsey (1956) based on the determination of total pigments by means of extraction with acetone as the principal solvent (Hornsey, 1956).

Sensorial evaluation of the ham colour: The red colour of cooked ham was assessed by two-experienced panellists according to a 10 point scale for red colour (1 =very pale; 10 = very dark).
Statistical analysis: Data were analysed using the General Linear Model procedure of the Statistical Analysis System (SAS., 1988).

Results and discussion

Table 1 shows the Least Squares Means and Standard Errors of the meat quality variables for the three groups of hams selected on the basis of pH and EC at 15 h p.m. in the SM muscle.
Meat quality: pHu was significantly lower in groups 2 and 3 than in group 1 (5.55, 5.54 and 5.67 respectively) as expected. With respect to EC, group 1 showed lower values (4.35) than groups 2 and 3 (15,81 and 13,84). Although significant differences were found between groups 2 and 3, they were considered not important from the meat quality point of view: both groups showed a very high mean value of EC indicating exudative meat (Oliver et al., 1991).
Colour: The variable related to colour (L*) measured in the SM of the fresh hams was significantly different in group 2 in relation to groups 1 and 3. No significant differences have been observed in L* value in the BF muscle although there was a tendency to have pale meat in group 2. With respect to pigment content (acid haematin) no significant differences in fresh or cooked hams were found. However, the cooked hams of group 2 presented lower concentration of acid haematin (28.97) than the other groups (34.06 and 33.19). This result could indicate a positive effect of APRORED in the colour of the final cooked ham obtained from exudative hams. This effect could also be observed in the visual assessment of the cooked hams: there was an increase in visual redness (VS) in both muscles in group 3 (Table 1), which in BF was statistically significant. The correlation between L* value and Visual assessment in SM and BF muscles was -0,60.
Colour stability: The L* value after slicing was higher in group 2 for both muscles at all times (Fig. 1), but the difference was not significant. The a* value was significantly higher for group 3 than for groups 1 and 2 at all times, due to the Aprored addition. Regarding b* value, no significant differences were found in SM and BF muscles at any time.

Conclusions

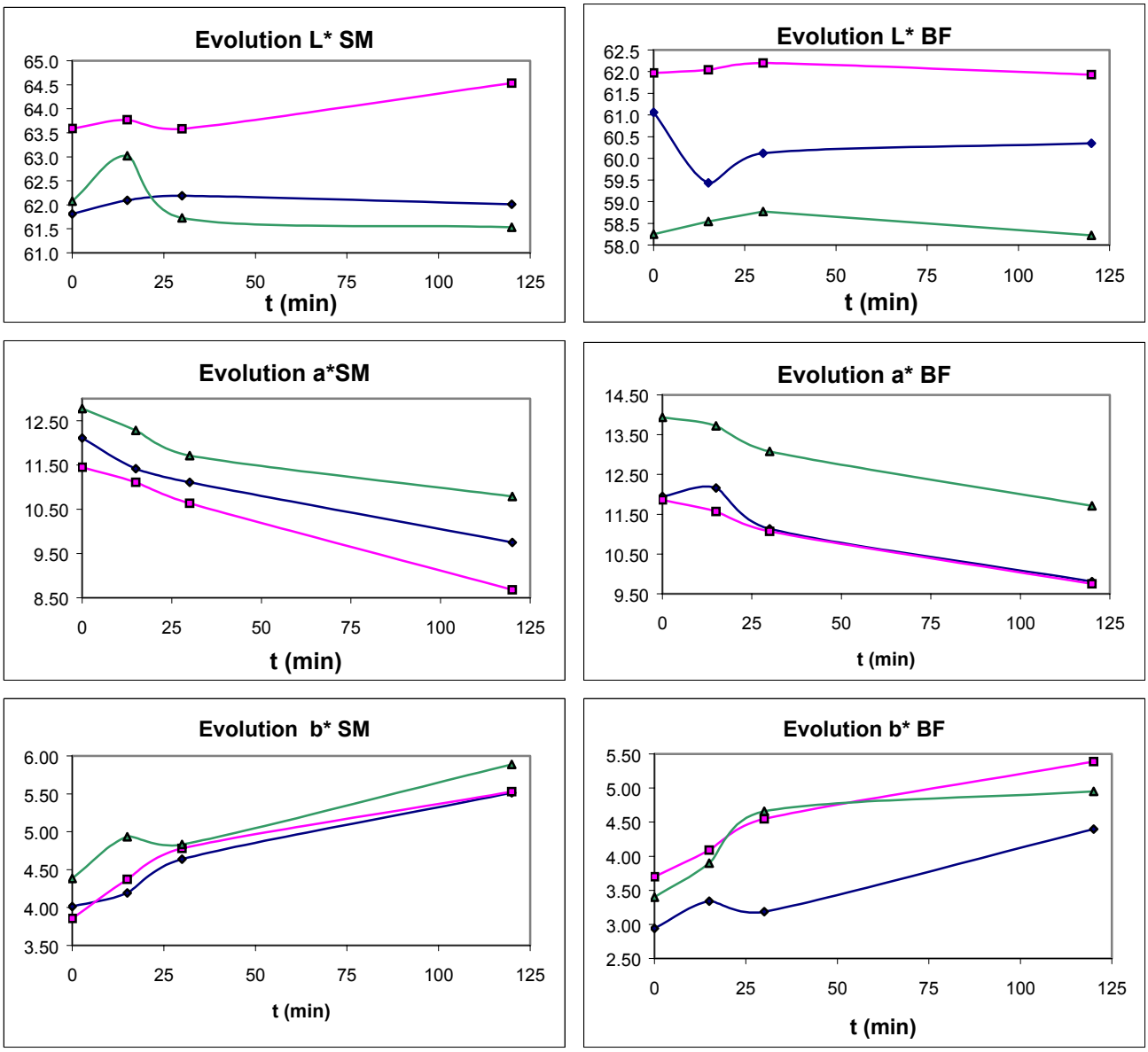
The study of the colour in cooked hams from normal and exudative meat indicated that the group of hams treated with APRORED had L*and a* values and visual redness assessment more similar to normal hams than to exudative hams, probably as a consequence of the effect of pigment addition on the colour of cooked ham. However, a second trial is in course to confirm these preliminary results.

Table 1. Least Squares Means and Standard Errors of meat quality variables for the three groups of hams studied in the *Semimembranosus* and *Biceps femoris* muscles.

	GROUP 1 (N)		GROUP 2 (PSE)		GROUP 3 (PSE+APRORED)	
	LSM	S.E.	LSM	S.E.	LSM	S.E.
Meat Quality variables in fresh hams						
pHu (in SM)	5.67[a]	0.03	5.55[b]	0.03	5.54[b]	0.03
EC (µs) (in SM)	4.35[c]	0.31	15.81[a]	0.31	13.84[b]	0.31
L* SM	45.25[b]	0.95	48.59[a]	0.945	46.61[b]	0.945
L* BF	45.25	0.74	46.80	0.74	45.52	0.77
Acid haematin (µg/g) in SM	27.70	2.85	29.12	2.99	29.30	2.86
Pigment content (acid haematin) and visual assessment of cooked hams						
Acid haematin (µg/g) in SM	34.06	3.03	28.97	3.18	33.19	3.03
Visual redness (VSM)[1]	2.75	0.13	2.64	0.13	3.04	0.13
Visual redness (VBF)[1]	3.11[b]	0.14	2.92[b]	0.14	3.59[a]	0.14

[1]:*Visual assessment of the red colour in the SM and BF muscles from 1 (very pale) to 10 (very red).*
[a, b, c]:*Least Squares Means with different superscripts within a row differ significantly (p<0,05)*

Fig.1. Evolution of CIELAB variables in *Semimembranosus* (SM) and *Biceps femoris* (BF) muscles at different times after slicing for the three groups of hams studied.



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EFFECT OF SUPPLEMENTATION WITH MAGNESIUM AND TRYPTOPHAN ON THE WELFARE AND MEAT QUALITY OF PIGS

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Background

Handling of animals during transport, lairage and slaughter is one of the most important causes of stress in pigs and that, together with genotype, most influence the final carcass and meat quality. A supplementation of magnesium (Mg) in the diet of pigs may reduce the response of the animals to stressful stimuli by reducing the levels of cortisol and cathecolamines in plasma (Kietzman & Jablonski, 1985). An increase of tryptophan (Trp) concentration in the diet enhances the synthesis of brain serotonin and may have sedative effects (Leathwood, 1987). Some authors report an improvement of pork meat quality as a result of the supplementation of Mg (Schaefer et al., 1993; D'Souza et al., 1998, 1999, 2000; Apple et al., 2000; Hamilton et al., 2003; Swigert et al., 2004) or Trp (Adeola & Ball, 1992; Henry et al, 1996). However, other studies contradict these findings or obtain inconsistent results (Caine et al., 2000; O'Quinn et al., 2000; Apple et al., 2002; Hamilton et al., 2002). The effects of the supplement of Mg on meat quality seem to be related to the stress-susceptibility of the pigs, that is to their halothane genotype (Apple et al., 2000; Caine et al., 2000). On the other hand, Souza et al. (2000) found that under a negative antemortem handling treatment, a dietary Mg supplementation improves significantly pork quality and reduces the incidence of PSE carcasses. Accordingly, Geesink et al. (2004) report that a short-term supplementation of the diet with magnesium acetate or with a combination of this salt with Trp and vitamins does not improve pork quality when pigs are not stressed beyond levels associated with routine slaughter procedures.

Objectives

The aim of this study was to evaluate the effect of the supplementation of the diet with Mg and Trp on the response to stressful factors and on meat quality of pigs. Animals homozygous with respect to the halothane gene (positive, nn, and negative, NN) were used to achieve this objective.

Materials and methods

Animals, diets and pre-slaughter treatment: Seventy-one entire male pigs (36 NN and 35 nn) from Pietrain, Landrace and Large White lines, with an average live weight of 108.51± 8.32 kg, were used in this study. All animals were housed individually in pens with a space allowance of 4 m^2, permitting visual and olfactory contact between each other. They were fed the same diet until 5 days before slaughtering, when three diet groups were established for each genotype: diet 1 group, same diet supplemented with 1.2 g elemental magnesium (Mg) and 8 g L-tryptophan (Trp) per kg; diet 2 group, same diet supplemented with 8 g Trp per kg, and control group, with no supplement. Two batches of 35 and 36 animals were slaughtered in 2 different days. The animals of each batch were mixed in 6 different groups according to diet and genotype and transported from the farm to the experimental abbatoir for one hour on rougher secondary roads. Lairage time for the first pigs slaughtered was about 30 min and for the last ones was of 7 h. They were all stunned with 90 % CO_2 and slaughtered. In each batch, the animals from the 6 groups were slaughtered alternatively. Hot carcass weight was measured at 45 min. *postmortem* (pm), and used to calculate the killing-out (%).
Skin lesions: After sticking, and when the carcasses were hoisted on the bleeding rail, fresh scratches on the skin were inspected and subjectively scored according to their location (head/neck, flanks/back and hindquarter) and occurrence (0= No occurrence, 1=1 occurrence, 2=2 to 5 occurrence, and 3=more than 5).
Meat quality measurements: The left side of the carcass was used to perform meat quality measurements on the *Longissimus thoracis* (LT) and *Semimembranosus* (SM) muscles. Muscle pH at 45 min (pH45) and at 24 h (pHu) pm were measured using a portable pH meter equipped with a Xerolyt electrode. Electrical conductivity at 24 h pm (ECu) was measured at the last rib level using a Pork Quality Meater. The muscle water holding capacity (WHC) was determined in the LT muscle as drip losses following the reference method supported by OECD (Honikel, 1997).

Colour measurements: Colour measurements were carried out with a Minolta Chroma Meter CR-200 using the white tile provided by the manufacturer as the internal standard and set to illuminant C. Triplicate measurements were taken on LT muscle. The measurements were expressed as CIE L*a*b* (CIE, 1976).
Statistical analysis: The GLM procedure of SAS software (SAS, 1999) was used. Genotype and diet were considered as fixed effects (the interaction was not significant in any of the variables studied) and when the slaughter day effect was significant ($P<0.20$) it was included in the model as a blocking variable.

Results and discussion

Five animals died before being slaughtered (during transport or while unloading the lorry), all of them from halothane positive pigs (nn): three corresponded to the control and the other two to the diet 1 group (Mg + Trp). The sedative effect of the diet with Trp (diet 2) seemed to facilitate the coping of the animals to stressful factors, such as new environments. The presence and severity of skin lesions indicative of aggressive behaviour are presented in Table 1. Skin lesions in the head/neck, flanks/back and hindquarters were significantly higher in the group of animals fed diet 1 compared with the animals of the control group. This was in agreement with the results of Caine et al. (2000), who found a higher frequency of pre-transport aggression in pigs fed on a supplement of 40 mg Mg (in the magnesium aspartate hydrochloride form)/kg of live weight during the 7 days prior to slaughter, with respect to the controls. No differences were found in the occurrence of skin lesions between diet 1 and diet 2, except in the flanks. That increase in skin lesions of the animals of diet 1 and 2 would suggest an increase of activity due to fighting and a depletion of the glycogen resource before slaughter. That was reflected in the ultimate pH and other meat quality variables. Table 2 shows the effect of the diet on several meat quality variables: pH_u SM tended to be higher ($P=0.080$) in the animals fed diet 1 and diet 2 than in the control. The incidence of DFD carcasses (dark, firm and dry), established as $pH_uSM \geq 6.0$, was 12.5% in the diet 1 group and 16.7% in the diet 2 group, and 0% in the control. The ECuLT tended to be slightly lower ($P=0.088$) when animals were fed diet 2. The most important differences among groups were found for the L* (lightness) and the drip loss variables. L* values were lower ($P<0.05$), indicating darker muscle colour, in the LT of pigs fed the supplemented diets than in the LT of controls, whereas drip losses were lower ($P<0.05$) in the LT of animals fed with diets 1 and 2 than the control. Thus, the supplementation with Mg+Trp or with Trp improved the muscle colour and the WHC. These results are in accordance with the ones reported by Hamilton et al. (2002), with a supplement of 3.2 g Mg per day and 2 days of treatment, and by D'Souza et al. (1998) who studied the effect of a supplement of 3.2 g Mg per day using magnesium aspartate for 5 days. Hamilton et al. (2002, 2003) and Frederick et al. (2004) evaluate the effect of different times of feeding the Mg supplement prior to slaughter and report that the shortest time studied (1-2 days) may be effective in improving pork colour and muscle WHC. From the present preliminary results and in the conditions of this experiment, it would seem that the darker colour and the decrease in drip losses of the muscle were a consequence of the inclusion of a supplement of (Mg+Trp) and Trp in the diet.

Conclusions

The mixture of Mg and Trp increased the frequency of skin lesions due to fighting during transport and lairage. The aggressive behaviour due to mixing contribute to an increase in pH_u in the meat from the animals fed diet 1 and diet 2 and, as a consequence, some meat quality characteristics (colour and water holding capacity) improved. However, to ascertain if these supplements are adequate to be used in animal production in order to improve meat quality, other factors such as food intake and daily growth should be considered.

Table 1. Least squares means and standard errors of skin lesions mean values in pigs from three groups of diets

	CONTROL (n= 19)		DIET 1* (n= 22)		DIET 2* (n= 23)	
	LSM	S.E.	LSM	S.E.	LSM	S.E.
Head/neck	0.93 [b]	0.195	1.58 [a]	0.180	1.17 [ab]	0.176
Flanks/back	0.82 [b]	0.205	1.80 [a]	0.190	1.17 [b]	0.186
hindquaters	0.34 [b]	0.161	1.06 [a]	0.149	0.83 [a]	0.146

[a,b]: Different superscripts within a row indicated significant differences (P<0.05).
*DIET 1: Magnesium and Tryptophan; DIET 2: Tryptophan.
(0= No occurrence, 1=1 occurrence, 2=2 to 5 occurrence, and 3=more than 5).

Table 2. Least squares means and standard errors of meat quality variables in the LT and SM muscles of pigs from the two genotypes fed the three groups of diets

	CONTROL (n=20)		DIET 1* (n= 22)		DIET 2* (n= 24)	
	LSM	S.E.	LSM	S.E.	LSM	S.E.
Killing out (%)	77.67	0.391	78.22	0.371	77.47	0.355
Carcass weight at 24 h (Kg)	43.18	0.580	41.95	0.536	42.04	0.5151
pH45LT	6.01	0.059	6.04	0.055	6.10	0.053
pH45SM	6.12	0.071	6.18	0.066	6.10	0.063
pHuLT	5.47	0.043	5.53	0.041	5.58	0.039
pHuSM	5.59	0.065	5.79	0.062	5.67	0.059
ECuLT	6.82	0.409	6.84	0.388	5.79	0.371
ECuSM	8.16	0.513	7.49	0.487	7.18	0.466
L* (lightness)	54.06[a]	0.999	50.82[b]	0.948	51.04[b]	0.907
a (redness)	0.79	0.231	1.22	0.214	1.22	0.206
b (yellowness)	5.10	0.394	4.45	0.374	4.41	0.357
Drip loss (%)	8.72[a]	0.460	7.34[b]	0.436	7.35[b]	0.417

[a,b]: Different superscripts within a row indicated significant differences (P<0.05).
*DIET 1: Magnesium and Tryptophan; DIET 2: Tryptophan.

Acknowledgements

This work was financed by CICYT AGL2002-00777. The authors thank the *Associació Catalana de Criadors de Bestiar Porcí Selecte* - that includes 8 pig breeders – for the availability of the pigs evaluated in this trial.

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MEAT QUALITY CHARACTERISTICS OF HIGHLTY MARBLED BEEF IMPORTED TO KOREA WITH REFERENCE TO HANWOO

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Background

It has been shown that intramuscular fat improves eating quality upon 15% in its extractable level (Thompson, 2001), and explains approximately 10-15% of variations in palatability (Dikeman, 1987). However, underlying mechanisms by which the fat improve palatability have been controversial. Miller et al. (1994) postulated that intramuscular fat acts as lubricant in chewing action, and reduces the relative number of muscle fibers within a certain amount of meat. On the other hand, Rymill et al. (1997) demonstrated that intramuscular fat indirectly improved eating quality through its effect on the prevention of over-cooking (e.g., burning), and raised a fundamental question whether the fat content is a direct cause of improved palatability in highly marbled meat. Similarly, Thompson (2001) believed that intramuscular fat increases consumer satisfaction by stimulating salivary gland, which consequently brings into being high juiciness in mouth feeling.

Our previous survey indicated that Korean consumer preferred highly marbled meat (Cho et al., 1999), and that was associated with more favorable palatability (Park et al., 2000). The latter study, using 229 Hanwoo *m. longissimus* with a large range of intramuscular fat content, demonstrated that meat containing high intramuscular fat received significantly higher sensory scores in tenderness, juiciness and flavor intensity. For the reason, Korean beef industry has made a long-term effort to improve the degree of marbling. On the other hand, highly marbled foreign product has been imported; while a total of imported beef took approximately 66% share in Korean beef market in 2003 (Korean Ministry of Agriculture and Forest, 2003).

Objectives

To evaluate objective and subjective meat quality traits for highly marbled imported beef *longissimus* muscle in comparison with Hanwoo beef.

Materials and methods

Sampling and treatment: A total of eight imported and seven Hanwoo *longissimus* muscles (visually from the 6th thoracic vertebrae to the last lumbar vertebrae) were purchased from HaNaRo Nong-Hyup Mart in Seoul. Breed, feeding regime and day of ageing were identified on the basis of labels on the whole cut box, as well as data from importing company. The imported products comprised four Wagyu (grain feeding for 450 days and aged for 31 days) and four Angus (grain feeding for 150 days and aged for 71 days). Seven Hanwoo samples were composed of four carcass quality grade (QG) 1 and three QG 3. As the domestic meat samples were collected following day of slaughter, the samples were aged at 1°C for 14 days.

Quality determination: WB-shear force was measured on cooked steaks (2.54 cm thick) in a pre-heated water bath for 60 min until the core temperature reached 70º and then cooled in running water (ca. 18ºC) for 30 min to reach a core temperature below 30ºC. Eight cores of 1.27-cm diameter were made for each sample, and peak force was determined using a V-shaped shear blade with a cross-head speed of 400 mm/min (Wheeler et al., 2000). Cooking loss was calculated as percent of weight changes during cooking for WB-shear force measurement. Non-trained eight panelists evaluated sensory characteristics of tenderness, juiciness and flavor intensity on a six-point scale. The panelists were asked to score from one (1) for extremely dislike to six for extremely like. Thin slice samples (ca. 4mm in thickness, and 50 x 70 mm in size) were cooked by placing on the tin plate (ca. 245-255°C) with turning at the first pooling of liquid on the surface of the strip, or at the start of shrinkage. The cooked strip was immediately served to each panelist for evaluation. Intramuscular fat content was determined by a microwave-solvent extraction method described by AOAC (2000). Fatty acid composition was determined by the Folch solvent extraction method (Folch et al., 1957), according to the method described by Chae et al. (2002). Least square means were estimated using

a general liner model (SAS, 1997), and difference in objective and subjective meat quality traits between the four groups of meat were evaluated by a pair-different test at 0.05 %.

Results and discussion

By definition, sensory characteristics of cooked meat is an overall mouth feel perceived by interactions between multi–parameters including deformation and breakdown during a consecutive action of chewing, and stimulation of salivary gland (Jowitt, 1974). This can be explained by the so-called 'hole effect', by which the sensory panelists tended to perceive higher juiciness and flavor intensity with tender meat and, *vice versa* (Shorthose and Harris, 1991). For the reason, the effect of individual sensory trait such as tenderness, juiciness and flavor on consumer acceptability cannot be considered separately. In other words, toughness of fiber component, intramuscular fat content and water-holding capacity simultaneously influence overall eating quality and consumer satisfaction.

Given the facts, the current result cannot be a direct indication of meat quality associated with either breed or origin of products because ageing time considerably varied from 14 to 71 days (Table 1), but that mirrors its quality *per se* on the market. The current analysis showed that the imported Wagyu after 450 days of grain feeding had a 22 % of intramuscular fat content in *longissimus* muscle (Table 1). Based on our unpublished data, that was a considerably higher percentage than that for average Hanwoo QG 1^{+}. On the other hand, the grain-fed Angus for 150 days had a significantly ($P<0.05$) lower intramuscular fat than Hanwoo QG 1, but greatly ($P<0.05$) higher than Hanwoo QG 3. This implies that the production strategy for highly marbled Hanwoo can be no longer sole solution for Korean beef industry. At the first glance, there was no difference in WB-shear force and sensory characteristics between Hanwoo QG 1 and 3, and between Wagyu and Angus. However, the imported products showed a significantly ($P<0.05$) lower WB-shear force than Hanwoo. This was likely attributed to the considerably longer ageing period, and was rather anticipatable. It is general consensus that difference in meat quality is largely equalized by approximately 14 days of chiller ageing (Hwang et al., 2003), and the changes take place at a relatively slower rate with extended ageing time (Lee and Lee, 1998). On the other hand, the instrument measurement was not reflected on sensory characteristics whereby sensory tenderness and juiciness did not differ between the domestic and foreign products.

Sole difference between Hanwoo and imported meats was observed in flavor intensity in that Hanwoo received significantly ($P<0.05$) desirable scores. It has been shown that chiller ageing at 1°C significantly increased 'off-odour' after approximately 35-60 days, depending on initial quality (Lee and Lee, 1998). However, it was unsure that the result was associated with differences in breed (Raes et al., 2003), feeding regime (Wood et al., 2003), ageing period (Lee and Lee, 1998), or interactions. Nevertheless, the result was an indication of superior eating quality of Hanwoo to the imported beef. An early study (Kim et al., 1999) showed that Hanwoo *longissimus* muscle was more acceptable than imported one when the Hanwoo meat contained significantly higher intramuscular fat. In addition to the study, the current result demonstrated that imported meat was less acceptable for Korean consumers, in spite of higher marbling than Hanwoo.

As Hanwoo QG 1 showed a significantly ($P<0.05$) less cooking loss than Hanwoo QG 3, more favorable sensory traits were somehow expected. However, difference was undetectable. Park et al. (2000) indicated that high intramuscular fat improved tenderness, juiciness and flavor when that was evaluated the following day after slaughter. The current result, on the other hand, indicated that the carcass quality grade had no detectable effect on sensory characteristics as well as objective texture measurement, when meats were aged for 14 days at 1°C.

Apart from nutritional aspect, composition of fatty acids is a decisive component in meat quality due largely to their variations in melting points and oxidation capacity which has significant effects on palatability and flavor development during cooking (Wood et al., 2003). Numerous studies (e.g., Raes et al., 2003; Laborde et al., 2001) have demonstrated that fatty acid composition affected sensory characteristics. Table 2 presents differences in the fatty acids between the meat quality groups, and their relations to tenderness, flavor and juiciness. Significant difference in individual fatty acid between the groups varied. The result was rather expected as fatty acid compositions were significantly affected by breed, carcass quality, and chiller ageing, and these were in a similar trend with previous studies (Laborde et al., 2001; Insausti et al., 2004; Wood et al., 2003). The current manuscript was not intended to discuss details about these effects on fatty acid composition. However, it was particular noticeable that the level of total n-3 PUFA was significantly ($P<0.05$) lower in Hanwoo QG 1, while C18:0 were significantly ($P<0.05$) lower in both the QGs 1 and 3 than these for the rest meats. Even though sensory tenderness and juiciness did not differ between imported

and Hanwoo beef, C20:4n-6 had a significant relationship with juiciness, while C18:1n-9, C20:3n-6 and C20:4n-6 were significantly ($P<0.05$) correlated with tenderness. In addition, flavor intensity was significantly correlated to C18:0 ($P<0.05$) and C19:1n-7 ($P<0.0$). The result indicated that fatty acids were associated with eating quality through their effects on various sensory characteristics.

Conclusions

Chilled beef with a high degree of marbling has been imported, and currently takes a large share in beef market. This was somehow perturbing to Korean beef industry because highly marbled Hanwoo meat has been considered as a sturdy characteristic versus imported beef. The current study demonstrated that Korean consumers found more favorable flavor intensity in Hanwoo beef in spite of lower marbling score and higher WB-shear force. However, it was unsure whether the result was related to difference in breed, feeding regime and/or ageing time. The result coincided with difference in fatty acids such as C18:0.

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Table 1. Least square means and significant difference in objective and subjective meat quality traits between various carcass quality of domestic and imported beef

	Hanwoo beef		Imported beef		Av. SE$^{\Sigma}$
Carcass quality $^{\Omega}$	QG 1	QG 3	Wagyu	Angus	
Day of ageing	14	14	31	71	
Intramuscular fat (%)	12.8^{a}	4.4^{b}	21.9^{c}	7.9^{d}	0.74
Shear force (kg)	4.1^{a}	3.7^{a}	2.4^{b}	2.4^{b}	0.41
Cooking loss (%)	19.6^{a}	25.7^{b}	20.2^{a}	24.8^{b}	0.19
Juiciness $^{\Psi}$	4.5^{a}	4.1^{a}	4.6^{a}	4.4^{a}	0.28
Tenderness $^{\Psi}$	4.8^{a}	4.5^{a}	5.3^{a}	5.3^{a}	0.31
Flavor intensity $^{\Psi}$	4.9^{a}	4.9^{a}	4.0^{b}	4.1^{b}	0.22

$^{\Sigma}$ Average standard error, numerous/denominator degree of freedom: 1/14.
$^{\Omega}$ QG 1 and QG 3: Carcass quality grade 1 and 3, respectively, Wagyu and Angus were fed in a grain feeding regime for 450 and 15 days, respectively.
$^{\Psi}$ 6: Extremely like, 1: Extremely dislike.

Table 2. Least square means and significant difference in fatty acids between various carcass quality of domestic and imported beef, and simple correlation coefficient between fatty acid and sensory characteristics

	Hanwoo beef		Imported beef		Av. SE$^{\Sigma}$
Carcass quality $^{\Omega}$	QG 1	QG 3	Wagyu	Angus	
C18:0 (stearic)	11.5^{a}	13.7^{b}	14.3^{c}	16.2^{c}	0.66
C18:1n-7 (vaccenic)	0.07^{a}	0.04^{a}	0.17^{a}	0.08^{a}	0.05
C18:1n-9 (oleic)	42.3^{a}	46.7^{a}	47.2^{a}	44.1^{a}	3.95
C20:3n-6 (Dihomo-γ-linolenic)	0.18^{a}	0.11^{ab}	0.04^{b}	0.14^{a}	0.03
C20:4n-6 (arachidonic)	0.31^{a}	0.46^{a}	0.16^{b}	0.32^{a}	0.05
Total n-3 PUFA	0.21^{a}	0.17^{b}	0.16^{b}	0.43^{b}	1.02

	Simple correlation coefficient (n=17)					
	C18:0	C18: 1n-7	C18: 1n-9	C20: 3n-6	C20: 4n-6	n3
Flavor intensity	-0.59*	-0.48†	-0.03	0.21	0.31	-0.30
Juiciness	-0.12	-0.35	0.36	-0.38	-0.61*	-0.18
Tenderness	0.18	-0.14	0.56*	-0.53*	-0.69*	0.10

$^{\Sigma}$ Average standard error, numerous/denominator degree of freedom: 1/14.
$^{\Omega}$ QG 1 and QG 3: Carcass quality grade 1 and 3, respectively, Wagyu and Angus were fed in a grain feeding regime for 450 and 15 days, respectively.
† P<0.1, *P<0.05.

BENCHMARKING VALUE IN THE PORK SUPPLY CHAIN: PROCESSING CHARACTERISTICS OF HAMS MANUFACTURED FROM DIFFERENT QUALITY RAW MATERIALS

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Background

In 1992, economic losses associated with low pork quality were estimated to be only 3% of the unrealized revenue lost from nonconformities in carcass quality. Today, low pork quality accounts for nearly 24% of the unrealized revenue lost from nonconformities in carcass quality, with most of those losses associated with pale, soft, and exudative (PSE) pork, and muscles containing low water-holding properties (Stetzer and McKeith, 2003). The 2003 estimate of PSE incidence was 15.5% (Stetzer and McKeith, 2003) compared to 10.2% reported for 1992 by Cannon *et al.* (1996).

It is often difficult to define the extent of PSE within a muscle; in many cases, muscles will exhibit varying degrees of PSE tissue. For example, the medial portion of a ham muscle may be entirely PSE, whereas the lateral portion of that same ham muscle may appear normal. The weight of hams increased 19% compared to 1992 (Stetzer and McKeith, 2003), making the aforementioned scenario more likely to occur because as the mass of a ham increases, it becomes more difficult to chill rapidly.

The ham processing industry uses a majority of the ham muscles produced by the packing sector, however, little research has been conducted to look at the production inefficiencies, such as decreased processing yields, increased rework, and decreased consumer demand from inferior products, that result from using raw materials that contain muscles with poor color and water-holding properties.

Objectives

The purpose of the study was to determine the impact of different quality raw materials on the processing characteristics of hams.

Materials and methods

Pork leg (fresh ham), insides (IMPS #402F; NAMP, 1997; USDA, 1997), containing both *M. semimembranosus* and *M. gracilis*, were characterized as having a low, intermediate or high incidence of pale, soft, and exudative (PSE) muscle tissue according to NPB (1999) guidelines and were sorted accordingly. Objective color measures (CIE L*-, a*-, and b*-values) and pH values were collected at the time of sorting using a Hunter MiniScan XE (HunterLab Associates, Inc., Reston, VA) and a handheld pH meter (pHStar, SFK Technologies, Inc, Cedar Rapids, IA). Objective measures of color and pH (n = 100 per group) were collected in two locations on the medial side of selected muscles. Groups also were characterized for the percentage of PSE in the muscle tissue by removing and collecting weights on PSE portions in 45 kg samples from each group. Muscles in the “Low PSE” group contained < 5% PSE tissue, whereas muscles in the “Intermediate PSE” group ranged between 20% to 30% PSE, and muscles from the “High PSE” group ranged between 40% to 60% PSE. Approximately 3,000 kg of raw materials were collected for each group and an additional 3,000 kg of commodity muscles were collected to serve as a control. All raw materials were shipped by refrigerated carrier to a commercial ham processing facility.

Upon arrival, raw materials were unloaded and purge was collected and weighed before it was reintroduced into the process. Muscles were injected with a curing solution, macerated, tumbled, formed, and cooked using normal procedures within the commercial processing facility to produce a 10.2 × 15.2 cm ham, water added product. After cooking, ham logs were chilled and stored for approximately 3 weeks before slicing. Ham logs were crust-frozen, sliced, and packaged in 454 g packages. Hams slices were evaluated for defects

after slicing, which were classified as normal rework (i.e., small tears, end pieces, or other minor imperfections) or PSE rework (i.e., severe product defects associated with low functionality muscle). Ham slices were evaluated for color uniformity during packaging. Slices containing nominal variations in color were classified as normal or no defect, those containing slight contrasts in color uniformity or small pale spots were classified as having minor defects, and those exhibiting substantial contrasts in color uniformity, large pale spots or small pockets were considered to have major defects. Packages (n = 100 per group) also were evaluated for objective color measures (CIE L*-, a*-, and b*-values) using a Hunter MiniScan XE (HunterLab Associates, Inc., Reston, VA).

Packaged, finished ham products from each group were selected randomly and shipped to Texas A&M University. Upon arrival, ham packages were placed in a 2°C cooler for storage. Packages of ham (n = 17 per group) were selected after 15-, 30-, 45-, 60- or 75-days of storage, objective color measures (CIE L*-, a*-, and b*-values) were collected as previously described, and the weight of purge was measured from each package. On day 45, ham slices also were evaluated for consumer sensory evaluations. Consumer evaluations were conducted at the sensory testing facility at Texas A&M University and participants were recruited from the faculty, students, and staff. Participants were asked to rate samples from each ham group for flavor intensity, overall like of flavor, visual appeal, and color. Participants were placed in individual panelist booths and given samples one at a time in random order. Ballots consisted of an 8-point scale for each trait evaluated (1 = extremely dislike; 8 = extremely like). In addition, purchase intent was determined by asking participants to select three packages of ham from an assortment of 12 packages. All groups of hams were represented equally and their arrangement was randomized. Packages that participants selected and the order that they selected packages was recorded.

Data were analyzed using SAS (SAS Institute, Cary, NC). Descriptive statistics and frequency distributions were generated using the PROC Means and PROC Freq procedures, respectively. Analysis of variance was performed using the PROC GLM procedure with product quality group tested as the main effect. When main effects were determined to be significant ($P < 0.05$), least squares means were generated and separated using a pairwise t-test (pdiff option).

Results and discussion

Sorting was effective in stratifying quality groups as the group containing the lowest amount of PSE had the highest mean pH value (6.14) and the group containing the most PSE muscle had the lowest mean pH value (5.62). Furthermore, pH differences between groups were incremental, with approximately 0.2 units separating each group. Kauffman *et al.* (1978) found that hams classified as “PSE” had a pH of 5.5, whereas “normal” hams had a pH of 5.9 and dark, firm, and dry hams had a pH of 6.3 (all as measured in *M. gluteus medius*).

Differences in objective color measures between raw material quality groups were as expected. The “Low PSE” group had the lowest L*-values and b*-values and the highest a*-values, whereas the “High PSE” group had the highest L*-values and b*-values and the lowest a*-values. Color and pH values for the control group were situated between the values recorded for the “Low PSE” group and the “Intermediate PSE” group. We estimated that the incidence of PSE was less than 5% and 20 to 30% in the “Low PSE” and “Intermediate PSE” groups, respectively. Because color and pH data indicate that raw materials in the control group fit between those groups, we expect that the control group contained 10 to 15% PSE muscle, which is similar to incidence rates reported by Stetzer and McKeith (2003), Cannon *et al.* (1995) and Kauffman *et al.* (1992).

Stratification of L*- and a*- values for boneless hams from different quality groups was identical to stratification of L*- and a*-values observed in raw materials. Hams manufactured from the control group had the lowest b*-values, which was unexpected because raw material data indicated that the “Low PSE” group had the lowest b*-values. This finding is probably not important because numerical differences in b*-values were extremely small.

Hams manufactured from the “Low PSE” group had the lowest purge loss, followed by hams from the control group. No difference in purge loss was observed between hams manufactured from the

"Intermediate PSE" group and the "High PSE" group. O'Neill *et al.* (2003) reported that drip loss in cooked hams manufactured from PSE pork was four times greater than hams manufactured from normal pork. Differences observed in this study were not as severe as those described by O'Neill *et al.* (2003), however, this may be because drip and purge loss were measured using different methods. The discrepancy in purge loss differences between hams manufactured from the different quality groups may partially be explained by the extent of purge loss in the raw materials before processing. Control and "Low PSE" raw materials possessed the ability to hold water more effectively as they had 0.7% and 0.6% purge loss, respectively, in storage before processing. In contrast, raw materials from the "Intermediate PSE" group had 1.0% purge loss and raw materials from the "High PSE" group had 2.6% purge loss indicating much lower water-holding capacity. This is not unexpected as Offer (1991) previously described lower water-holding capacities in muscle containing greater denaturation. After processing, it was not expected that hams manufactured from the "Intermediate PSE" and "High PSE" groups would have similar purge loss percentages. We expected the "High PSE" group would have the greatest purge loss because greater quantities of PSE muscle should have less functional protein and lower water-holding capacities.

Boneless hams were sliced, sorted into 454 g samples, and vacuum-packaged. Before packaging, ham groups were assessed for quality defects that would be considered "normal rework" (i.e., ends and pieces, small pockets, etc.) or "PSE-outs" (i.e., severe holes caused by a lack of functional protein). Boneless hams manufactured from the "Low PSE" group had the lowest incidence of quality defects and lowest total yield loss. Incidence rates for "PSE-outs" and total yield loss were three times greater in control product than were observed in hams manufactured from the "Low PSE" group. Incidence rates for "PSE-outs" in the "Intermediate PSE" and 'High PSE" groups were 5 to 6 times greater than those observed in the "Low PSE" group. Moreover, total yield losses observed in the "Intermediate PSE" and "High PSE" groups were four to five times greater than the yield losses observed in the "Low PSE" group. O'Neill et al (2003) reported the importance of water retention and cohesiveness in slicing. As greater percentages of PSE in the raw materials were incorporated into the ham formulations, water retention and cohesiveness would be reduced because of the incorporation of less functional protein. Surprisingly, incidence rates for "normal rework," and "PSE-outs," and thus total yield losses were greatest in hams manufactured from the "Intermediate PSE" group. We expected incremental increases in slicing defects as the percentage of PSE pork included in the hams increased; clearly this was not the case. Raw materials in the "Intermediate PSE" group may have been the most heterogeneous and therefore presented the greatest challenge of merging "functional protein" with "non-functional" protein (i.e., PSE muscle).

After packaging, finished hams (after "normal rework" and "PSE-outs" were sorted out) were assessed for minor and major appearance defects. Generally, percentage of defects increased incrementally with increasing levels of PSE product in the raw materials. Hams manufactured from the "Low-PSE" group had the highest percentage of packages with no defects and virtually no packages with major defects. In contrast, only half of all packages containing ham manufactured from the "High PSE" group had no defects, whereas 43.1% contained a minor defect.

Demographic data provided by consumers indicated that nearly 64% of participants were between the ages of 20 and 39 and approximately 45% were male and 55% were female. Consumers indicated no preference for "flavor" or "overall like" of ham from any of the quality groups. Consumers gave the lowest color ratings for ham manufactured from the "High PSE" group, but gave similar color ratings for hams manufactured from the other quality groups. Ratings for visual appeal were similar to ratings for color as consumers gave the lowest ratings for ham from the "High PSE" group, but did not differentiate ham manufactured from the other quality groups. Purchase intent for packages of ham manufactured from the different quality groups showed even greater consumer discrimination against hams manufactured from different quality groups than color or visual appeal responses. Overall selection showed that nearly 60% of consumer selections were packages of ham manufactured from the "Low PSE" quality group with 64% of consumers making one of those packages their first selection. Packages of ham manufactured from the "Intermediate PSE" quality group had the second most frequent selection, whereas packages of ham manufactured from the control group and the "High PSE" quality group were selected the least frequently. We expected selection frequencies for packages of ham from the control group and "Intermediate PSE" group to have similar selection responses, however, clearly this was not the case. It is unclear why consumers discriminated

against ham packages from the control group. Consumer data showed that color and visual appeal responses for ham from control, "Low PSE," and "High PSE" products were similar.

It is interesting to note that when consumers were asked to make independent ratings of hams, they gave responses that indicated very little difference in the color and visual properties of the hams manufactured from the various quality groups. Nonetheless, when consumers were in a situation that they could directly compare packages of ham (i.e., similar to a retail environment), there was distinct discrimination against hams manufactured with greater amounts of PSE in the raw materials. Whether or not consumers would be willing to spend more for hams manufactured with low quantities of PSE will require further research.

Conclusions

Sorting pork raw materials according to quality parameters impacts the processing yields and consumer appeal of products manufactured from those raw materials. For boneless ham manufacturing, processing yields and defects were minimized when muscles containing high levels of PSE tissue were eliminated. Further research is needed to determine the optimal ratio of allowable PSE product in formulation that enables processors to maximize consumer appeal with the economic realities of sorting out PSE pork.

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BENCHMARKING VALUE IN THE PORK SUPPLY CHAIN: PROCESSING CHARACTERISTICS AND CONSUMER EVALUATIONS OF PORK BELLIES OF DIFFERENT THICKNESSES WHEN MANUFACTURED INTO BACON

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Background

Bacon consumption and use has seen extensive growth throughout the 1990's. This trend has had an important effect on the pork industry by increasing demand and value of fresh pork bellies. The 1992 Pork Chain Quality Audit (Cannon *et al.*, 1992) reported that 10% of bellies were too thin for bacon production and an additional 2% were too soft/oily to be used in bacon manufacturing. Quality defects identified by packers, such as belly thickness, often manifest substantial losses in profitability at the processor level due to reduced processing yields and a greater percentage of inferior products when sliced.

Improvements in swine genetics, nutrition, and management decisions have produced pork carcasses with 19% heavier hams and 21% heavier loins than carcasses from 10 years ago, whereas belly weights have remained constant (Stetzer and McKeith, 2003). Mandigo (2002) reported that bellies from current market swine contain approximately 29% less fat compared to bellies from swine 40 years ago. These changes have resulted in decreases in the thickness of bellies. This is a cause of concern because thicker bellies have been found to have higher processing yields than thinner bellies (Jabaay *et al.*, 1976). Nonetheless, West *et al.* (1973) and Jabaay *et al.* (1976) have shown that consumers prefer leaner bacon, which is often derived from thinner bellies that contain less fat.

Objectives

To understand the relationship between belly thickness, processing yields and consumer preferences so that bacon processors may develop raw material specifications that maintain processing efficiency without compromising customer satisfaction.

Materials and methods

Fresh pork bellies (IMPS #408; NAMP, 1997; USDA, 1997) were selected from 500 kg lots consisting of bellies ranging in weight from 5.4 to 7.3 kg at a commercial bacon manufacturing company. Bellies (n = 96 per group) were sorted subjectively into "thin" (approximately 2.0 cm), "average" (approximately 2.5 cm), and "thick" (approximately 3.0 cm) belly thickness classifications by three evaluators. Sorting procedures were validated by measuring the thickness of all bellies using digital calipers at three locations (blade end, center, and ham end) along the dorsal and ventral edges. Bellies were skinned, and skin weights were recorded to calculate skinning yield. After skinning, bellies were injected to 110% of raw weight with a curing solution specific for that commercial facility and chilled at 2°C for 24 hrs. Following storage, bellies were smoked, chilled, and pressed into uniform rectangles according to standard industry procedures. Weights were recorded during all phases of production and used to calculate processing yields.

Bellies were sliced into uniform strips (approximately 3 mm and weighing 28 g) using standard industry equipment. During slicing, bacon slices were classified according to their characteristics for secondary lean and slice thickness. The most valuable slices, "#1 slices," were those that met requirements for secondary lean characteristics (*M. cutaneous trunci* greater than 50% of the width of the slice) and appropriate slice profile thickness (no measurement less than 1.9 cm in profile thickness at any point). The less valuable slices, "#2 slices," were those that had insufficient secondary lean characteristics (*M. cutaneous trunci* less than 50% of the width of the slice) or inappropriate slice thickness (a measurement less than 1.9 cm in profile thickness at any point). Those slices not meeting "#1 slice" or "#2 slice" characteristics and slices

from the cranial or caudal ends of bellies were classified as "ends and pieces." Weights of each component were recorded and slicing yields were calculated. During slicing, approximately 500 g of bacon slices were collected from the center portion of every third belly (n = 32 per group). Bacon slices were vacuum-packaged and shipped to the University of Illinois for further testing.

Consumer evaluations of bacon were conducted at the University of Illinois. Bacon slices were placed on racks and cooked in convection ovens set at 232°C for 12 min. To ensure even cooking throughout slices, racks were rotated after 6 min. Bacon slices were removed from racks, blotted with paper towels to remove excess grease, and placed on paper plates as individual slices. Participants were recruited from residents of the Champaign-Urbana, Illinois area and asked to complete a basic demographic worksheet. Consumers (n = 120) were placed in individual sensory evaluation booths and asked to evaluate bacon slices, served in a random order, for flavor, fattiness, saltiness, crispiness, leanness, and pinkness. In addition, consumers were asked to evaluate the visual appearance of uncooked bacon slices. Bacon slices were laid out individually under cool white fluorescent light against a white background and placed in separate evaluation cubicles to prevent visual comparison between slices. Slices were selected randomly from packages representing each thickness group, and slices were replaced periodically throughout the evaluation timeframe. Also, purchase intent was recorded for both taste and appearance. Sensory and visual evaluations were scored on 5-point scales. For flavor, fattiness, saltiness, crispiness, leanness, and pinkness, scales were 1 = much too little, 2 = somewhat too little, 3 = just right; 4 = somewhat too much, 5 = much too much. For taste and visual appearance, scales were 1 = extremely unacceptable, 2 = moderately unacceptable, 3 = neither, 4 = moderately acceptable, 5 = extremely acceptable. For purchase intent for taste and appearance, scales were 1 = definitely, 2 = probably, 3 = might, 4 = probably not, 5 = definitely not.

Proximate composition was determined using raw, and cooked (n = 5 slices per group) bacon slices. Samples were ground and moisture content determined using AOAC (1995) approved methodology, and fat content of samples was determined by a chloroform-methanol method.

Data were analyzed using SAS (SAS Institute, Cary, NC). Descriptive statistics and frequency distributions were generated using the PROC Means and PROC Freq procedures, respectively. Frequency distributions for consumer sensory responses were tested for significance ($P < 0.05$) using chi-square analysis. Analysis of variance was performed using the PROC GLM procedure with belly thickness group tested as the main effect. When main effects were determined to be significant ($P < 0.05$), least squares means were generated and separated using a pairwise t-test (pdiff option).

Results and discussion

Objective measures of belly thickness were recorded to validate subjective selection procedures. Mean thickness between groups were different with approximately 4 mm separating "thin" from "average" bellies, and 5 mm separating "average" from "thick" bellies. "Thin" bellies had slightly higher skinning losses than "average" or "thick" bellies, but this is likely a function of green weight as the weight of skins collected from each thickness group was nearly identical. Likewise, bellies from the "thin" thickness group had the highest cooking shrink, which also was probably related to lighter belly weights because all bellies were subjected to the same smoking and cooking cycle. Brewer *et al.* (1995) reported a strong correlation (r = 0.70) between belly thickness and raw belly weight. Currently, most bacon processors purchase bellies by weight categories. This possibly accounts for a portion of the variation in belly thickness, but there still is substantial variation within weight ranges that may be best controlled by sorting bellies based upon thickness measures in lieu of or in concert with weight. By sorting bellies on thickness, processors may be able to regulate cooking shrink if all bellies in a cook cycle are of a similar thickness. Within the weight ranges included in this study (approximately 5 to 7 kg), which accounts for approximately 42% of available bellies (Stetzer and McKeith, 2003), we were able to find the variation in raw materials. "Thick" bellies had the highest final yield, which was approximately 2.3 percentage points higher than the yields for bellies from the "thin" and "average" groups. No differences in final yields were observed between bellies from the "thin" and "average" groups.

Bellies from the "thin" group had the lowest slicing yields as evidenced by the lowest percentage of #1 slices (i.e., more valuable product), and the highest percentage of #2 slices (i.e., less valuable product), and "ends

and pieces." Thickness groups had an equal percentage of slices classified as #2 product because of inadequate secondary lean (7.2% for "thin," 7.5% for "average," and 7.1% for "thick"). This is not surprising because we would expect the *M. cutaneous trunci* to remain relatively proportional to the width of the belly. Thus, "thin" bellies had a higher yield of #2 slices because those slices were too thin in profile to be classified as #1 slices (1.5% for "thin," 0.3% for "average," and 0.2% for "thick").

Demographic information provided by consumers showed that 80% of participants were 24 years old or older, 43% had an income of $35,000 or higher, and 60% were female. Consumer evaluations of bacon flavor showed that consumers gave similar responses for "thin" and "average" thickness bellies, whereas respondents indicated that slices of bacon from "thick" bellies lacked bacon flavor. Nearly 60% of consumers indicated that the saltiness of bacon slices from "thick" bellies was appropriate, whereas 38% and 43% of consumers indicated that slices of bacon from "thin" and "average" thickness bellies were too salty. Belly thickness may have an effect on flavor attributes with thicker bellies diluting flavors more than "average" or "thin" bellies. In contrast, Brewer *et al.* (1995) found no differences in bacon flavor or saltiness as belly thickness increased. A consumer panel was used in this study, whereas a trained sensory panel was used by Brewer *et al.* (1995). This may explain why a discrepancy in findings exists. Generally, consumers gave similar responses for fattiness evaluations for each thickness group, which may be expected because bacon is approximately 50% fat.

"Thin" bellies had a greater frequency of responses indicating less crispiness compared to bacon slices from "average" and "thick" bellies. It appears bacon from "thin" bellies may require greater cooking times to achieve the crispiness level consumers desire. Compositional data showed that bacon slices from "thin" bellies contained approximately 7% higher moisture than bacon slices from "average" or "thick" bellies, which may have provided extended evaporative cooling during cooking, and prevented slices from becoming as crispy as slices from "average " and "thick" bellies. This could have ramifications on precooked bacon slices because processors may need to adjust cooking parameters for slices from "thin" bellies to meet the crispiness expectations of consumers.

Generally, responses for overall taste were similar for all belly thickness groups. "Thin" and "thick" bellies had a slightly greater frequency of responses indicating unacceptable taste than slices from "average" bellies. This may be related to fatty acid composition of "thin" bellies, which are usually softer and/or oilier, may have a higher percentage of unsaturated fatty acids, whereas "thick" bellies, which are usually firmer, may contain a greater percentage of saturated fatty acids. In agreement with overall taste data, consumers showed slightly increased likelihood of purchasing bacon from "average" thickness bellies based on taste compared to "thin" or "thick" bellies, with 65.6% of consumers indicating they would "definitely" or "probably" purchase bacon from "average" thickness bellies compared to 50.5% and 48.3% for bacon slices from "thin" or "thick" bellies, respectively. Likewise, greater than 25% of consumers indicated they would be unlikely (i.e., "probably not" or "definitely not") to purchase bacon manufactured from "thin" or "thick" bellies, respectively, because of taste compared to 17.3% for "average" bellies.

Most consumers (73.7%) gave responses indicating that the lean to fat ratio for bacon slices from "thick" bellies was too low (i.e., too much fat in relationship to the amount of lean), whereas greater than 50% of participants indicated that the lean to fat ratio in bacon slices from "thin" and "average" bellies was "just right". Similarly, Brewer *et al.* (1995) observed that as belly thickness increased, sensory assessments of lean-to-fat ratio decreased.

Approximately 70% of consumers indicated that the pinkness of lean in bacon slices from "thin" and "average" bellies was "just right," whereas 56.6% of consumers indicated that the pinkness of bacon slices from "thick" bellies was less than ideal (i.e., "much too little" and "somewhat too little"). It is unclear if the fatness of bacon slices from "thick" bellies "diluted" the pinkness of color in those slices or if it is more difficult to distribute a curing solution in those bellies, resulting in less pinkness.

Consumer responses for visual acceptability indicated that raw bacon slices from "average" (72.4%) and "thin" (78.7%) bellies were acceptable (i.e., "moderately acceptable" or "extremely acceptable"). In contrast, a majority of consumers (56.3%) found slices of bacon from "thick" bellies visually unacceptable. Purchase intent based on visual appearance closely mimicked consumer responses for visual appearance.

For bacon slices from "thin" and "average" bellies, 62.3% and 59.4%, respectively, of consumers indicated that they were likely to purchase those products. In contrast, 62.4% of consumers indicated that they were unlikely to purchase bacon manufactured from "thick" bellies. Our findings agree with the results of Jabaay *et al.* (1976) and West *et al.* (1973) who reported that consumers preferred the appearance of leaner bacon.

Compositional differences were observed in bacon slices from different thickness groups. Raw bacon manufactured from "thin" bellies had the highest moisture content (47.9%) and less fat (36.2%) than raw bacon from "average" (40.5% moisture; 46.4% fat) and "thick" (40.4% moisture; 46.3% fat) bellies. Brewer et al. (1995) reported that as bellies became thicker, moisture content decreased and fat content increased. Compositional differences between thickness groups were similar for cooked bacon. Cooked bacon from "thin" bellies contained less fat (34.7%) than cooked bacon from "average" (42.5%) and "thick" (43.5%) bellies. It is surprising that the composition of bacon from "average" and "thick" bellies was so similar because consumers identified bacon from "thick" bellies as having the lowest lean-to-fat ratio and discriminated against it because of its excessive fatness.

Conclusions

Belly thickness influenced the processing yields and consumer reactions to the acceptability of bacon. "Thick" bellies offer processors the greatest processing yields, however, it is likely that consumers will discriminate against the resulting products because of excessive fatness. Consumers viewed bacon from "average" and "thin" bellies very favorably, however, working with "thin" bellies may reduce industry profitability because of lower processing yields. Swine producers and geneticists should focus on producing swine with bellies of "average" thickness.

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EFFECT OF VACUUM PACKAGE STORAGE ON PORK PURGE AND COLOR

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Background

Vacuum packaging is used to extend the shelf life of fresh pork and is known to preserve the microbiological integrity of the product for up to 12 weeks (Jeremiah et al, 1995). Purge is a consequence of chilled storage and is seen as a defect when the Japanese customer opens the vacuum package. Factors such as pre-slaughter management, stunning procedures, chilling rates and holding temperatures will affect drip and color. Genetics play an important role in this regard and this data set will investigate the effect that breed has on the meat quality of vacuum packaged loins.

Objectives

The objective of this study was to investigate the effect of background genetics on meat quality of vacuum packaged loins as assessed by measuring purge and color of the meat.

Materials and methods

Pig breeds used in this trial originated from the PIC genetic nucleus in Kipling Saskatchewan, Canada and included the following basic genotypes: Landrace (Land), Large White (LW), Duroc, White Duroc (Wdur), Berkshire (Berk), Hampshire (Hamp), Synthetic line (Syn), Berkshire x Hampshire Cross (BH), hal-negative Pietrain ($Piet^-$), hal-positive Pietrain ($Piet^+$). A section of the longissimus muscle from the right side between the 5th and 10th ribs was cut into four equal size portions and vacuum packaged. Packages were then randomly allocated to one of four storage intervals (0, 4, 6 and 8 weeks) and stored at 2ºC (± 0.5ºC) for the designated storage interval. Following storage, the packages were weighed and opened. The meat samples were removed, blotted dry and weighed. The packages were air dried and weighed. Purge was calculated by difference. Samples were then placed on Styrofoam trays, over-wrapped with an oxygen permeable film (Vitafilm Choice Wrap), and allowed to bloom for 1 hour prior to being evaluated by a 5-member trained and experienced sensory panel for muscle color (5 point descriptive scale), surface discoloration (7 point descriptive scale), and retail appearance (7 point hedonic scale). The packages were then opened and the samples were evaluated for off-odors (5 point descriptive scale) and odor desirability (5 point hedonic scale) (Jeremiah and Gibson 1995). CIE L*, a* and b* were measured in triplicate on each muscle section at each storage interval using a Minolta CR-300 reflectance meter (illuminant - C, observer angle - 2º). Statistical analysis was performed using the GLM procedure of SAS (2001).

Results and discussion

Percent purge loss measurements by genotype over storage time are presented in Figure 1. In general, there is known to be a positive relationship between percent purge and the length of storage (Apple et al. 2001; Apple et al. 2002; Kim et al. 1998,). Statistically there is a significant interaction between genotype and week in storage. Overall, the Berk shows the lowest level of purge while the $Piet^+$ has the highest percent purge values. The high percent of purge of the $Piet^+$ was expected due the presence of the halothane gene (Apple et al., 2002). The low purge level of the Berk is expected since it usually displays low rates of muscle lactic acid production that would result in a minimum amount of protein denaturation and better WHC (NPPC, 1995). In terms of time dependent effects on purge, the Dur, Syn and BH are the only lines that show a significant increase between week 4 and week 6. All the other genotypes show no significant increase in purge after after week 4. This observation indicates that accumulated purge has reached a maximum at week 4 and that in order to measure rates of accumulation in packages, purge may well be better evaluated within the first 4 weeks of packaging.

L* measurements are presented in Figure 2. In general, as the storage time increases the meat tends to have a paler appearance. This trend is generally and clearly displayed in the results where, at packaging time (Time

0), L values for all genotypes were below 50, and then rose sharply to above 51 by Week 4 ($P<0.05$). The magnitude of increase after 4 weeks in storage was then minor for all genotypes. Overall, Piet$^+$ was the palest and Syn along with Land and LW were on the other end of the spectrum, showing the darkest loins. In general, these evaluations confirm the trend towards lighter meat color for all genotypes (Apple et al. 2001; Apple et al. 2002). Along with this increase in L* (paleness), both a* and b* increased with storage time for all genotypes (results not shown). The increases in a* and b* translate to increases in chroma and hue angle, which implies an increase in color intensity and a shift toward the yellow but within the red part of the spectrum.

The changes in purge and color during vacuum packaged storage for up to 8 weeks were accompanied by changes in a number of sensory traits (results not shown). Discoloration increased by up to 10% and retail acceptability decrease from "desirable" to "neither desirable nor undesirable". Piet+ and piet- lines were poorer at all storage times and approached "undesirable" at 8 weeks. By 8 weeks storage all genotypes had a moderate off odor which resulted in a decrease in odor acceptability (from "acceptable" to "slightly unacceptable").

A detailed characterization of the quality (pH, drip loss, etc) of the longissimus muscles of these genotypic lines can be found in the paper of Pommier et al. (2004).

Conclusions

Genotype had a large effect on both the amount of purge in the vacuum package and on the meat color. The effects of length of storage in vacuum packages on percent pork purge loss and color are consistent with basic literature (Kim et al, 1998). In addition, the results indicate that majority of purge in the package accumulates during the first 4 weeks post packaging. Increasing storage time increased the amount of muscle discoloration, paleness, chroma, hue angle and prevalence of off odors, and after 8 weeks of storage, muscle samples decreased in retail desirability and odor acceptability as observed previously (Jeremiah et al, 1992). Any attempt to determine differences between genotypes in the rate of fluid accumulation should focus on the initial 4-week period because in most cases maximum purge is attained at that time.

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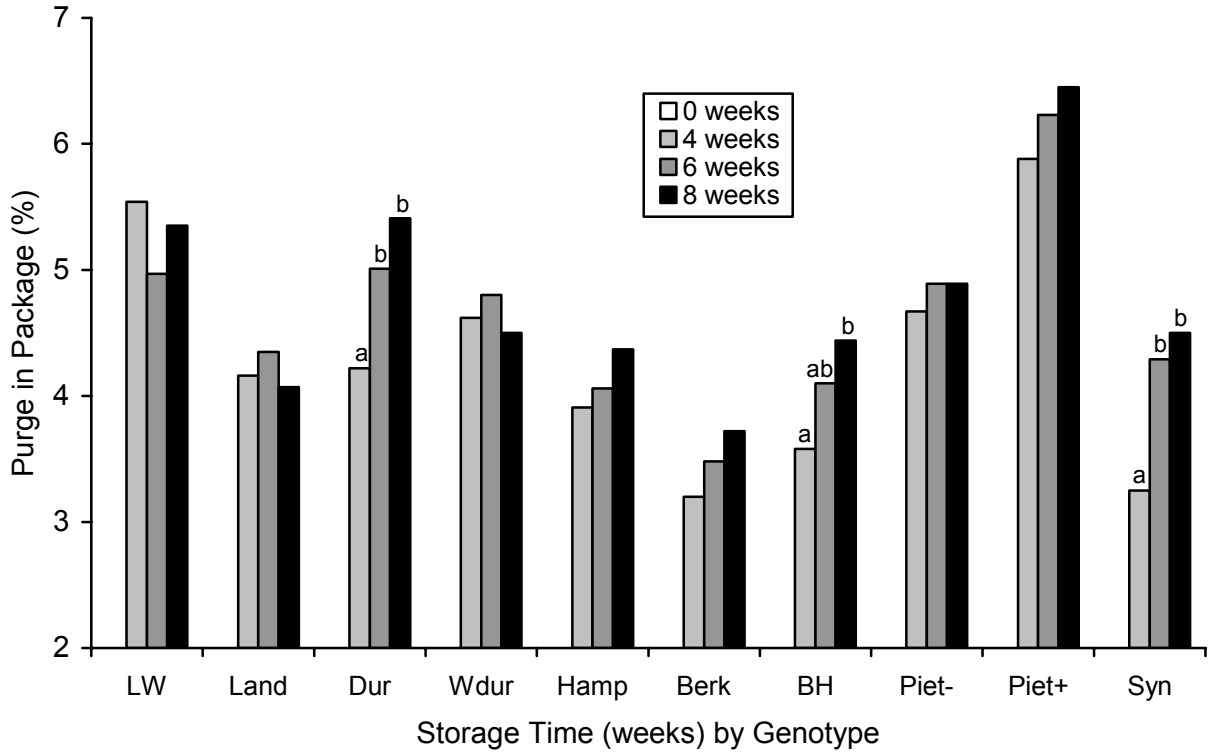

Figure 1: Effect of genotype and storage time on purge of vacuum packaged loins. (Columns within genotype with different letters differ significantly ($P < 0.05$)).

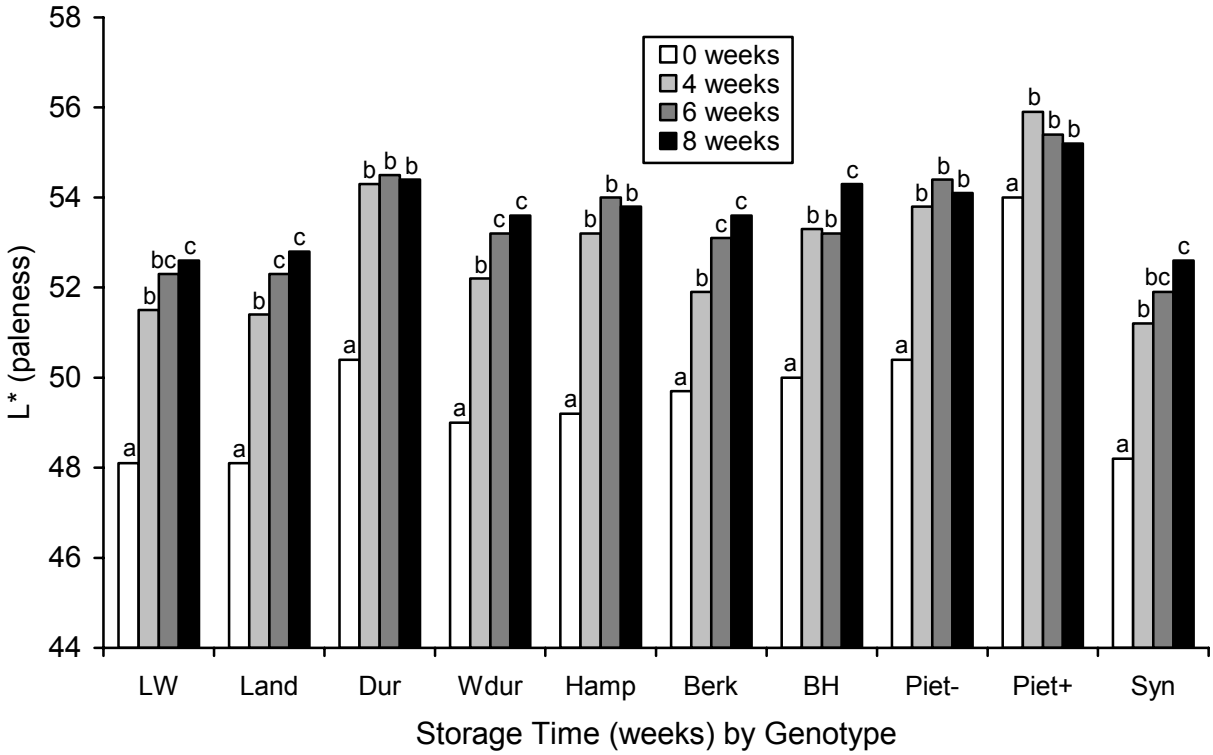

Figure 2: Effect of genotype and storage time on L* of vacuum packaged loins. (Columns within genotype with different letters differ significantly ($P < 0.05$)).

REARING OF PARTLY OUTDOOR PIGS IN ESTONIA AND THE QUALITY OF THE PORK

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Background

Meat quality depends on many factors – rearing, feeding, pre-slaughter handling, transportation, slaughtering, stress etc. Meat quality defects such as PSE-meat can be caused by poor transport and unsuitable pre-slaughter conditions.

This paper discusses studies made to investigate two different feeding and rearing systems and the pork quality in different stress conditions (low stress and high stress). Research is completed as part of the 5th Framework project "Susporkqual" and financed through EC contract No QLK 5-CT-2000-00162. Countries involved in the research were: Denmark, Poland, France, Sweden, Ireland, England and Estonia.

Objectives

The aim of the project for Partner 9 and 9A (Estonia) was to study: a) the interactive effects of genotype and rearing methods on stress reactivity at slaughter and on meat quality choosing country specific breeds and rearing methods, b) the effect of rearing methods on reactivity to transport; c) to find out if it is possible to produce pork more effectively by using partly out-door rearing and different feeds.

Materials and methods

The main difference between pigsties was, that the conventional pigsty had only an inside area (15,22 m²), whilst the partly outdoor pigsty had two areas: an inside area and an outside area. Compared to conventional pigsty (0,7m² per animal) stocking density was higher in partly outdoor pigsty (1,1 m² per animal).

Piglets were from sows of Estonian Landrace breed and boars from crossbreed Landrace and Large White. All piglets were female. We selected stress-negative sows and boars on the bases of a DNA – test. Two different compositions of feed were used for indoor and outdoor pigs: a) import with soya (diet a); b) domestic (diet b) with pea + rape corn. Diets a1, b2 were given to pigs with a live weight of 30 to 60 kg and diets a3, b4 to pigs with a live weight of 60 to slaughtering. The diets were based on the general principles of pigs feed content in Estonia /5/.

Live weights (~ 30, 60 kg and pre - slaughter) of pigs, feed conversation rate (FCR) and weight gain were determined during rearing period.

Slaughtering of 80 pigs was proceeded in two different conditions: half of the pigs (n = 40) were slaughtered in Linnamäe Peekon (LP) at low transportation stress conditions (distance from farm to slaughterhouse ~ 500 m) and the other half (n = 40) in Saaremaa Meat Plant (SMP) after having left behind a distance of 200 km by lorry and ferry – high transportation stress conditions. So we had eight different groups in slaughtering: e.g. pigs from pen 1 (n = 20) were divided into two: 10 pigs were slaughtered in LP and another 10 – in SMP (Scheme).

Methods

I. Carcass weight. Two measurements of carcass weight were made: 1.warm carcass weight; 2. cold carcass weight:

II. Back fat measurements. Linear measurements of the back fat thickness were made the day after slaughter (24 hours) at four locations.

- One measurement was made on the shoulder area (at the cut between the third and forth vertebra in sternum) over the middle of M. trapeziu.s (backfat 1).
- One measurement was made on the loin area (at the cut behind the last rib) over the middle of M. Longissimus dorsi(LD).(backfat 2)

- Two measurements were made on the ham cut: one measuring over the middle of M. gluteus medius (backfat 3) and one measuring over the two fat layers at the thickest point at the cartilage of the hip bone. (backfat 4).

III. pH. All the pH-measurements were made in LD at the last rib with a combined electrode. The electrode was calibrated at 35 °C for the measurements in hot carcass (pH45 min) and at low temperatures for measurements in cold carcass (pH24h with ARGUS X /Sentron pH – meter.
IV. Temperature. All the temperature measurements were made with ARGUS X/Sentron pH – meter. In LD at the last rib: measurement 45 minutes after sticking – temp 45 min and 20 – 24 hours after slaughtering temp 24h.
IV. Chemical composition of meat. Chemical composition of meat: protein, fat, ash and moisture content were determinated in the 4 cm sample from LD at the last rib towards the ham. Intercalibrated methods were used. Analyses was made in the Tartu Department of the Estonian Food and Veterinary Laboratory.
V. Lean meat content in SMP was determined with Ultra – FOM 100 and in Linnamäe Peekon by the ZP – method.

Results and discussion

Compared to conventional rearing partly outdoor rearing resulted in 19,45% bigger average feed intake; 9,9 % higher FCR; faster growth, described by the growth rate, higher dressing % and faster alacrity of pre slaughter live weight. All back fat measurements (except nr 4, conventional) were higher in partly outdoor reared pigs (Table 1).
The present work involves studies made to investigate how pork quality is conditioned by different stress conditions (low stress and high stress) in connection with two different feeding and rearing systems. To avoid the appearing of PSE-meat during the journey from farm to slaughterhouse, the transportation distance should be as short as possible. Great attention has to be paid to the transportation vehicle and pre-slaughter handling. During transportation the quality of the vehicle, ventilation, stocking densities and the travel distance are important factors influencing the stress-level of the pigs /1,3,4,/. Poor on farm handling increases the susceptibility to pre-slaughter stress /2/.
The carcass quality is affected by several factors, of which pre-slaughter handling in general seems to be one of the most important. Carcass quality (especially skin damages) depends on the type of rearing. The skin of partly outdoor pigs was damaged by mosquitos. Transport and pre-slaughter handling also affected carcass quality. The pigs slaughtered in Linnamäe Peekon had less skin damage. The pigs` intravitalis activity did not affect carcass quality (except mosquito'-bites). When comparing the skin damage by slaughter condition, it becomes obvious that the middle part of the carcass was less damaged in LP, but the front part and the fore shank were less damaged in SMP. Carcasses had less scratches and bone fractures in LP. The technological process in small slaughterhouses brings about a lower amount of defects. Big slaughterhouses like Saaremaa MP have a high mechanization level. In such slaughterhouses the carcass quality depends greatly on the machinery. Experimental results indicate better carcass quality of pigs slaughtered in LP.

Conclusions

In partly out-door conditions the pigs grow faster and the amount of used feed per pig and per kg of pork is higher.
Dressing % is higher in pigs produced in partly out-door conditions and is also depending on slaughter conditions. Higher dressing % was indicated in pigs slaughtered in Linnamäe Peekon (low stress, no long transportation).
The results of the research show that transport was the most stressful procedure for pigs.
It is important to load the pigs (especially the outdoor ones) very slowly and calmly.
Rearing, feed and place of slaughter had a significant influence on the carcass temperature.
Rearing methods had great influence on the back fat thickness.
Rearing animal welfare aspects, pigs and pork characteristics, partly out-door rearing is recommended in some piggeries in Estonia.

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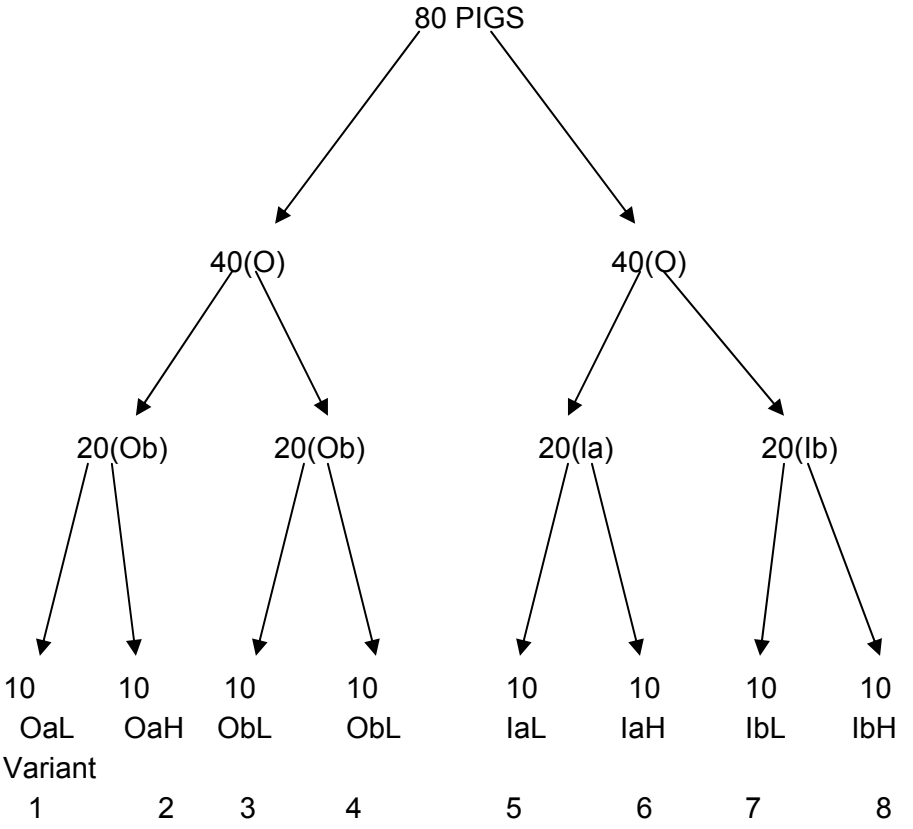
Scheme of production and slaughtering of pigs Partner 9 (Estonia)



(Estonian Landrace x Landrace & Large White crossbreed)
11 weeks old, 25-30 kg, females

2 rearing systems:

* partly out - door (O)

*in - door (I)

2 feeding rations:
a) conventional (diet a) = import
b) with pea + rape corn (diet b) = domestic
Diets: a1,b2 weight 30 - 60 kg
Diets: a3,b4 live weight 60 -105 kg

slaughter live weight 96 -122 kg
2 slaughter stress variants:
low stress (L) in LP transport
high stress (H) in Saaremaa Meat Plant

Table 1. Statistical results

Trait	Place Least Square Mean		P - value	Rearing Least Square Mean		P - value	Feed Least Square Mean		P - value
	LP	SLT		LP	SLT		LP	SLT	
pH45min	6,115	5,962	0,0124	6,117	5,960	0,0099	6,075	6,002	0,2269
pH 24h	5,792	5,947	0,0001	5,835	5,905	0,0003	5,900	5,840	0,0017
t° 45 min	39,082	37,192	0,0001	38,392	37,882	0,0621	38,00	38,275	0,3105
t° 24h	3,55	3,329	0,0001	2,702	4,176	0,0001	3,391	3,487	0,0409
Backfat1, mm	15,050	15,275	0,7786	13,775	16,550	0,0009	15,900	14,425	0,0684
Backfat2, mm	15,275	16,500	0,1103	14,675	17,100	0,0020	15,675	16,100	0,5766
Backfat3, mm	13,425	24,725	0,0001	12,900	25,250	0,0001	18,475	19,675	0,1746
Backfat4, mm	33,225	23,950	0,0001	31,100	26,075	0,0001	28,225	28,950	0,5168
Moisture, %	69,093	70,480	0,0004	69,653	69,919	0,4771	69,717	69,856	0,7099
Protein, %	22,262	22,430	0,4320	22,622	22,070	0,0111	22,347	22,345	0,9906
Fat, %	5,878	5,401	0,2759	5,805	5,474	0,4496	5,732	5,547	0,6707
Ash, %	1,147	1,153	0,6941	1,163	1,137	0,1118	1,139	1,160	0,2061

EFFECT OF FREE AND RUMINALLY-PROTECTED FISH OILS ON FATTY ACID COMPOSITION, SENSORY AND OXIDATIVE CHARACTERISTICS OF BEEF LOIN MUSCLE

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Background

It is recommended that the concentrations of long chain polyunsaturated fatty acids (PUFA) such as eicosapentaenoic acid (C20:5*n-3*, EPA) and docosahexaenoic acid (C22:6*n-3*, DHA), for which oily fish are an excellent source, should be increased in the human diet (Simopoulos, 1998). Rumen biohydrogenation in cattle limits the amount of long chain PUFA which can be transferred into ruminant muscle, but this process also results in the production of *trans*-C18:1 and conjugated linoleic acid (CLA; particularly the trans-10, cis-12 CLA) intermediates in the rumen. It is recognised that the main sources of CLA, which may confer a health advantage to humans, are meat and milk from ruminants (Chin et al., 1992). The amount of CLA in muscles of animals given increased amounts of unprotected n-3 fatty acids was increased two- to three-fold whilst *trans*-C18:1 increased four- to seven-fold (Enser et al., 1999) with free fish oil being more effective at inhibiting rumen reductases than linseed oil.

We have previously demonstrated that feeding concentrates containing free fish oil, in comparison to megalac (a saturated fat source), increased the content of long chain n-3 PUFA in beef muscle and lowered the n-6:n-3 ratio (Scollan et al., 2001). However, the P:S ratio was unchanged, reflecting the high degree of ruminal biohydrogenation. This might be overcome by protecting the fish oils against ruminal biohydrogenation, yet some free fish oil should be available in order to encourage CLA production. The increase in long chain PUFA in beef meat had significant negative effects on colour shelf life and organoleptic properties in previous work (Vatansever et al., 2000), hence it is important to feed supra-nutritional concentrations of the antioxidant, Vitamin E, and to monitor lipid and colour oxidation and sensory aspects of the meat produced.

It is hypothesised that the use of protected fish oil, in conjunction with some unprotected fish oil, in the diet of ruminants represents a useful method for simultaneously targeting the three major objectives of producing healthy beef meat, which are (1) increase the P:S ratio, (2) reduce the n-6:n-3 ratio and (3) increase CLA content.

Objectives

The current study was designed to manipulate the long chain PUFA and CLA content of beef fat and muscle by feeding free and ruminally-protected fish oil. The effect of the level of feeding of the protected fish oil on beef muscle fatty acid composition and its effects upon sensory characteristics and colour and lipid oxidative stability during simulated retail display were also determined.

Materials and Methods

Thirty two Charolais steers with an initial mean liveweight of 619kg (s.e 7.9) were allocated on age and live weight to one of four dietary treatments, each consisting of eight animals. The 4 diets consisted of *ad libitum* grass silage plus one of four concentrates in which the lipid source was either Megalac (high in saturated palmitic acid; 16:0 from palm oil) and 100g free fish oil (FFO; rich in EPA and DHA) or megalac/FFO supplemented with a ruminally protected fish oil supplement (PFO) which was fed separately. Diet 1 megalac/FFO (control); Diet 2, megalac/FFO and 50g/d PFO (PFO1), Diet 3 megalac/FFO and 100g/d PFO (PFO2) and Diet 4 megalac/FFO and 200g/d PFO(PFO3). The PFO supplement comprised soyabean and fish oil (tuna rich in DHA), and was prepared and protected from ruminal biohydrogenation by encapsulating the lipids in a matrix of rumen inert protein (Scott et al., 1971). The PFO was considered as part of the

overall concentrate allocation per day in maintaining an overall forage:concentrate ratio of 60:40 on a DM basis. The diets were formulated so that the total dietary oil intake was approximately 5.5% (45% of which was the test oil) of total diet. Vitamin E was added to the concentrates at a level of 350IU/kg.
Animals were slaughtered conventionally and carcasses chilled overnight. At 48h post-mortem, samples of m. *longissimus thoracis* at the 11th rib were removed and blast frozen and stored at –80ºC for subsequent fatty acid analysis. Lipid was extracted using chloroform/methanol and the neutral and polar lipids separated by silicic acid column chromatography. Fatty acid methyl esters were prepared by alkaline hydrolysis followed by methylation with diazomethane and analysed on a CP Sil 88, 100m x 0.25mm ID column (Chrompack, UK). An additional 180mm section of m. *longissimus lumborum* was conditioned at 1°C for 12 days in vacuum pack. A 100mm section was then frozen at -20°C for sensory analysis. After overnight thawing at 1°C, 20mm thick steaks were cut and grilled to 74°C internal temperature. The meat was assessed by a 10 person trained taste panel using 100mm unstructured line scales. Four steaks 20mm thick were cut from the remaining sample, packed in modified atmosphere trays (O_2:CO_2, 75:25) and subjected to simulated retail display (700lux lighting for 16h a day, 4ºC±1ºC). Colour (L*a*b*) was measured on the surface of two steaks at three points, daily with a Minolta Chromameter. The remaining steaks were taken at 10d of display and analysed for thiobarbituric acid reacting substances (TBARS) by the methods of Tarladgis et al. (1960) as a measure of lipid oxidation.

Results and discussion

There were no effects of diet on total DM intake, forage or concentrate intake, liveweight gain during the feeding period, carcass weight, conformation or fatness (results not shown). There were also no significant differences in total, neutral or phospholipid contents in muscle between diets (Table 2).
The 22:6*n*-3 content of muscle total lipid increased by 3.5 fold from the control to the highest level of supplementation (Table 1), all of which came from the phospholipid fraction. The content of 20:5*n*-3 also increased whilst 22:5*n*-3 decreased. CLA did not change and *cis*-18:1*n*-9 and 16:1 decreased. There were no changes in the main long chain PUFA in the neutral lipid fraction, which is very low in these fatty acids. CLA did not increase with increasing PFO, but the concentration in total lipid was similar to that found in our previous studies when free fish oil was present in the diet (Enser et al., 1999).
Total saturated fatty acids fell non-significantly by a small amount, in the PFO diets, and the total PUFA increased only slightly in the total lipid, which gave no change in the P:S ratio whether calculated by incorporating the long chain PUFA or not (Table 2). The n-6:n-3 ratio, as expected, did not change when calculated as 18:2*n*-6:18:3*n*-3, but when calculated as total n-6 PUFA to total n-3 PUFA showed a small but significant decrease with increasing PFO (Table 2). There was little change in total PUFA, the increasing EPA and DHA displacing mainly monounsaturated fatty acids, but at nearly 200mg/100g of muscle, a 100g serving would make a substantial contribution to daily requirements.
Despite the fairly small changes in PUFA content of the muscle, there were large significant effects on lipid stability. TBARS rose significantly between the control and the highest concentration of PFO in the diet. The long chain PUFA have more unsaturated double bonds and are correspondingly more susceptible to oxidation than those fatty acids with only one or two double bonds. It should also be noted that there was sufficient incorporation of EPA and DHA from the control diet, which contained free fish oil, to give a higher value for TBARS than is normally seen with this control diet (Vatansever et al., 2000). From day 7 of display it is clear that the control diet gave slightly more colour stable meat than those with PFO, but that the 7d display required by UK supermarkets is achieved by most samples (Figure 1). The highest level of PFO produced meat that was more tender than the control (Table 3), but which developed significantly higher abnormal flavours. Despite this there was no significant effect on overall acceptability.

Conclusions

Feeding protected fish oils increased the nutritionally important long chain *n*-3 PUFA in muscle. Since these PUFA are predominantly deposited in phospholipids, which are diluted by the more saturated fatty acids in neutral lipids results in only small effects on the usual indices of healthy nutrition such as n-6:n-3 and P:S ratios. The incorporation of some free fish oil produced an expected concentration of CLA, which did not increase with increasing PFO, despite its imperfect protection. Long chain PUFA are sufficiently, oxidatively unstable that even small increases in their concentration made the total lipid more unstable and had a small effect on colour stability, as seen previously (Vatansever et al., 2000). Despite producing more

apparent off-flavours there was some evidence that the highest level of PFO incorporation produced more tender meat and overall hedonic acceptability was unchanged.

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Table 1. Effect of diet on composition of total fatty acids (mg/100g muscle) of m. *longissimus thoracis*.

	Mega	PFO1	PFO2	PFO3	s.e.d.	P
14:0 myristic	143.3	129.2	115.3	132.1	30.60	ns
16:0 palmitic	1321	1167	1096	1189	207.4	ns
18:0 stearic	647.3	549.3	519.8	569.8	86.7	ns
18:1 *trans*	100.9	103.3	97.2	99.0	17.69	ns
18:1*n*-9 oleic	1669	1391	1305	1486	207.1	ns
18:1 *cis*-11	72.2	62.4	57.7	63.1	7.93	ns
18:2*n*-6 linoleic	87.8	81.3	87.0	92.9	6.67	ns
18:3*n*-3 α-linolenic	24.7	23.2	23.2	25.5	1.940	ns
CLA *cis*-9, *trans*-11 C18:2	20.7	20.8	20.1	20.9	3.84	ns
20:4*n*-6 arachidonic	23.7	22.2	24.6	24.6	1.98	ns
20:5*n*-3 eicosapentaenoic (EPA)	13.70[a]	14.09[a]	14.93[a]	18.39[b]	1.622	0.035
22:5*n*-3 docosapentaenoic (DPA)	22.46[b]	20.71[ab]	19.73[a]	18.61[a]	1.145	0.016
22:6*n*-3 docosahexaenoic (DHA)	3.36[a]	7.04[b]	9.76[c]	12.01[d]	0.801	<0.001

Table 2. Total fatty acids (mg/100g muscle), nutritional indices of total lipid of m. *longissimus thoracis* and Thiobarbituric acid reacting substances (TBARS, mg malonaldehyde/Kg meat) values of m. *longissimus lumborum* after 10d display in MAP

	Mega	PFO1	PFO2	PFO3	s.e.d.	p
Total fatty acids	4698	4092	3858	4258	614.2	ns
Total neutral lipids	4191	3632	3390	3785	611	ns
Total phospholipids	507	460	467	473	33	ns
18:2*n*-6:18:3*n*-3	3.58	3.52	3.78	3.66	0.142	ns
Total n-6:Total n-3	1.70[a]	1.55[b]	1.61[ab]	1.56[b]	0.060	0.043
P:S†	0.06	0.06	0.07	0.07	0.007	ns
P:S‡	0.107	0.118	0.131	0.128	0.015	ns
TBARS	1.64[a]	2.24[a]	2.33[a]	3.12[b]	0.361	0.004

[abc]Figures with the same superscript do not differ significantly

† 18:2*n*-6+18:3*n*-3:(C12+C14+C16+C18)

‡ Total PUFA:(C12+C14+C16+C18)

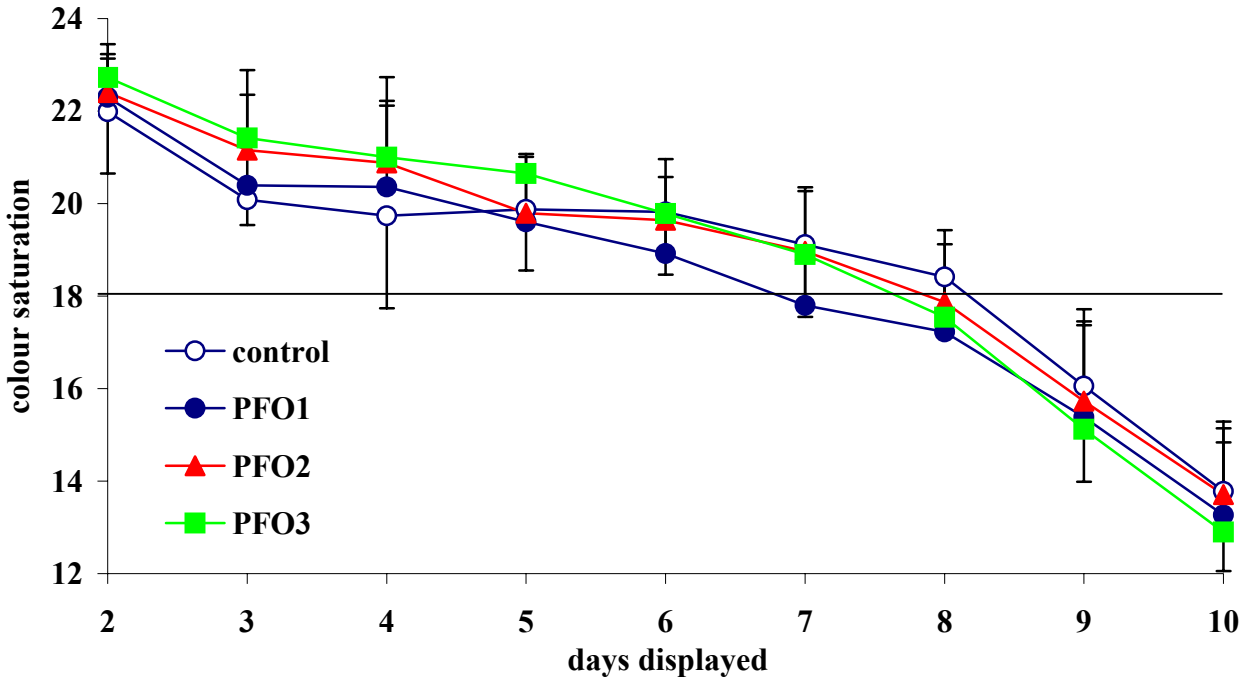

Figure 1. The effect of days displayed upon the change in colour saturation (±stdev) of modified atmosphere packed loin steaks, from animals fed varying levels of protected lipid supplement.

Table 3. Effect of Protected Fish Oil Supplement on sensory values of grilled loin steaks
Values are the means derived from analysis of variance with Supplement and Assessor as factors; panels are treated as a 'block structure' with 8 replications.

	Control	PFO1	PFO2	PFO3	sed	sig
Attributes						
Toughness	43.8^{ab}	45.8^{b}	47.4^{b}	38.4^{a}	32.81	0.05
Juiciness	40.4^{bc}	41.8^{c}	34.2^{a}	35.7^{ab}	32.65	0.05
Beef	23.7	24.7	22.3	19.8	21.68	ns
Abnormal	14.9^{a}	14.0^{a}	15.7^{a}	21.2^{b}	32.40	0.05
Greasy	13.6	12.6	14.9	14.1	01.68	ns
Bloody	3.2	7.1	4.7	4.8	21.46	ns
Livery	5.7	3.9	5.9	5.9	01.99	ns
Metallic	3.4	6.2	5.4	3.3	21.40	ns
Bitter	4.2	3.1	2.3	2.4	01.31	ns
Sweet	1.9	2.5	3.4	1.4	21.80	ns
Rancid†	1.6	1.8	2.5	3.6	10.99	ns
Fishy	1.3	1.2	2.6	3.9	21.18	ns
Acidic	9.7	11.3	9.9	7.8	02.19	ns
Cardboard	4.4	4.1	6.1	2.6	11.57	ns
Vegetable/grassy	6.2	6.1	6.1	8.4	01.85	ns
Dairy	6.9	6.1	5.7	9.0	02.27	ns
Hedonic						
Overall liking	22.5	22.3	19.2	19.1	2.42	ns

Acknowledgments

This work was supported by the Department for Environment Food and Rural Affairs and the European Commission (HealthyBeef QLRT-2000-31423).

INFLUENCE OF SEX, SEASON, WEIGHT AT SLAUGHTER AND MUSCLE TYPE ON BARROSÃ VEAL CHARACTERISTICS

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Background

The colour of fresh meat is an extremely important quality issue influencing the consumer´s purchase decision. That characteristic is influenced by the pigment content (Lindahl *et al*., 2001), the chemical state of the pigment and the microstructure of muscle tissue determined by the rate and extense of the pH fall. The relative influence of each of these factors depends on the muscle considered (Guignot *et al*., 1992).

Barrosã calves are produced according to a traditional extensive production system in a region well known for its mountains and uplands where the winter is the main period of feed scarceness. Since they have not suffered as much genetic pressure as other breeds, the biochemical characteristics of their muscles could be different. No work was done therein to characterize the meat of Barrosã breed from the production point of view.

Objectives

The main objective of this study was to investigate the relationship between fibre metabolic/contractile profile and some other traits veal characteristics evaluated on *Longissimus dorsi*, *Supra spinatus* and *Biceps femoris* muscles.

Materials and methods

Around 1 hour after slaughter, samples from *Longissimus dorsi* (Ld), *Supra Spinatus* (Ss) and *Biceps femoris (Bf)* of twenty Barrosã calf carcasses of both sexes, with ages ranging from 6 to 10 months were taken and frozen in liquid nitrogen for histochemical analysis as described by Roseiro *et al.* (2003). Sixty carcasses were selected for pH, colour and haem pigment analyses. Samples of the three muscles were taken and frozen at -18ºC until assessment of haem pigments (Hornsey, 1956). Results were expressed as µg haematin/g wet tissue, using a standard curve of haematin instead of the factor used by Hornsey (1956). The pH was measured at 24h after slaughter using a glass electrode (Mettler Toledo LoT406-M6-DXK-S7/25). The colour parameters (Lab) were measured 24h *post-mortem* on the carcass surface using a Minolta CR300 in the CIE L* a* b* space.

Vitamin E was determined according to European Standard-EN 12822 with minor modifications. 20g of sample trimmed of visible fat and connective tissue was weighed into a dark flask added of 1g of pyrogallic acid and 200 mL of saponification solution. This solution was prepared fresh each day and comprised 50 mL of 60% potassium hydroxide solution and 150 mL of ethanol absolute. The mixture was heated at reflux for 40 min at 100º C and then cooled and extracted 3 times with petroleum ether. After the evaporation of the solvent under vacuum, the residue was redissolved in 5 ml of hexane HPLC grade and filtrated through a membrane Acrodisc 25 mm GHP, GF 0.20 µm (Gelman Sciences, Inc.). α-Tocopherol was measured by HPLC (Spectra-Physics, model Spectra 100) using a Spherisorb S 5W silica, 5µm, 4.0x125 mm cartridge (Waters PSS 845549) at 292 nm. Hexane/1,4-dioxane (99:1) was used as mobile phase at a flow rate of 0.8 mL min^{-1}. Recovery of α-tocopherol from meat was determined by the addition of an internal standard to samples before saponification. Detector signals were quantified using peak areas and a calibration curve. The α-tocopherol content was expressed as mg/100 g of muscle

Data were analysed using one-way and two-way analysis of variance (ANOVA) and significant differences were determined using Tukey´s HSD post hoc test (Statistica 6.0-StatSoft Inc., 2001) Differences were considered different at $p<0.05$.

Results and discussion

Muscle traits

Least square means of fibre type composition, colour parameters, pH_{24}, α-tocoherol and haem pigment concentration obtained from *Longissimus dorsi*, *Biceps femoris* and *Supra spinatus* muscles are presented in Table 1. Significant differences were found among muscles for all traits studied except for b* value and βR fibres percentage. Ss muscle presented higher mean α-tocopherol ($P<0.05$) and haem pigment ($P<0.001$) than Ld and Bf muscles, which did not differed. Regarding pH_{24}, Ss and Ld muscles showed significantly higher mean values than Bf muscle. There were no significant differences between Ld and Bf muscles in regarding to fibre metabolic and contractile profiles, except for αW fibres. Bf muscle showed the highest frequency of αW fibres, but not presented the highest glycolytic profile which belong to Ld. Ss muscle presented a metabolic profile significantly less glycolytic than Ld, but did not differ from Bf.
Guignot *et al* (1992) reported a contractile profile in Friesen-Holstein calves, similar results to those observed in this study, for the same muscle.

Table 1 – Compositional characteristics of *Longissimus dorsi*, *Biceps femoris* and *Supra spinatus* from Barrosã veal.

	MUSCLE			
	Ld	**Bf**	**Ss**	**F value**
α-tocopherol ($mg.g^{-1}$)	0.172 [b]	0,165 [b]	0,216 [a]	4,049*
Haem pigment ($mg.100g^{-1}$)	16.36 [b]	16,66 [b]	19,69 [a]	24,023***
PH_{24}	5.67 [a]	5,46 [b]	5,72 [a]	23,50***
L*	35.22 [b]	39,51 [a]	ND	44,83***
a*	11.69 [b]	13,73 [a]	ND	49,37***
b*	2.76	2,53	ND	1,54[ns]
βR fibres (%)	25.35	22,35	24,27	0,65[ns]
αR fibres (%)	22.89 [ab]	17,56 [b]	26,81 [a]	8,98***
αW fibres (%)	51.76 [b]	60,10 [a]	48,93 [b]	6,21**
Oxidative fibres (%)	48.52 [b]	52,20 [ab]	53,96 [a]	4,55*
Glycolytic fibres (%)	51.48 [a]	47,80 [ab]	46,04 [b]	4,55*

In same row, means with different letters are significantly different. * P<0.05; ** P< 0.01; ***P<0.00; ns-not significant; ND-not determined; L*,a*,b*- colour parameters.

The number of fibres in bovine muscles is almost determined at birth. However, the relative fibre type composition is affected to a number of genetic and environmental factors such as age, sex, breed and exercise performance. The main fibre type change occurs in the first few months of life. Based on different breeds, which varied in age between 0 and 12 months, Wegner *et al*. (2000) stated that such fibre type conversion occurred as early as 6 months old and was characterized by an increase and decrease frequency of type IIb and type IIa fibres, respectively, and no alterations in the type I incidence.
All Barrosã calves used in our study were more than 6 months old and the age effect on muscle fibre profile was not evaluated. Nevertheless, there was a remarkable effect of the weight at slaughter on contractile ($P<0.001$) metabolism (Table 2). Attending to the low correlation between the animal age and weight at slaughter, that influence could be attributed to differences in diet and animal handling. Listrat *et al* (1999) referred that muscle fibre characteristics could be related to the energy level of the diet and to daily dry matter intake. However, the diet effect on muscle fibre type profile of calves are not easily explained since during the most important period for fibre type changes, they are basically fed with mother's milk. The impact of diet on muscle fibre profile of calves reared in an extensive system needs more attention.

The influence of sex on meat traits was generally less expressive inducing significant changes only on βR and αW fibres and pH_{24}. Dreyer *et al.* (1977) reported that muscles of bulls contained more red fibres than those of steers. Johnston *et al.* (1981) found a significant higher percentage of αW fibres in Ld muscle of heifers than in steers. In our studied population, male muscles showed significantly ($P<0.001$) higher and lower percentages of βR and αW fibres, respectively, than females (data not shown).

Table 2 – Effect of weight, sex and slaughter season on muscle characteristics.

	F VALUE		
	Weight	**Sex**	**Season**
α-tocopherol	0,49 ns	0,006 ns	2,79 ns
Haem pigment	14,03***	0,519 ns	4,01*
pH_{24}	0,40 ns	3,90*	10,70**
L*	1,81 ns	0,90 ns	1,23 ns
a*	0,22 ns	0,80 ns	0,03 ns
b*	6,04*	1,34 ns	4,39*
βR fibres (%)	7,97**	5,24*	ND
αR fibres (%)	11,77***	2,68 ns	ND
αW fibres (%)	24,44***	9,39**	ND
Oxidative fibres (%)	2,77 ns	0,47 ns	ND
Glycolytic fibres (%)	2,77 ns	0,47 ns	ND

In same row, means with different letters are significantly different. * P<0.05; ** P< 0.0; ***P<0.001; ns-not significant. ND-not determined; L*,a*,b*- colour parameters

Relations between pigment content, muscle traits and production factors

Correlations between the haem pigment concentration and the other Barrosã meat parameters are depicted on Table 3, and were not significant.

The highest haem pigment content was found, as expected, in the most oxidative muscle corroborating the trend observed by Meynier & Gandemer (1991). This parameter was affected by the animal weight at slaughter (P<0.001) and the slaughter season (P<0.05) but not by sex. Irrespective of the carcass weight, the highest pigment value was measured in older animals (data not shown).

Table 3 – Correlations between muscular traits.

	1	**2**	**3**	**4**	**5**
1 α-tocopherol	1,00				
2 Haem pigment	-0,23 ns	1,00			
3 pH_{24}	-0,27 ns	0,10 ns	1,00		
4 L*	0,24 ns	-0,27 ns	-0,36*	1,00	
5 a*	-0,07 ns	0,29 ns	-0,29*	0,12 ns	1,00
6 b*	0,23 ns	-0,11 ns	-0,52***	0,44**	0,51***

* P<0.05; ** P< 0.01; ***P<0.00; ns-not significant. L*,a*,b*- colour parameters.

The effect of slaughter season on haem pigment content as well as on pH_{24} is not easily comprehensible, but it could possibly be related to animal handling, diet composition and feed availability. Although animals are slaughtered throughout the year, spring and autumn can be described as stronger production seasons. The grass availability and its composition are not regular all over the year and this fact could have implications in growth traits and influence the carcass and meat quality. The pigment concentration referred in our study for Ld muscle was similar to those reported by Gil *et al.* (2001), in older animals of Retinta breed.

Relations between colour, pH, muscle traits and production factors

Due to its difficult access, colour parameters were not measured on Ss muscle. Comparing the other two muscles, L* and a* values were significantly higher in Bf than in Ld muscle (P<0.001). The absence of a significant correlation with the pigment content confirms the results obtained by Hunt & Hedrick (1977) and Tam *et al*. (1998) and disagreed with the findings of Lindahl *et al.* (2001). Although McDougall (1982) claimed that the pigment content was the main factor affecting muscle colour, that relationship depended on the muscle location (Guignot *et al*., 1992).

A study of Priolo *et al.* (2001) have shown that meat from animals finished on pasture is darker than those finished on concentrate, with the formers presenting a L value about 5% lower. In accordance to Page *et al.* (2001) a significant correlation between muscle pH_{24} and colour values was also observed. This correlation can be explained in that colour in muscle tissue is based on the reflectance of light off free water and on

oxygenation of the myoglobin At a higher muscle pH, proteins are bound strongly with water, allowing less free water to escape to interstitial space around cells and the muscle fibres to swell. Therefore, meat with higher pH will be darker in colour because there is less free water to reflect light. These authors also stated that a higher pH_{24} produced less red and yellow beef. Our results agreed with Page's experiment. The Ld muscle showed a significant higher mean pH_{24}value ($P<0.001$) and lower L* and a* values ($P<0.001$) than Bf muscle.

Acknowledgements

This research was supported by the Fundation for the Science and Technology (FCT). The authors are grateful to Cooperativa de Boticas (CAPOLIB) and to Hospital de Santa Maria.

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IMPORTED AUSSIE BEEF QUALITY AND COMPARISON WITH US BEEF

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Background

Meat consumption in Japan is considerable at present, with beef imports particularly high from the USA and Australia. Certain brands are quite popular and have become standards of choice. But this merely reflects personal preferences and attitudes, without basis in objective data. The Japanese domestic beef (black cattle) is of course best known to the people in Japan. The breeding periods and types of feed differ for this domestic brand. But little research has been conducted of the meat quality of imported beef.

Objectives

Consequently, the present study was conducted to address this matter using Aussie beef frequently to be found in stores in Japan. Based on the results, comparison is made with American brand (US) beef considered as equivalent in quality to or even better than the former.

Materials and methods

Beef sample: Strip loin samples of Aussie and US beef (N=4) were chilled and aged for 30 days for sensory and physicochemical analysis. All samples of either brand were graded as Choice or better. The Australian species, Angus cross, is primarily fed barley while the American Angus cross is given feed whose major constituent is corn.

Sensory evaluation: Evaluation in all cases was made using beef (thigh side) sliced at 8 mm in thickness. Raw meat and meat grilled for 2 min, (1 min for each side) at 180˚C by an electric plate were evaluated by a team of experts from Nippon Meat Packers using the two-point comparison method.

Color of meat and fat: Lab-values were obtained for lean and fat of the 8 mm slices using a color difference meter (Minolta CR-200). Lean color was assessed after 2 days store-front storage under fluorescent lighting for meat packed with plastic O_2-permeable film at 4˚C.

Myoglobin content: Beef samples were cut up into pieces for homogenization and myoglobin (Mb) content as well as the met-Mb (MetMb) ratio in minced meat were determined by the extraction method (Sakata and Nagata, 1992; Trout, 2003).

TBA value: Distillation was carried out for determination of malonaldehyde (MA, Yamauchi and Ando, 1973) in the above minced meat sample. TBA value was expressed as MA mg/1000g meat.

Water and fat contents: Using minced meat, water and fat contents were determined by the freeze-dried and Soxhlet extraction methods, respectively.

Water holding capacity: Five cm thick beef cuts were vacuum-packed and cooked at 70˚C for 90 min. Cooked meat yield (%) of meat following water loss due to cooking was designated as water holding capacity. Using a press-machine, beef (10×10×5 mm) was pressed at 15kgw/cm^2 for 40 sec. The amount of water released and pressed meat area were determined based on the water holding capacity of raw meat.

Hardness assessment: Beef hardness of the 8 mm slices was assessed by the multi-bite method using a Tensipressor (Taketomo TTP-50BXII).

Melting point and area % of muscular fat: Muscular fat extracted by the Soxhlet method was used to determine fat melting point using apparatus specifically designed for this purpose.

Ratio of fat size in lean muscle: The 8 mm meat slices were photographed and monochrome figures were incorporated into computer provided with an image analysis system (Pias LA525). Using this system, fat marbling and lean portions were divided into black or white sample groups and on the basis of the size ratio, fat size (% of whole meat area) and number were determined.

Composition of fatty and amino acids in lean muscle: Fatty acid composition was clarified by gas - chromatography (Hewlett-Packard 6890) using muscle specimens. Amino acid analysis on minced beef was carried out for clarification of free and peptide-forms using an amino acid analyzer (JEOL JLC-500/V) and carnosine were determined at the same time.

Results and discussion

Sensory evaluation: Table 1 shows the results for sensory evaluation of the 2 beef brands. Aussie raw beef exhibited a darker red and the fat was white, in obvious contrast to the US beef. The grilled samples were assessed as better aroma, more tasty and more tender. But these qualities differed according to the sample. US beef was generally considered more tender.

Color of meat and fat: Table 2 shows changes in color with storage. On 0 day (1 hr after slicing), the L value was greater in US beef, with a and b values essentially the same. This would account for its brighter red color. On day 2, L had decreased and a value was greater. Changes in Aussie beef were fewer during this period. US beef thus has greater tendency to undergo discoloration. Table 3 indicates fat color for the 2 brands and L to be higher and a lower in Aussie beef. It thus follows that Aussie beef has greater white fat content.

Myoglobin content: Mb content and MetMb% during storage are shown in Table 4. Mb content was greater in Aussie beef. At 2 days storage, Mb content of both brands had decreased, suggesting the possibility of drip from meat surface during storage. MetMb increased with the period of display, more so in US than Aussie beef.

TBA value and carnosine content: Tables 5 and 6 show respectively values for TBA and carnosine. TBA value was higher in US beef on day 0 and 2 while Aussie beef contained greater carnosine.

Water content , cooking yield and water holding capacity: Table 7 shows water content for lean meat and Table 8, water holding capacity (WHC), The values were basically the same for the two brands in cooked meat yield (Table 8-1), released water (-2) and area of pressed meat (-3).

Hardness assessment: Table 9 shows the value of this parameter as determined using a Tensipressor. The US beef was generally significantly more tender.

Fat content in lean muscle and melting point: Table 10 shows fat content in lean muscle and its melting point. The values were essentially the same for the 2 brands. Aussie beef had greater fat content (Table 11), this being consistent with greater degree of fat marbling as seen on the computer image display. US beef exhibited significantly lower fat melting point.

Fat and amino acid composition: Myristoleic acid (C14:1) and arachidic acid (C20:1) were higher in US beef and stearic acid (C18:0) was significantly abundant in Aussie beef. Ratios of unsaturated fatty acids were significantly lower in Aussie beef, this possibly causing higher fat melting point. Glutamic acid was higher, through not significantly, in Aussie beef (data not shown).

The results for sensory evaluation and color measurement of raw beef indicated Aussie beef fat to be lighter in color and meat color darker red compared to US beef. This reflects the chromatic character of Aussie beef, which is superior in quality owing to its fatness character. US beef appears to incur more discoloration owing to fluorescent lighting in the display case, while that of the former is stable maintained. The MetMb ratio was higher in US beef subsequent to display, causing the meat to become brownish. TBA was lower in Aussie beef with consequently less rancidity. This feature and discoloration are due to oxidation which has been shown to occur less in Aussie beef. Aussie beef capacity for preservation is thus greater. The presence of relatively more carnosine, an antioxidant, may be one reason for this. The sensory evaluation of grilled beef, aroma, taste and tenderness showed essentially the same results for the two brands. The evaluation team gave the same scores for eating quality. US beef was considered a little more tender than the Aussie brand. No relationships could be detected among tenderness, water holding capacity and fat marbling. US beef in this study showed looser muscle fibers and this possibly may explain its somewhat greater tenderness.

Conclusions

Based on the results of this study, the following conclusions were drawn: Subcutaneous fat in Australian meat cattle was at one time noted to be almost entirely yellow with little marbling. This was due to the cattle feeding mainly on grass. But grain feed with special formula for fattening has resulted in improved meat quality and greater meat exports to Japan. Aussie beef is shown by the present study to be superior in quality owing to its greater water holding capacity. The Japanese are grateful for this high quality beef. Aussie beef imports will continue to increase until BSE inspections in the USA is fully implemented with satisfactory results.

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Table 1. Results of sensory evaluation of beef

		Aus. beef	US beef	No difference
Raw	Darker red (meat)	67	26	8***
	More white (fat)	88	7	6***
	Greater aroma	39	53	9
Cooked	Greater aroma	41	55	5
	More tasty	41	55	5
	More tender	34	63	4*
	More delicious	45	41	5

Figures indicate numbers of evaluation personnel.
Showed significant differences at $p<0.05$ (*) and $p<0.001$ (***).

Table 2. Changes in beef color with storage

	0 day		2 days	
	Aus. beef	US beef	Aus. beef	US beef
L	38.74	40.73*	38.46	38.77
a	20.11	20.75	20.80	19.72*
b	6.56	6.90	6.39	7.20

* $p<0.05$

Table 3. Fat color of beef

	Aus. beef	US beef
L	74.27	70.70*
a	7.46	9.12*
b	9.00	9.18

* $p<0.05$

Table 4. Mb content (mg/g meat) and MetMb (%).

Mb content (mg/g meat)				MetMb (%)			
0 day		2 days		0 day		2 days	
Aus. beef	US beef	Aus. beef	US beef	Aus. beef	US beef	Aus. beef	US beef
7.15	6.33*	6.72	5.83*	8.71	6.55	39.48	45.89**

* $p<0.05$, ** $p<0.01$

Table 5. TBA values during storage

0 day		2 days	
Aus. beef	US beef	Aus. beef	US beef
0.342	1.390*	0.767	2.422*

* p<0.05

Table 6. Carnosine content (μmoles/g meat)

Aus. beef	US beef
26.5	22.14*

* p<0.05

Table 7. Water content (%)

Aus. beef	US beef
69.60	69.08

Table 8-1. WHC: Cooked meat yield (%)

Aus. beef	US beef
78.92	79.88

Table 8-2. WHC: Released water (%)

Aus. beef	US beef
23.07	24.65

Table 8-3. WHC: Area of pressed meat (cm^2/g meat)

Aus. beef	US beef
21.27	21.92

Table 9. Hardness (kgw/m^2)

Aus. beef	US beef
24.73	22.67*

* p<0.05

Table 10. Muscular fat content and its melting point

Fat content (%)		Melting point (˚C)	
Aus. beef	US beef	Aus. beef	US beef
6.81	6.88	41.5	40.2*

* p<0.05

Table 11. Fat size and fat numbers of muscle

Fat size (%)		Fat number	
Aus. beef	US beef	Aus. beef	US beef
10.03	6.85	155	182

Measured by computer provided with image analysis system.

THE EFFECT OF REFRIGERATION CONDITIONS ON THE QUALITY OF LAMB MEAT

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Background

In Portugal the traditional lamb meat production systems are based on local breeds, raised on pasture for most of the year. Many of these products are protected by the EU with denominations of origin (PDO) or geographic indications (PGI). Consumer's normally consider certified meat as healthy food, produced in natural conditions and have high expectations concerning eating satisfaction. Certified meat is marketed at higher prices than undifferentiated meat and is required by high quality segments of the market. Particularly for these products, it is essential to control all the production processes in order to obtain the highest quality standards and to reduce variability. In Portugal, in spite of being recommended for several lambs with PDO or PGI the practice of low carcass cooling rates, the common practice is to cool carcasses as soon as possible in order to reduce evaporative weight loss and prevent spoilage.

Tenderness is one of the most important organoleptic characteristics, and only tender meat can assure consumer's satisfaction. The main factors affecting tenderness are sarcomere length, proteolysis of myofibrillar proteins and connective tissue (Koohmaraie *et al*. 2002). The rates of temperature and pH decline in the early *post mortem* play an important role in meat tenderness. A slow cooling rate before the onset of *rigor mortis* prevent cold shortening and stimulate the proteolysis of myofibrils and associated proteins (Lockner *et al*., 1980; May *et al*., 1992), increasing meat tenderness. The effect of rapid cooling of carcasses may be particularly important in small carcasses, such as lamb, and Geesink *et al*. (2000), in a trial with lamb *longissimus* muscle concluded that optimal temperature at the onset of *rigor mortis* is around 15 ºC. In these conditions muscle shortening was minimized and proteolysis during storage was unaffected.

Objectives

The objective of this trial was to evaluate if 4 and 8 hours at 12 ºC, before cooling lamb carcasses at 2º C affects tenderness, histological, biochemical, physical and microbiological traits of meat.

Materials and methods

Animals and sampling

Thirty Merino Branco ram lamb carcasses were used to conduct this trial. Lambs were slaughtered with four months of age in the abattoir of the Estação Zootécnica Nacional. After dressing and weighing, three groups of ten carcasses were assigned to three refrigeration conditions: 0 (cooling at 2 ºC immediately after slaughter), 4 (cooling at 2 ºC after four hours at 12 ºC) and 8 (cooling at 2 ºC after eight hours at 12 ºC). Temperature and pH of the *longissimus lumborum* (LL) and *semimembranosus* (SM) muscles were measured 4, 8 and 24 hours after slaughter, using a penetration probe electrode. Eight hours after slaughter, and at the third, seventh and tenth days, sampling to microbiological analysis were performed on carcass shoulder region by destructive method (2,5cm^2) according to the Directive 64/433/CEE. Three days after slaughter, carcasses were split in two halves and the muscles LL and SM of the right sides were vacuum packed and frozen at – 20 ºC until shear force determinations. The colour of *longissimus thoracis* (LT) was estimated in the L*, a* and b* system (Minolta CR-300 chromometer) at the level of the 13th dorsal vertebra, after one hour of exposition to the air. Samples of LT and SM were collected, vacuum packed and frozen at – 80 ºC to determine sarcomere length. A small portion of LT muscle was collected to determine water-holding capacity (WHC). In the left halves of the carcasses, LL was vacuum packed and frozen at – 20 ºC until sensory analysis.

Analytical procedures

Sarcomere length was determined by the method described by Sañudo *et al*. (2003). Water holding capacity was determined by the method proposed by Santos-Silva *et al*. (2002).

Concerning microbiological analysis, total psycrotrophic aerobic counts at 7ºC for 10 days (Plate Count Agar, Merck, Germany), *Enterobactereaceae* on VRBD agar (Merck, Germany) for 48h at 37ºC, *Escherichia.coli* at 44,5ºC in Tergitol BCIG agar (Biokar Diagnostics) for 24h, lactic acid bacteria counts on Man Rogosa Sharpe Agar (Oxoid, UK) incubated at 30ºC for 3 days and *Brochothrix termosphacta* count in streptomycin, actidione, thallous acetate agar (STAA, Oxoid, UK) incubated for 2 days at 30ºC. Counts were expressed as log cfu/cm^2.

For shear force determination, samples were thawed for 24 hours at 2ºC and cooked in an electric oven until the meat internal temperature reached 65 ± 5 ºC. Cores with a section of about 1 cm^2 were prepared in the muscle fibres direction. Shear force was determined using a texture analyser (TA-XT2i Texture Analyser, Stable Micro Systems) equipped with a Warner-Bratzler shear device and data were collected with specific software (Texture Expert Exceed, Stable Micro Systems). Measurements were recorded as the average value of a minimum of six replicates. For sensory analysis a trained panel of six members was used to compare tenderness of meat of the three experimental groups using a structured scale of ten points (0 – very tough; 10 – very tender). Meat was prepared as described for shear force determination. Three cores corresponding to the three cooling conditions were presented to the panellists simultaneously and served in hot plates in a total of 51 comparisons.

The data were analysed using the GLM procedure of SAS (1989). When the F-test was significant, the least-squares means were compared. For shear force, muscle temperature, pH and sarcomere length, muscle and refrigeration conditions were used as fix effects and cold carcass weight (CCW) as covariate. For muscle colour parameters and WHC, the refrigeration condition was the fix effect and CCW the covariate. For tenderness and microbiological traits both models included the refrigeration conditions, besides panellist (tenderness) and days after slaughter (microbiological traits).

Results and discussion

Shear force was higher ($p<0.05$) when carcasses were immediately cooled (group 0) and no significant differences were found between groups 4 and 8. Data for LL are in accordance to those obtained for sensory evaluation, as panellists considered meat of group 0 the tougher and of group 4 the most tender. These results agree with those of Marques de Almeida *et al*. (2003), suggesting that 3 or 4 hours delay of carcass cooling at 2 º C are enough to improve meat tenderness 3 days after slaughter. Jaime *et al*. (1993), comparing the effects of 3 cooling conditions on tenderness of lamb *longissimus* muscle, concluded that toughness 2 days after slaughter was higher in carcasses with faster temperature decline, although no differences were found after eight days of ageing. Thatcher and Gaunt (1992) observed higher shear force of meat from lambs chilled at 2º C compared to others chilled at 10 ºC.

As expected, the rate of temperature decline was higher, intermediate and lower for groups 0, 4 and 8 respectively, and in LL than in SM. These results for temperature explain the differences in the rates of pH decline, which was higher in groups 8 and 4 than in group 0 and in SM than in LD.

When muscle temperature reaches values below 10 ºC and pH is still above 6, cold shortening may occur. The values observed for pH and temperature could suggest a higher muscle shortening, particularly in group 0. However, the values of sarcomere length indicate that shortening was very moderate in the 3 groups and no differences were found between the three refrigeration conditions or the two muscles. The correlation coefficient between shear force and sarcomere length was low ($r=-0.06$) and not significant ($p>0.05$). Marsh and Leet (1966) demonstrated that up to 20 % of muscle shortening there is no effect in tenderness of muscle *sternomandibularis*.

Shear force values showed correlations coefficients of -0.40 ($p<0.05$) and -0.42 ($p<0.05$) with muscle temperature 4 hours after slaughter in LL and SM, respectively. Other authors have reported that temperature in the initial period after slaughter is the factor most highly correlated with tenderness (Lockner *et al*., 1980; May *et al*., 1992), which is probably related to a higher extension of proteolysis by endogenous peptidases (Yates *et al*., 1983). Although we do not dispose of data to confirm this hypothesis, the differences in *in situ* proteolytic activity may explain the results of this trial.

In the range of pH values above 5.5, meat with higher pH may have higher WHC (Purchas, 1990) and sow lower values of L* (Priolo *et al*., 2001). Also, higher rates of glycolysis, associated to lower rates of temperature decline, may affect negatively meat WHC (Hood and Joseph, 1985). In this trial the refrigeration conditions had no effect on LT colour and WHC. Thatcher and Gaunt (1992), also found minor effects on meat colour of ewe lambs chilled at 2 or 10 ºC.

The results for microbiological traits are presented in table 2. The counts on the day of slaughter (0) for total psycrotrofic bacteria (± 3 log cfu/cm^2) and the results obtained for *E. coli*, that was not detected at day 0 and showed low values during conditioning, indicate good hygiene in the abattoir. On day 0 the dominant Gram+ flora seems to be lactic bacteria. Only for this group of microbes, the counts were higher ($p<0,001$) when temperature decline was lower (groups 4 and 8) and this effect was still observed on day 3. However, on the 7th and 10th day the results were similar for the 3 refrigeration conditions, suggesting that the increase of population reached a stationary state corresponding to values of 3-4log cfu/cm^2. After the 7th day, the dominant flora is *Brochothrix thermosphacta* and the Gram– psycrotrophic bacteria. These populations are responsible for the spoilage of meat that is already evident at the 10th day, when the counts for total psycrotrophic are higher than 8 log cfu/cm^2, independently of the refrigeration conditions. The shelf life period, in the conditions of slaughter and refrigeration of this trial, was up to 7 days.

Conclusions

According to the results of this trial the rapid cooling of lamb carcasses results in tougher meat. The 4 hours delay in carcass cooling at 2 ºC, seems to improve tenderness, and have no negative effects on the physical traits of meat (colour and water-holding capacity). Although the delay of cooling increased the counts of lactic acid bacteria at the day of slaughter, it did not affect the hygienic conditions of meat after 7 days. In the conditions of slaughter and refrigeration of this trial, the meat microbial shelf life period was up to 7 days.

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Table 1. Meat quality traits of *longissimus* and *semimembranosus* muscles of lamb carcasses submitted to three refrigeration conditions

	longissimus			*semimembranosus*			SEM	Effects		
	0	4	8	0	4	8		M	G	M*G
Shear force (kg)	6.65 b	4.51 a	5.97 ab	7.18 b	5.82 a	5.77 a	0.552	ns	*	ns
Tenderness	5.81 a	7.32 c	6.67 b				0.231		***	
Temperature 4h (ºC)	6.7 a	13.5 c	13.5 c	9.6 b	15.5 d	16.6 e	0.21	***	***	**
Temperature 8h (ºC)	4.0 a	5.6 c	11.8 e	5.0 b	6.6 d	12.4 f	0.22	***	***	**
Temperature 24h (ºC)	2.9 a	2.9 a	3.9 b	2.6 a	2.8 a	3.6 ab	0.33	*	ns	***
pH 4h	6.70	6.50	6.67	6.46	6.26	6.46	0.053	***	***	ns
pH 8h	5.98	5.97	5.76	5.97	5.87	5.76	0.059	**	ns	ns
pH 24h	5.72 b	5.69 ab	5.61 ab	5.71 b	5.65 ab	5.58 a	0.038	*	ns	*
L*	41.5	42.7	40.1				0.74		ns	
a*	11.7	11.8	12.4				0.34		ns	
b*	3.34	3.79	3.43				0.244		ns	
Water-holding capacity (%)	30.0	26.4	30.6				1.11		ns	
Sarcomere length (μ)	1.99	2.09	1.96	1.69	1.72	1.76	0.047	***	ns	ns

SEM- standard error of means; M - muscle; G - group

Table 2. Effects of refrigeration conditions and days after slaughter on microbial proliferation at the surface of lamb carcasses (cfu/cm^2)

Group	0				4				8				SEM	Effects		
Days *pm*	0	3	7	10	0	3	7	10	0	3	7	10		G	D	G*D
Bt	a 0.308	a 0.100	cde 5.008	cde 5.023	a 0.290	b 1.262	cde 4.917	e 6.247	a 0.000	b 1.747	c 4.364	de 5.657	0.2957	*	***	**
Ent	ab 0.715	ab 0.755	de 2.873	e 3.270	a 0.460	bc 1.648	cd 2.306	f 4.474	ab 0.742	a .580	de 3.139	de 3.144	0.3387	ns	***	*
Tp	3.377	3.887	6.501	8.250	3.265	4.248	6.163	8.997	3.579	4.381	6.032	8.094	0.3138	ns	***	ns
E. coli	0.000	0.230	0.396	0.390	0.000	0.278	0.360	0.360	0.000	0.130	0.130	0.317	0.0838	ns	ns	ns
Lb	0.341	0.818	3.359	3.568	1.221	1.856	3.880	4.518	1.084	1.611	3.955	3.612	0.1326	***	***	ns

SEM - standard error of means; G - group; D - days after slaughter; *Bt* - *Brochothrix termosphacta; Ent* - *Enterobactereaceae;* Tp - Total psicrotrophic; *E. coli* - *Escherichia coli;* Lb - Lactic bacteria

CARCASS QUALITY OF SEVERAL EUROPEAN CATTLE BREEDS: PRELIMINARY RESULTS

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Background

Selective breeding in cattle has been very successful in increasing production indicators, but until now there is little information and subsequently limited knowledge to allow selection programs to be designed to improve product quality. Identification of the genes involved in aspects of carcass and meat quality and the quantification of their effects will provide the basic information to devise breeding programmes that enhance quality and take account of regional variations in preference.

The present study is part of an ongoing EU project, which will examine a total of 450 animals from 15 different breeds representing the genetic diversity of European cattle. This project will undertake a rigorous comparison of carcass and meat quality from this range of cattle breeds with animals raised under similar management conditions in order to minimise environmental variation and to identify and define genetically determined component of that variation.

Objectives

To assess the variability among 15 different European cattle breeds in carcass traits.

Materials and methods

The material consisted of 337 young entire males from 15 different European cattle breeds. The breeds used were as follows: From United Kingdom, Jersey, South Devon, Aberdeen Angus and Highland; from Denmark, Holstein, Danish Red Cattle and Simmental; from Spain, Asturiana de los Valles, Asturiana de la Montaña, Avileña-Negra Ibérica and Pirenaica; from Italy, Piemontese and Marchigiana and from France, Limousin and Charolais. All animals were intact bulls, which were fed *ad libitum* with a standardised diet composed of high barley proportion (about 80%), soya (9%) and chopped straw (10%) with appropriate minerals and vitamins. Energy density was approximately of 12.5 kJ/Kg dry matter.

The welfare regulations were taken into account when handling the animals. The bulls were slaughtered at the nearest EU licensed abattoir, to minimise the effect of the transport stress on meat quality. Slaughter was at 75% of mature bull weight, which in most cases was about 15 months of age. Stunning was by captive bolt pistol. Carcass dressing was carried out using a standardised project protocol that corresponded to commercial practice. Carcasses were chilled at 4 ± 1º C for 24 hours.

Immediately after slaughter, the following variables were recorded:

- Kidney fat weight.
- Hot carcass weight, measured without removing the subcutaneous fat.
- Dressing percentage, calculated according to the following formula: 100*hot carcass weight/slaughter live weight.
- Conformation score was graded according to the SEUROP classification (R. (CEE) 1026/91, R. (CEE) 2237/91 and R. (CEE) 2930/81) with a scale ranging from 1 (very bad conformation) to 18 (very good conformation).

- Fatness score was measured by UE classification, with a 15 points scale (1, very low fat to 15, very high fat).

Also, several standard measurements were taken on the left half carcass to evaluate carcass morphology, according to the methodology described by De Boer, Dumont, Pomeroy & Weniger (1974). Variables recorded were carcass length, internal depth of breast, limb length and limb thickness. The blockiness index was calculated. This index expresses the relationship between carcass weight (kg) and carcass length (cm). High values indicate high muscular development (Albertí *et al.,* 2001). Limb index expresses the relationship between limb thickness (cm) and limb length (cm).

The area of the *Longissimus thoracis* (LT) muscle, at 5^{th}-6^{th} rib level, was recorded by tracing: an acetate sheet was placed on the surface of the loin, and the border of the muscle marked on the sheet using glass marker. The area of the muscle was calculated by planimetry. Medium-lateral and dorso-ventral diameters (A and B, respectively), were also measured (Cañeque & Sañudo, 2000).

The 6^{th} thoracic rib joint was collected at 24 h post-mortem, and its' weight was recorded together with the *Longissimus thoracis* muscle, which was separated for instrumental analysis. The rest of the rib joint was dissected. Tissue composition for muscle, bone, fat and other components (tendons and noticeable blood vessels) were estimated from the rib joint according to the method described by Robelin & Geay (1975). Results are expressed as percentage of the entire rib weight.

Statistical analysis was performed using SPSS 11.0 software. An ANOVA procedure was carried out with breed as an unique effect.

Results and discussion

Global results are shown in Table 1.

Breed was a very important factor determining carcass quality, especially in carcass blockiness index (F=96.7), carcass weight (F=96.5), dressing percentage (F= 93.2), fatness score (F= 86.6) and limb length (F=84.8). Carcass weights ranged from 189.9 kg for Jersey to 386.6 kg for Charolais. In general, dairy breeds, such as Jersey, Holstein or Danish Cattle, or local breeds, such as Highland or Asturiana de la Montaña, produced small carcasses, while specialised beef breeds, as Charolais or Pirenaica produced higher carcass weights. Similarly, dressing percentage was lowest for Jersey (46.6%) while five specialised beef breeds (Charolais, Pirenaica, Asturiana de los Valles, Piemontese and Limousin) had values up to 60%. The breed purpose was also reflected in conformation scores, the highest score was for Piemontese (14.6) and the lowest for Jersey (4.4). On the other hand, dairy and local breeds showed the highest values for kidney fat weight, which was maximum for Highland (9.2 kg) and minimum for both Italian breeds (less than 1.5 kg). Nevertheless, fatness scores did not follow this behaviour, since Charolais or Limousin had the same fatness scores as some dairy or double purpose breeds such as Simmental, Holstein or Red Danish Cattle (about 8 points). The breed with the highest fatness score was the Aberdeen Angus (11.7) and at the lowest was Piemontese (3.6), indicating that fat deposition occurs in different ways in different breeds (Robelin, 1986, Micol *et al.,* 1993).

Biometric measurements on the carcass also showed significant differences among breeds, especially for limb length, which varied from 64.4 cm in Highland to 86.3 cm in Holstein, and for internal depth of breast, which varied from 33.3 cm in Asturiana de los Valles to 43.3 cm in Holstein. There were also, significant differences in the limb index ($F=30.9$; $p<0.001$) and in the carcass blockiness index ($F=96.7$; $p<0.001$). The loin area was greatest for Asturiana de los Valles breed (52.7 cm^2), followed by Piemontese (52.2 cm^2), while Holstein presented the lowest value (35.0 cm^2). These differences in the loin area were mainly due to differences in the dorso-ventral diameter, rather than in the medium-lateral dimension. Data for British breeds were not available.

From the dissection of the 6^{th} rib it can be seen that different breeds show important differences in muscle (ranged between 58.1% in Holstein and 79.9% in Piemontese breeds) and fat percentages (ranged between 3.2% in Piemontese and 23.0 % in Angus animals). These findings are in accordance with the carcass

measurements and conformation scores. The differences found in bone plus others were significant, but lower (F= 18.5) than those for fat and muscle (F= 52.4 and 56.9 respectively) thus, bone plus others ranged between 15.0% in Limousin and 23.1% in Holstein breeds. These results agree with the more precocious development of the bone tissue and of the "other" components, and subsequent their higher bio-stability, compared to fat and muscle tissues.

Conclusions

We can conclude that breed is an important factor in determining carcass quality. The information that is being generated by this project will be important for devising breeding strategies to meet the demands of the market.

Acknowledgements

Authors are grateful for the financial support to the EU (QLK5-CT-2000-00147) and for their technical assistance to the Breeders Associations and the rest of people of the different groups.

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Table 1. Carcass quality traits in 15 European cattle breeds. Means, standard deviation and F for breed as fix effect

	JER	SOD	AA	HIG	HOLS	RED	SIMM	ASV	ASM	AV-NI	PI	PIE	MARC	LIM	CHAR	F
n	*	*	*	*	13	*	3	30	31	30	31	30	28	31	30	(p)
Carcass weight (kg)	189.81 (11.55) [25]	347.24 (14.16) [11]	350.74 (15.80) [17]	248.64 (11.55) [20]	322.15 (29.22)	317.14 (18.26) [7]	345.00 (19.70)	348.74 (34.43)	244.69 (22.94)	324.70 (49.16)	371.45 (30.14)	335.86 (28.59)	307.52 (23.24)	360.68 (20.85)	386.64 (27.42)	96.39 ***
Dressing percentage	46.59 (3.02) [25]	57.62 (2.62) [11]	57.31 (1.7) [17]	55.31 (1.55) [20]	55.54 (1.83)	55.78 (1.29) [7]	57.36 (1.07)	63.57 (3.21)	57.50 (1.89)	59.54 (2.03)	63.06 (2.31)	63.67 (1.81)	58.75 (1.84)	64.61 (0.98)	61.90 (1.65)	93.16 ***
Kidney fat weight (kg)	7.67 [23] (2.27)	7.64 [8] (3.21)	2.29 [16] (0.63)	9.25 [20] (2.42)	5.52 (1.77)	3.57 [7] (0.72)	2.15 (0.40)	2.32 (1.47)	3.86 (1.28)	3.85 (1.18)	3.24 (1.79)	0.77 (0.28)	1.36 (0.49)	5.09 (1.53)	5.84 (1.24)	56.74 ***
Conformation score (1-18)	4.36 [22] (1.26)	10.43 [7] (1.40)	10.53 [17] (2.00)	8.33 [3] (0.58)	4.92 (0.76)	5.00 [7] (0.58)	7.33 (1.15)	12.10 (2.80)	7.58 (1.03)	8.00 (1.23)	11.48 (1.31)	14.57 (1.28)	11.11 (1.31)	10.20 (1.16)	9.83 (1.23)	77.62 ***
Fatness score (1-15)	4.55 [22] (1.10)	7.29 [7] (2.69)	11.71 [17] (1.96)	6.67 [3] (1.15)	8.23 (.83)	8.43 [7] (1.13)	8.00 (0.00)	4.07 (1.53)	5.90 (1.16)	5.77 (1.17)	4.94 (0.57)	3.60 (0.62)	4.96 (0.96)	8.37 (0.56)	8.87 (0.61)	86.22 ***
Carcass length (cm)	123.14 [24] (16.67)	132.73 [11] (3.51)	131.44 [17] (5.93)	120.03 [13] (4.49)	136.12 (3.91)	131.00 [7] (3.42)	133.00 (6.56)	127.13 (4.20)	122.35 (7.50)	133.07 (6.45)	132.42 (4.87)	123.33 (3.26)	123.87 (3.50)	126.66 (2.74)	133.02 (2.94)	12.43 ***
Internal depth of breast (cm)	39.27 [24] (1.47)	35.94 [11] (2.90)	41.09 [17] (1.57)	37.42 [13] (2.37)	43.31 (1.79)	42.57 [7] (0.93)	43.00 (2.00)	33.35 (1.82)	33.81 (1.91)	37.03 (1.92)	34.68 (2.04)	37.32 (1.85)	39.61 (3.53)	34.69 (1.82)	34.43 (1.62)	44.76 ***
Limb length (cm)	67.27 [24] (1.36)	70.64 [11] (2.52)	69.79 [17] (2.28)	64.38 [13] (2.30)	86.27 (2.31)	81.86 [7] (3.72)	82.67 (2.52)	81.02 (3.27)	75.31 (2.79)	83.35 (3.23)	82.11 (2.99)	69.00 (7.86)	72.79 (2.36)	81.56 (1.62)	83.16 (1.87)	84.75 ***
Limb thickness (cm)	-	-	-	-	26.54 (1.03)	25.93 [7] (0.67)	28.00 (0.87)	30.22 (2.62)	25.73 (2.43)	27.77 (2.38)	31.05 (1.19)	33.88 (2.55)	31.13 (1.06)	31.57 (0.79)	31.81 (1.12)	48.92 ***
Blockiness index (kg/cm)	1.56 [24] (0.16)	2.62 [11] (0.10)	2.68 [17] (0.22)	2.11 [13] (0.15)	2.36 (0.17)	2.42 [7] (0.10)	2.60 (0.18)	2.74 (0.24)	2.00 (0.17)	2.43 (0.27)	2.80 (0.19)	2.72 (0.18)	2.48 (0.14)	2.85 (0.14)	2.91 (0.18)	96.69 ***
Limb index (cm/cm)	-	-	-	-	0.31 (0.01)	0.32 [7] (0.02)	0.34 (0.02)	0.37 (0.03)	0.34 (0.03)	0.33 (0.03)	0.38 (0.02)	0.50 (0.13)	0.43 (0.02)	0.39 (0.01)	0.38 (0.01)	30.87 ***
Maximum diameter of the LT at 6th rib level (cm)	-	-	-	-	9.51 (0.76)	9.99 [7] (0.80)	9.77 (0.40)	9.31 (0.91)	8.57 (0.59)	8.99 (1.15)	9.23 (0.79)	10.33 (0.77)	10.24 (0.49)	9.63 (0.59)	10.24 (0.88)	14.39 ***
Minimum diameter of the LT muscle at 6th rib level (cm)	-	-	-	-	4.46 (0.46)	4.53 [7] (0.48)	4.80 (0.52)	6.73 (0.81)	5.19 (0.65)	5.31 (0.85)	6.19 (0.66)	6.10 (0.86)	5.53 (0.84)	6.07 (0.50)	5.82 (0.78)	17.25 ***
LT area at the 6th rib level (cm^2)	-	-	-	-	35.03 (5.31)	36.58 [7] (7.66)	38.67 (2.07)	52.71 (8.25)	35.85 (5.78)	40.31 (6.65)	48.68 (5.41)	52.17 (6.79)	47.83 (5.35)	44.86 (4.12)	43.91 (6.77)	23.65 ***
Fat percentage (6th rib dissection)	14.84 [2] (1.02)	16.30 [4] (6.35)	22.98 [16] (4.26)	17.92 [3] (2.13)	17.06 (4.22)	18.45 [3] (2.72)	-	7.77 (4.56)	14.75 (3.67)	12.63 (2.43)	9.67 (2.83)	3.23 (1.09)	8.94 (1.90)	13.20 (2.33)	15.43 (2.68)	52.43 ***
Muscle percentage (6th rib dissection)	67.09 [2] (1.53)	66.80 [4] (3.95)	60.40 [16] (3.08)	64.22 [3] (1.93)	58.13 (3.69)	58.73 [3] (3.43)	-	75.04 (5.74)	66.31 (3.30)	69.05 (2.59)	72.87 (3.24)	79.91 (2.12)	70.07 (2.42)	71.82 (2.87)	67.69 (3.55)	56.97 ***
Bone and others percentage (6th rib dissection)	18.07 [2] (0.51)	16.90 [4] (3.31)	16.62 [16] (2.67)	17.50 [3] (0.39)	23.06 (1.92)	22.02 [3] (2.02)	-	17.19 (2.61)	18.94 (2.15)	18.32 (2.47)	17.46 (1.74)	16.86 (1.90)	20.99 (2.03)	14.99 (1.58)	16.88 (1.89)	18.52 ***

* *n varies in function of the considered variable because technical causes. Superscript indicates the n in each case.*

JER - *Jersey; SOD - South Devon; AA - Aberdeen Angus; HIG - Highland; HOLS - Holstein; RED - Danish Red Cattle; SIMM - Simmental; ASV - Asturiana de los Valles; ASM - Asturiana de la Montaña; AV-NI - Avileña-Negra Ibérica; PIE - Piemontese; MARC - Marchigiana; LIM - Limousin; CHAR - Charolais.*

EUROPEAN CONSUMER ACCEPTABILITY OF LAMB MEAT FROM DIFFERENT ORIGINS AND PRODUCTION SYSTEMS

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Background

The international market for lamb meat tends to increase the commercial exchanges between geographical regions and countries. Europe is the greatest importer of lamb meat (Sañudo *et al*., 1998a) with almost 50% of the world market. In this sense, South American countries are looking for new markets for exporting their red meat products. These products must compete with those produced locally. At the same time, each country or region in Europe produces a specific type of lamb according to its production system characteristics (breeds, environments, infrastructures, feeding resources, etc.). The particular characteristics of these systems determine the type of product that is commercialised in each region, according to liking, preferences and cooking habits of the consumers (Hernando *et al*., 1996). Mediterranean countries, in general, produce lamb with lower carcass weights, while Central-Northern European countries produce animals with higher carcass weights. All these products are clearly appreciated in their own regions and producing countries, but they may not be appreciated so much in the overseas markets by potential consumers. According to Jeremiah (1988), there are some reasons to believe that significant differences may exist in the palatability attributes of lamb from different geographical sources due to differences in genetics, nutrition, slaughter weight and age and the extent of post-mortem ageing.

Objectives

The main objective of this study was to evaluate Spanish, German and British consumers' acceptability of lamb meat sourced from Uruguay and compared with their locally produced lamb at two different ageing periods.

Materials and methods

Consumers

Consumer tests were conducted in Spain, Germany and United Kingdom. In each of these three countries, two hundred consumers (in two different places of 100 consumers each) evaluated 4 different lamb meat samples: 2 local and 2 from Uruguay. Overall consumers were stratified in terms of sex, age and education level. Gender classification was almost equal (male-51%, female-49%), with smaller differences within each country. On the other hand, consumers were mostly concentrated on the two medium ranges of age (26-40 and 41-60 years of age, 32% and 39 % respectively), with an important variation in the extreme groups in each country; in average 17% (18-25 years of age) and 12% (61-75 years of age). The education level of the sampled population was in general very high (56% of people with University or college education level), 26% was located at the school to age 16 and 18% was at the school to age 18.

Animals and meat samples

Uruguayan lambs comprised light and heavy Corriedale lambs (11.1±1.4 kg and 19.6±2.2 kg carcass weight, respectively) reared on a pure extensive improved sward system and the meat aged for 20 days. In the case of the European lambs only one type by country was used, representative of each country where the comparative consumer tests were performed, aged for either 7 or 20 days. Samples taken from United Kingdom represented a common commercial lamb type (22.8±1.7 kg carcass weight) reared on grass-based

system with strategic use of concentrate. German samples were taken from crossbreed between Suffolk or Schwarzköpfe x Merino Landschaf (23.2±3.65 kg carcass weight) reared on grass complemented with concentrates. In Spain, lambs came from the Rasa Aragonesa breed, produced under an intensive system using concentrates and cereal straw *ad libitum* (10.2±0.6 kg carcass weight).

All meat samples were taken from the loin (*M. longissimus lumborum*). After thawing, they were sliced into 2 cm-thick steaks and grilled until the internal temperature reached 72ºC. Consumers evaluated tenderness, flavour and overall acceptability on 8-points category scales.

Statistics

Consumer test results were separately analysed for each country. The analysis of variance was performed using the GLM procedure of SAS for Windows version 8.1 (SAS, 2000). Lamb type was included as a fixed effect and consumer as a random effect. The session effect had been previously corrected.

Results and discussion

Tables 1, 2 and 3 show the results of the consumer tests carried out in Spain, United Kingdom and Germany, respectively.

Table 1. - Tenderness, flavour and overall acceptability of lamb by Spanish consumers. Least Square Means and Root MSE.

Lamb type	Tenderness*	Flavour*	Overall acceptability*
Light Uruguay	6.1	5.7 ab	5.8
Heavy Uruguay	6.2	5.5 bc	5.6
Spain 7d ageing	6.3	5.8 a	5.8
Spain 20d ageing	6.3	5.4 c	5.6
RMSE	1.28	1.56	1.44

a, b, c: LSMeans with different letters within tenderness, flavour and overall acceptability are statistically different (P<0.05). *: from 1, very tough and extremely disliked flavour and acceptability, to 8, very tender and extremely liked flavour and acceptability.

In Table 1, it can be observed that Spanish consumers preferred the flavour of the Spanish lamb meat aged for 7 days and of the Uruguayan light lamb (P<0.05), without differences in tenderness and overall acceptability (P>0.05). This could partially be explained by the similarity of live weight and age between the Spanish and the Uruguayan light lamb, even though they came from very different production systems.

German consumers (Table 2) gave higher ratings of tenderness to the Uruguayan heavy lamb and the German lamb aged for 20 days, with higher flavour ratings to both German lamb samples, and the highest overall acceptability given to the German lamb type aged for 20 days (P<0.05). At the same time, the flavour and overall acceptability of Uruguayan light lambs were the least preferred (P<0.05). In general, it seems clear that German consumers preferred their own products and those that have some similar characteristics to them (Uruguayan heavy lamb), even being consumers without or with a very small experience in lamb meat consumption. They rejected meat from Uruguayan light lamb, which was the only unweaned type. Thus, Kemp *et al*. (1981) found that differences in flavour between two groups of lambs could be explained by differences in feeding regimens when comparing lambs fed exclusively with maternal milk versus lambs supplemented with commercial pellets and hay *ad libitum*.

Table 2. - Tenderness, flavour and overall acceptability of lamb by German consumers. Least Square Means and Root MSE.

Lamb type	Tenderness*	Flavour*	Overall Acceptability*
Light Uruguay	6.1 b	5.7 c	5.7 c
Heavy Uruguay	6.6 a	6.0 b	6.1 b
German 7d ageing	6.2 b	6.1 ab	6.1 b
German 20d ageing	6.7 a	6.3 a	6.4 a
RMSE	1.14	1.18	1.07

a, b, c: LSMeans with different letters within tenderness, flavour and overall acceptability are statistically different (P<0.05). *: from 1, very tough and extremely disliked flavour and acceptability, to 8, very tender and extremely liked flavour and acceptability.

For the UK consumers (Table 3), flavour and overall acceptability of the British lamb aged for either 7 or 20 days and of the Uruguayan heavy lamb were similar, and they obtained the higher ratings(P<0.05). The highest tenderness ratings were for the Uruguayan heavy lamb and British lamb aged for 7 or 20 days (P<0.05), while the lowest ratings were given to the Uruguayan light lamb (P<0.05). In general it can be suggested that British consumers were able to recognise their own products (grass fed) for lamb meat flavour and overall acceptability. These results were probably due to the strong influence that age (older animals) and production and feeding systems has on flavour, and the relationship between flavour and consumer acceptability. In the same way, Sañudo *et al*. (1989), working with lambs of different origins (Spanish light lamb, New Zealand and French light lambs) tested in Spain, found that New Zealand lamb meat obtained the highest values for flavour intensity and the lowest ratings for overall acceptability. Crouse *et al.* (1983) and Solomon (1980) reported similar results.

Table 3. - Tenderness, flavour and overall acceptability of lamb by British consumers. Least Square Means and Root MSE.

Lamb type	Tenderness*	Flavour*	Overall Acceptability*
Light Uruguay	5.6 c	5.4 b	5.3 b
Heavy Uruguay	6.9 a	6.1 a	6.3 a
UK 7d ageing	6.5 b	6.1 a	6.2 a
UK 20d ageing	6.7 ab	6.2 a	6.3 a
RMSE	1.28	1.42	1.35

a, b, c: LSMeans with different letters within tenderness, flavour and overall acceptability are statistically different (P<0.05). *: from 1, very tough and extremely disliked flavour and acceptability, to 8, very tender and extremely liked flavour and acceptability.

In general, these findings are probably related to the consumption habits of the different consumers evaluated, which determine a lower acceptance of unfamiliar products. This can be clearly seen when Uruguayan light lamb was compared to German and British lamb types or when Uruguayan heavy lamb was compared to Spanish lamb samples. In a previous report using British and Spanish lamb carcasses, Sañudo *et al.* (1998b) showed that both British and Spanish panels found the odour and flavour intensity higher in the British carcasses and juiciness higher in the Spanish lamb types. However, the panels differed in their ratings of flavour quality and overall appraisal. The British panel preferred the flavour and overall liking of British lamb, whereas the Spanish panel preferred the flavour and overall appreciation of Spanish lamb.

Conclusions

Lamb meat acceptability depends on the consumption habits of the consumers, at least in countries with relatively high lamb consumption such as Spain and United Kingdom. Also, lamb meat tenderness was improved by ageing period, although only significantly in Germany, and, in general, more tender lamb meat was associated with higher acceptability at longer ageing times (20 days), especially in the older and heavier lamb types.

Overall acceptability seems to be better related to flavour than tenderness scoring, and this is in agreement with previous European studies (Dransfield *et al*., 1984) that showed increased focus on flavour and overall acceptability when all the meat being compared was tender.

Acknowledgements

We thank to the AECI, INIA-Spain and INIA-Uruguay for their financial support, to the rest of the people of the different groups and to the IGER (Wales, U.K.) for their technical support

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THE RELATIONSHIP BETWEEN CIE L* AND PH AT 1 DAY POSTMORTEM IN PORCINE SEMIMEMBRANOSUS MUSCLES HARVESTED FROM NATIONAL PORK DEVELOPMENT HOGS

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Background

Pork quality is defined by CIE L* (lightness), ultimate muscle pH, and drip loss in Kauffmann et al. (1992). Pale, Soft, and Exudative (PSE) pork is a quality classification characterized as being very light colored, soft, and watery. Such meat is classified as low quality and undesirable to consumers due to its poor appearance, texture, and palatability (Pearson and Gillett, 1996). PSE meat also exhibits poor water holding capacity and texture in processed products (Solomon et al. 1998). PSE meat originates from a rapid decline in pH postmortem (45 min) at high muscle temperature (Briskey and Wismer-Pedersen, 1961). PSE meat was identified (Kauffmann et al, 1992) by ultimate pH< 5.6, CIE L* Value > 50, and drip loss > 5%, and red, firm, and non-exudative (RFN) or normal pork was classified as ultimate pH=5.6-5.9, 42<CIE L*< 50, and drip loss < 5 %. It is not guaranteed that a sample with a CIE L* value > 50 is PSE. However, in theory, the above-listed classifications would allow for two possible relationships between CIE L* value and ultimate pH. There would be either a strong linear relationship between CIE L* value and pH over the whole range of values or samples with a pH greater than 5.6 would generally have a CIE L* value greater than 50 and samples with a pH less than 5.6 would have a CIE L* value less than 50. For either example, it may be possible to utilize ultimate pH to predict with accuracy if a porcine *semimembranosus* muscle CIE L* value is greater than or less than 50. Unpublished data from the industry indicates that there may not be a clear relationship between ultimate pH and CIE L* in pork. This research was performed to first test the hypothesis that there is a relationship between CIE L* and ultimate pH for national pork development hogs and then to characterize that relationship if it exists.

Objectives

The main objective of this research is to demonstrate if there is a relationship between ultimate pH and lightness of porcine *semimembranosus* muscles in National Pork Development Hogs. A second objective is to utilize logistic regression to predict if the CIE L* value is greater or less than 50 based on ultimate pH.

Materials and Methods

Porcine *semimembranosus* and *adductor* muscles (n=384) were obtained from a pork processing plant in Virginia (United States) on 15 separate occasions to obtain a good representation of sample color. Samples were selected based on visual color as an attempt to obtain half of the samples as pale and half as normal. All samples were taken from National Pork Development (NPD) pork carcasses produced from market age pigs that weighed 110-125 kg. CIE L* values were measured using a chroma meter (Model CR-200, Minolta Camera Co., Ltd., Osaka, Japan) at three similar anatomical locations on each muscle. The chroma meter was calibrated using a standard Minolta calibration plate (white plate, No. 20933026; CIE L* 97.91, a* -0.70, b* +2.44) each time prior to testing. The pH of each *semimembranosus/adductor* muscle was also taken in triplicate by removing three 2-g samples from the three same similar anatomical locations that color was measured and homogenized (Virtishear Model.225318, The Virtis Company, Inc., Gardener, NY) for 10-20 s (3 short bursts) in 20 ml of distilled deionized water. pH was measured for the individual samples with a calibrated pH meter (Model AR25, Fisher Scientific, Pittsburgh, PA).

Statistical Analysis

The correlation coefficient was determined for the relationship between CIE L* and pH for all samples, those with CIE L* values less than 50, and CIE L* values greater than 50 (SAS 8.2, 2001). Logistic regression

was utilized to determine how well ultimate pH would predict if a sample had a CIE L* > 50 or a CIE L* value <50 (SAS 8.2, 2001).

Results and Discussion

There is a strong negative correlation (r= - 0.83) between ultimate pH and CIE L* values for porcine semimembranosus muscles (Figure 1). This relationship is accurate, but is also misleading. Separation of the data into two categories including ultimate pH>5.6 and ultimate pH<5.6, established a correlation of -0.45 and -0.45, respectively (Figures 2 and 3). This drastic decrease reveals that the relationship between CIE L* and ultimate pH is misleading. Furthermore, through the removal of outliers from the data, the correlation decreases from -0.45 to -0.40 for pH below 5.6 and from -0.45 to -0.046 for samples above a pH of 5.6. These results demonstrate that there is not a linear relationship between the data and that logistic regression may be appropriate to determine the relationship between a samples ultimate pH and CIE L* value.

The logistic regression analysis demonstrated that ultimate pH is effective ($p<0.05$) in determining whether the CIE L* value will be either greater or less than 50. The logistic regression model predicted that if the pH was less than 5.6, there is a 0.962 probability that the CIE L* would be above 50. At a pH above 5.6, the predicted probability that the CIE L* value is below 50 was 0.905. This research reveals that ultimate pH is a good predictor for whether CIE L* will be above or below 50. The relationship between these two variables is that of a categorical distribution where the probability that a sample would have a CIE L* value greater or less than 50 can be predicted based on pH value. Furthermore, predictions of whether CIE L * is greater or less than 50 can be predicted at any pH (Figure 4). Predictions can actually be made for any set of categories that are desired. Figure 4 reveals that as the ultimate pH approaches 5.4 and 5.9, the probability that a sample has a CIE L * value less or greater than 50 approaches 1. Therefore, from 5.9-7.0, the probability that the CIE L* value will be below 50 is 1, and the predicted probability that any sample with a pH below 5.4 having a CIE L* value is 1. At pH values of 5.6 and 5.7, the probabilities that a sample will be above 50 in CIE L* is 0.81 and 0.39, respectively (Figure 4). This evaluation reveals that there is a higher probability that a sample is pale than normal in this pH range. Therefore, ultimate pH is a useful indicator of product paleness when pH is higher than 5.8 and less than 5.5, but is ineffective in classifying porcine semimembranosus color from NPD hogs when the pH is between 5.5 and 5.8.

Conclusions

The ultimate pH of porcine *semimembranosus* muscles correlates well with CIE L* values. CIE L* values can be explained by ultimate pH when CIE L* values are divided into a categorical variable. Logistic regression is a useful tool in explaining the relationship between ultimate pH and CIE L* values.

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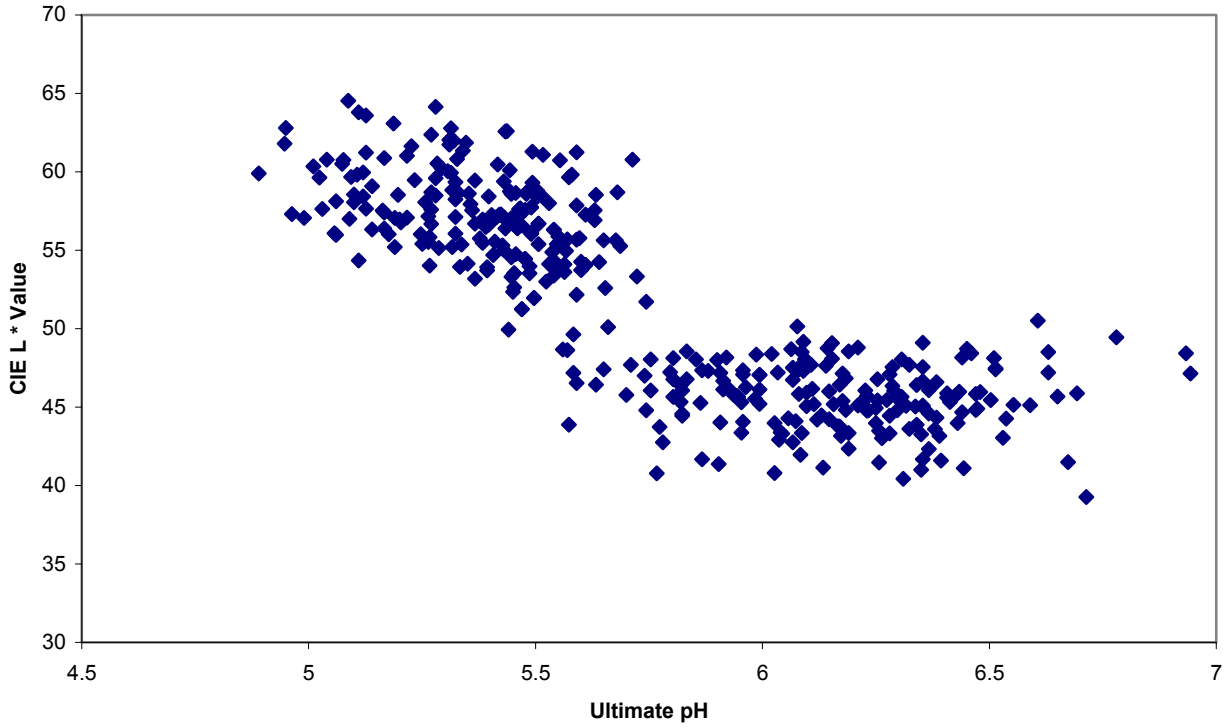

Figure 1: The Relationship Between ultimate pH and CIE L* value of porcine semimembranosus muscles (n=384) of National Pork Development (NPD) hogs.

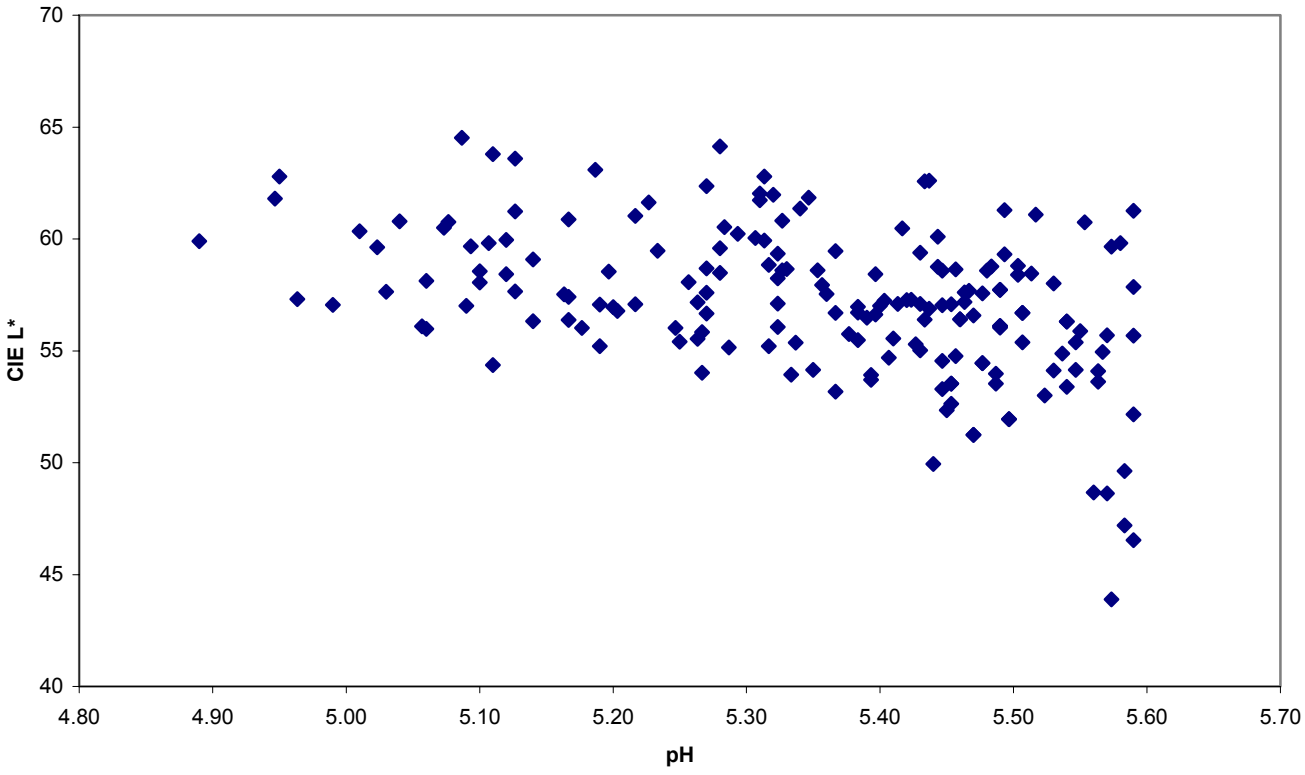

Figure 2: The relationship between ultimate pH and CIE L* for semimembranosus muscles (pH<5.6, n=184) from National Prk Development Hogs

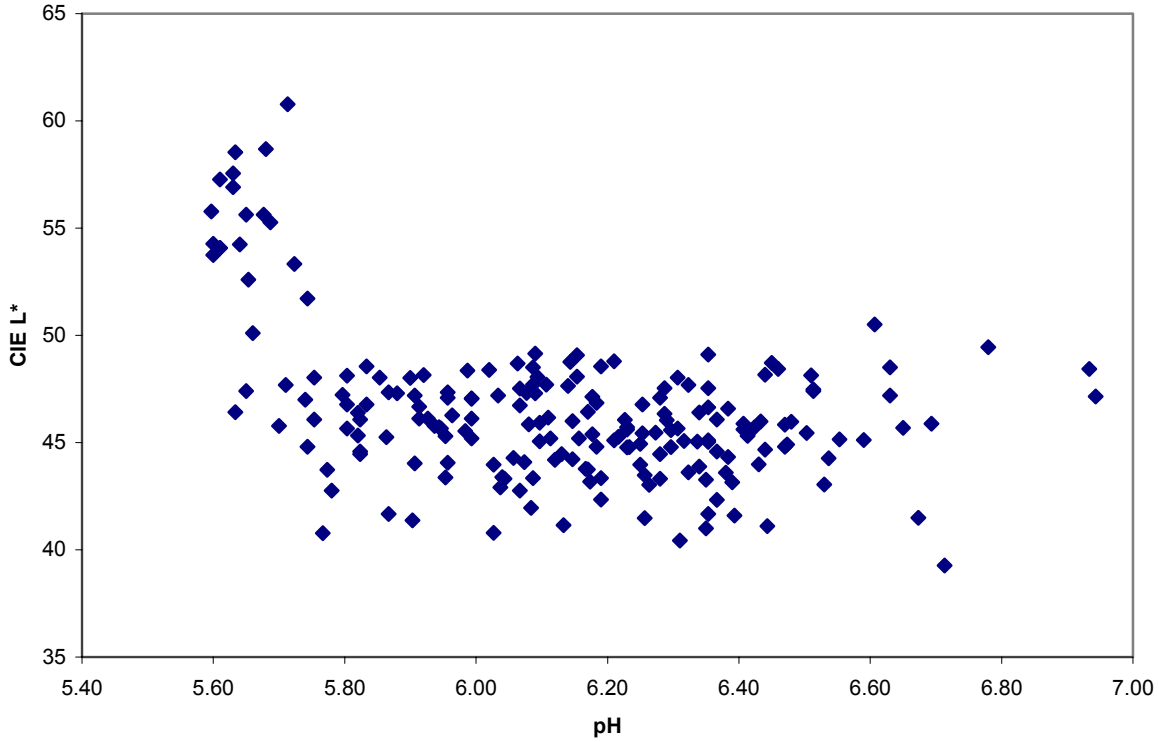

Figure 3: The relationship between ultimate pH and CIE L* for semimembranosus muscles (pH>5.6, n=200) from National Pork Development Hogs

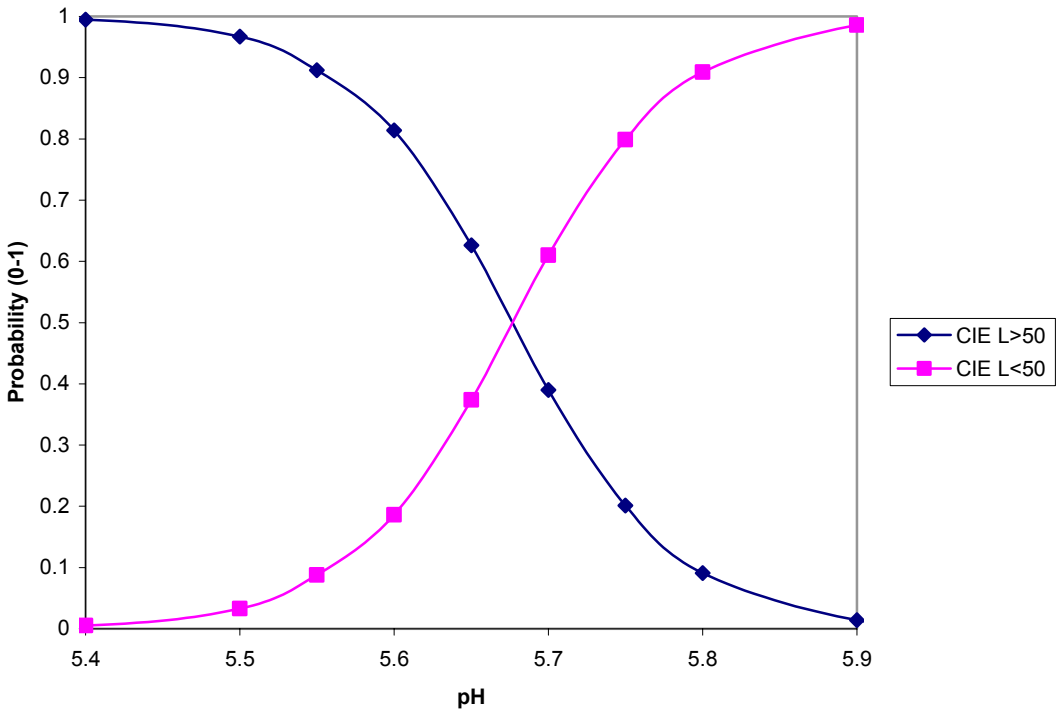

Figure 4: The Predicted probability that a porcine *semimembranosus* muscle from a National Pork Development (NPD) hog with a certain ultimate pH will have a CIE L* less than or greater than 50.

THE EFFECTS OF RUMINALLY-PROTECTED DIETARY LIPID ON THE FATTY ACID COMPOSITION AND QUALITY OF BEEF MUSCLE

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Background

Improving the nutritional value of beef by reducing saturated fat and increasing the content of polyunsaturated fatty acids (PUFA) is an important research target. Rumen biohydrogenation of dietary PUFA limits the ability to manipulate the ratio of polyunsaturated:saturated fatty acids (P:S), which for beef is typically 0.06 – 0.1 (Wood *et al*., 2003). However, feeding a ruminally-protected, PUFA-rich, lipid supplement produced from soya beans, linseed and sunflower oils mixed to give a 2.4:1 ratio of 18:2*n*-6:18:3*n*-3 resulted in major improvements in the P:S ratio (Scollan *et al*., 2003) with only small associated changes in the colour shelf life and sensory attributes of the meat (Enser *et al*., 2001). However, in that study, the protected lipid supplement (PLS, with an 18:2*n*-6:18:3*n*-3 ratio of 2:4:1) was less effective in increasing the deposition of *n*-3 relative to *n*-6 PUFA, resulting in a less favourable *n*-6:*n*-3 ratio in beef longissimus muscle. It is hypothesised that the ratio of *n*-6:*n*-3 PUFA in the lipid supplement has a large impact on the deposition of *n*-6 relative to *n*-3 PUFA in muscle.

Objectives

To determine the effects of including a ruminally protected lipid supplement, in the diet of beef cattle, with an 18:2*n*-6:18:3*n*-3 ratio of 1:1 on the fatty acid composition of muscle neutral and phospholipids and to relate this to meat shelf-life (colour and lipid oxidation) and flavour.

Materials and methods

Thirty two Charolais steers (initial live weight 507 (s.e. 10.3) kg) were fed on *ad libitum* grass silage plus one of four concentrates in which the lipid source was either Megalac (Mega, rich in palmitic acid; 16:0) or PLS (soya beans, linseed and sunflower oils resulting in a 1:1 ratio of 18:2*n*-6:18:3*n*-3): Concentrate 1, (Mega, control) contained 139g/kg Mega; Concentrate 2, (PLS1) contained 67g/kg Mega with 400 g/d PLS fed separately; Concentrate 3, (PLS2) contained 24g/kg Mega with 800 g/d PLS fed separately, Concentrate 4, (PLS3) contained no Mega and 1000 g/d PLS fed separately. Supra-nutritional levels of vitamin E were included in all diets (350 IU/kg concentrate). At 48h post-mortem, samples of m. *longissimus thoracis* at the 11th rib were removed and blast frozen for fatty acid analysis. Other samples of m. *longissimus lumborum* were removed and conditioned for 10 days in vacuum packs at 1ºC, then a joint was frozen for subsequent organoleptic assessment and steaks cut and packed in a modified atmosphere (O_2:CO_2, 75:25). These were displayed for 10 days at 4ºC under 700 lux for 16h out of each 24h to simulate commercial retail display. Colour was measured daily using a Minolta Chroma Meter. Lipid oxidation was determined as thiobarbituric acid reacting substances (TBARS) (Tarladgis *et al*., 1960) after 10d display. After thawing, steaks were cut from the frozen joint, grilled to an internal temperature of 74ºC and sensory assessments made by a 10 member trained taste panel using 100mm unstructured line scales. Lipid was extracted using chloroform/methanol and the neutral and polar lipids separated by silicic acid column chromatography. Fatty acid methyl esters were prepared by alkaline hydrolysis followed by methylation with diazomethane and analysed on a CP Sil 88, 100m x 0.25mm ID column (Chrompack, UK).

Results and discussion

Total lipid, total neutral lipid and total phospholipid fatty acids were not influenced by diet (Tables 1, 2 and 3). The content of the main saturated fatty acids (14:0, 16:0, 18:0) were not different in either lipid fraction, but 18:1*n*-9 was reduced in the neutral lipid (Table 1). On average, feeding PLS increased the content of

18:2*n*-6 and 18:3*n*-3 by a factor of 3.2 and 5.9, respectively in the neutral lipid. In phospholipid, on average, PLS increased the content of 18:2n-6 and 18:3n-3 by 1.86 and 2.31, respectively. Interestingly, in contrast to the neutral lipid, no further increases in either 18:2*n*-6 or 18:3*n*-3 were noted in phospholipid after the first increment of PLS (Table 2). These increases in C18 PUFA in phospholipid were associated with reductions in oleic acid, 18:1*n*-9 and DPA, 22:5*n*-3. The percentage of 18:2*n*-6 and 18:3*n*-3 in muscle total lipid on the highest level, PLS3, was 3.2 and 5.9%. This compares with 9.3 and 1.9% for 18:2n-6 and 18:3n-3 at the highest inclusion of a PLS (with a 2.4:1 18:2n-6 and 18:3n-3) in the study by Scollan *et al*. (2003). The P:S ratio increased markedly ($P < 0.001$) while the *n*-6:*n*-3 ratio was decreased ($P < 0.001$; Table 3).

The increase in PUFA in the meat, with increasing PLS fed, was associated with increased susceptibility to lipid oxidation as reflected in higher TBARS (Table 3) and colour deterioration (Figure 1). Colour acceptability (saturation index > 18) was decreased by approximately 2 days for PLS3 relative to Mega. The high levels of vitamin E fed to counteract the effects of increasing PUFA in the tissue were not sufficient to negate the negative effects on colour shelf life or lipid oxidation. There was a trend for the tenderness of meat samples to increase with an increase in the amount of protected lipid fed (Table 4). PLS2 and PLS3 samples were significantly juicier than samples from PLS1. There was a trend for beefy flavours to decline with increased supplementation. However, abnormal flavour increased significantly with increasing PLS. This was associated with a trend for greasy to increase, a significant rise in rancid and a trend for vegetable/grassy and dairy notes to increase. These trends in abnormal flavours resulted in a significant reduction in overall liking for PLS2 and PLS3 compared to PLS1 and control.

The fatty acid changes had marked effects on lipid oxidation, colour shelf life and beef flavour. These results provide very clear evidence that these characteristics are inter-related. Increasing concentrations of PUFA in meat to the extent seen here would therefore not be a commercial proposition without further antioxidant protection. This would include careful meat processing procedures as anything that increases oxidative susceptibility such as mincing or cooking would further increase lipid oxidation.

Conclusions

The protected lipid supplement rich in 18:2*n*-6 and 18:3*n*-3 resulted in large increases in these fatty acids in beef muscle. This resulted in a favourable increase in the P:S ratio and a beneficially lower *n*-6:*n*-3 ratio than that observed in our previous study (Scollan *et al*., 2003). The changes in fatty acid content of the meat had marked effects on lipid oxidation, colour shelf life and beef flavour. The high levels of vitamin E fed were insufficient to significantly reduce the negative effects of increasing PUFA in the meat on lipid stability and colour shelf life. There is a need to determine the role of antioxidants in enhancing the stability of meat which has enriched levels of PUFA.

Acknowledgments

This work was supported by Department for Environment Food and Rural Affairs and European Commission (HealthyBeef QLRT-2000-31423).

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Table 1. Fatty acid content (mg/100g muscle) of neutral lipid fraction of *M. longissimus thoracis*

	Mega	**PLS1**	**PLS2**	**PLS3**	**s.e.d.**	**P**
14:0 myristic	123.9	121.6	119.2	114.0	23.70	NS
16:0 palmitic	1216	1215	1155	1097	199.7	NS
18:0 stearic	661	666	712	697	112.0	NS
18:1 *trans*	102	106	109	128	18.1	NS
18:1n-9 oleic	1521	1595	1559	1563	269.0	NS
18:1 cis vaccenic	56	58	58	58	9.6	NS
18:2 n-6 linoleic	42^{a}	109^{b}	135^{b}	158^{b}	18.7	0.001
18:3 n-3 α-linolenic	15^{a}	69^{b}	88^{bc}	110^{c}	12.5	0.001
CLA cis-9, trans-11 C18:2	18.7	22.8	23.0	29.8	4.98	NS
Total fatty acids	4137	4370	4352	4370	715	NS

Table 2. Fatty acid content (mg/100g muscle) of phospholipid fraction of *M. longissimus thoracis*

	Mega	**PLS1**	**PLS2**	**PLS3**	**s.e.d.**	**P**
14:0 myristic	2.36	3.57	2.52	2.52	0.971	NS
16:0 palmitic	89	97	83	78	8.7	NS
18:0 stearic	56.4	64.8	60.8	61.5	4.51	NS
18:1 *trans*	3.7	4.6	4.2	4.4	0.55	NS
18:1n-9 oleic	123^{c}	74^{b}	51^{ab}	49^{a}	12.0	0.001
18:1 cis vaccenic	11.9^{b}	11.0^{b}	8.2^{a}	8.6^{a}	1.02	0.002
18:2 n-6 linoleic	78.9^{a}	146.7^{b}	144.4^{b}	146.9^{b}	9.46	0.001
18:3 n-3 α-linolenic	13.1^{a}	32.7^{b}	29.6^{b}	28.5^{b}	2.22	0.001
CLA cis-9, trans-11 C18:2	1.23	1.35	1.16	1.17	0.187	NS
20:4 n-6 arachidonic	26.9	27.5	24.5	24.5	1.97	NS
20:5 n-3 eicosapentaenoic (EPA)	12.4	14.8	13.5	14.8	1.10	NS
22:5 n-3 docosapentaenoic (DPA)	20.9^{b}	20.9^{b}	17.4^{a}	16.5^{a}	1.49	0.009
22:6 n-3 docosahexaenoic (DHA)	1.89	1.81	1.53	1.56	0.272	NS
Total fatty acids	548	606	528	525	42.0	NS

Table 3. Total fatty acids (mg/100g muscle), nutritional indices of total lipid of *M .longissimus thoracis* and thiobarbituric acid ((TBARS) mg malonaldehyde/Kg meat) values for *M .longissimus lumborum* after 10 days simulated retail display in modified atmosphere packs

	Mega	**PLS1**	**PLS2**	**PLS3**	**s.e.d.**	**P**
Total fatty acids	4685	4976	4880	4895	737	NS
n-6:*n*-3 ratio	2.27^{c}	2.02^{b}	2.00^{b}	1.88^{a}	0.055	0.001
P:S ratio	0.07^{a}	0.177^{b}	0.199^{c}	0.218^{d}	0.0179	0.001
TBARS Day 10	0.54^{a}	2.04^{b}	4.17^{c}	4.03^{c}	0.665	0.001

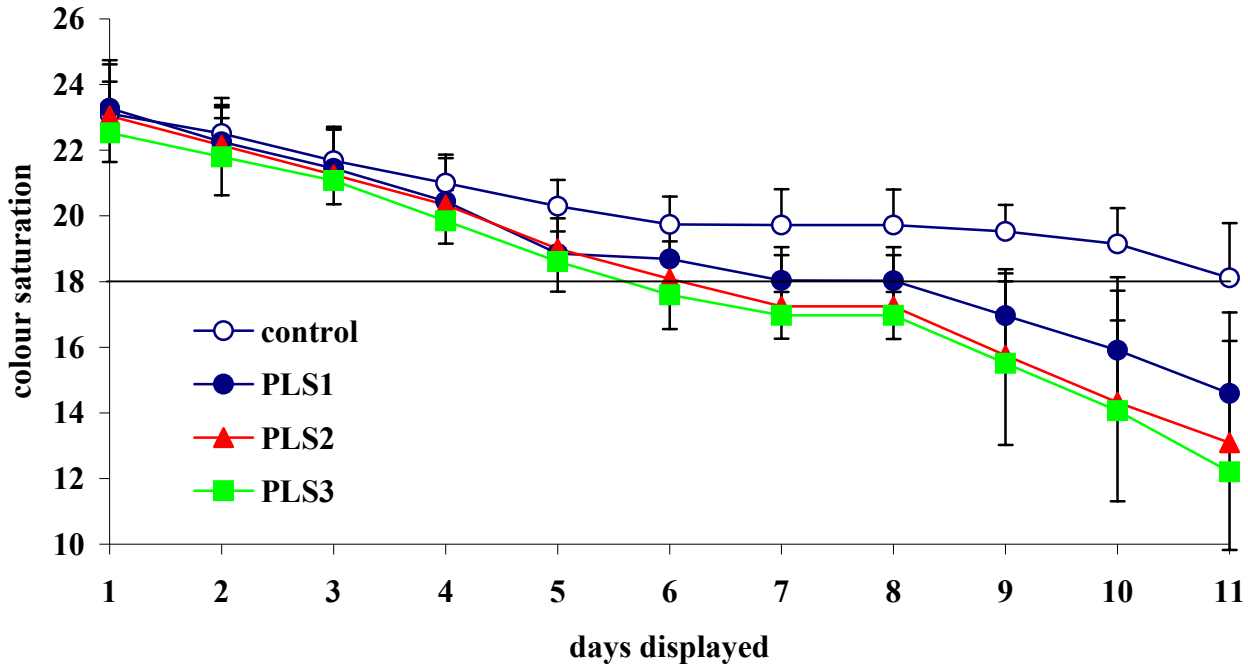

Figure 1. The effect of days displayed upon the change in colour saturation (±stdev) of modified atmosphere packed loin steaks, from animals fed varying levels of protected lipid supplement.

Table 4. Effect of Protected Lipid Supplement on sensory values of grilled loin steaks
Values are the means derived from analysis of variance with Supplement and Assessor as factors; panels are treated as a 'block structure' with 8 replications.

	Mega	PLS1	PLS2	PLS3	s.e.d.	P
Toughness	41.8	43.4	44.2	38.7	2.51	NS
Juiciness	36.5^{ab}	40.3^{b}	33.5^{a}	35.0^{a}	2.29	0.05
Beef	25.1	25.2	23.0	21.0	1.79	NS
Abnormal	18.5^{a}	17.9^{a}	20.9^{ab}	24.4^{b}	2.10	0.01
Greasy	15.3	14.4	16.5	16.2	1.78	NS
Bloody	4.7^{a}	8.6^{b}	6.4^{ab}	4.7^{a}	1.55	0.05
Livery	7.2	5.2	6.1	5.0	1.82	NS
Metallic	4.9	7.4	7.8	5.3	1.26	NS
Bitter	3.9	2.3	3.0	2.5	1.25	NS
Sweet	1.9	3.2	2.5	1.5	0.77	NS
Rancid	1.4^{a}	1.4^{a}	2.3^{ab}	3.8^{b}	0.76	0.01
Fishy†	1.5	1.3	2.2	4.2	1.34	NS
Acidic	10.8	10.8	10.3	9.0	2.02	NS
Cardboard	3.9	5.0	5.3	3.3	1.44	NS
Vegetable/grassy	7.5	8.6	8.6	10.5	1.90	NS
Dairy	8.0	8.6	5.5	11.1	2.11	NS
Hedonic						
Overall liking	23.2^{c}	22.8^{bc}	19.2^{a}	19.6^{ab}	1.69	0.05

Figures with the same superscript do not differ significantly
†Interaction between type and assessor, therefore vr, sed and sig are recalculated.

IMAGE ANALYSIS FOR CHARACTERIZATION OF THE INTRAMUSCULAR CONNECTIVE TISSUE IN MEAT

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Background

Image analysis is a promising approach to characterize the spatial organization of the intramuscular connective tissue (IMCT) using morphological features. A common approach is based on segmentation which consists in subdividing an image into its constituent parts (Pal and Pal, 1993). In meat science segmentation has been principally used to quantify fat distribution on steaks and to determine skeletal maturity of carcass (Tan, 2004). We developed and applied an adaptative segmentation for extracting IMCT thus permitting characterization of spatial organization from visible images of histological sections of muscles. It can also be used to quantify morphometrical parameters on segmented objects, such as size or network hierarchy parameters.

Objectives

This paper describes a practical approach, called Fuzzy Cards Thresholding (FCT), based on building reference maps: a) to set up thresholding rules based on the features calculated from the intensity histogram, b) to quantify the precision of the segmentation and to compare it with other methods. Once the optimal segmentation was obtained, morphometrical parameters characterizing the distribution of IMCT were quantified and compared within several muscle types.

Materials and methods

Animals and sample preparation Muscle samples were taken from 2-year-old Aubrac heifers (n=4). *Biceps femoris* (BF), *Infraspinatus* (IS), *Longissimus thoracis* (LT) and *Pectoralis profundus* (PP) were chosen for their different architectures, including fiber bundle size, IMCT thickness and hierarchy. Samples were aged 8 days *post mortem* at 4°C before cutting and staining. Muscles samples of 20×20×10 mm^3 were frozen with isopentane chilled by liquid nitrogen at -160°C. They were cut to make transversal sections of 0.01 mm thickness. Perimysial tissue was revealed with red Sirius stain, specific for collagen, which is the main connective tissue component. A second group of animals (8 Charolais cows) was submitted to the same treatment and used for collagen amount determination and to study the differences between muscles.

Optimization of thresholding using fuzzy reference maps The process is based on 4 steps: a) to build the fuzzy reference maps, b) to threshold the map at different confidence levels, c) to define the optimal threshold which minimizes the difference between the thresholded image and the reference card, and d) from n cards obtained on different animals and muscles, to define the thresholding rules according to the histogram features of the images.

Each sample image was digitalized with a transmitting light box and a CCD camera JAI CV-M300 coupled with a macroscopic objective leading to a pixel size of 6.8 $.10^{-4}$ mm² (figure 1). Visilog software (Noesis, France) has been used for image processing. The grey level histogram, in the whole field of view, was computed and features such as the mean, standard deviation, skewness and kurtosis were calculated from the histogram moments. Ten test images of 50 mm² of section were displayed at random to a panel of 20 non-trained judges. The judges had to draw (with a one-pixel-thick and 0 grey level line) what they thought to be IMCT (figure 2b). The thickest segments which everyone could identify had already been labelled by thresholding at a high level. The work of the judges was to trace the thinnest elements of the network which are more difficult to identify. Their digital drawings were averaged for each image to obtain the fuzzy reference maps for the perimysial network (figure 2c).

By construction, a fuzzy reference map is in grey levels. It has to be binarized in order to be compared with the result of our adaptive threshold method. When the reference map is thresholded, the resultant binary image (S_ε) is composed of the pixels selected by a percentage of judges called confidence level, ε. A high ε (ε

> 50%) corresponds to a consensual set of IMCT, which favours points selected by most of the judges. In this case, S_ε represents the thick segments, which are distinguished without ambiguity (figure 2d). On the contrary, for $\varepsilon < 50\%$, the thresholded reference map includes the thinner segments (figure 2e).
To evaluate the quality of the segmentation for a given ε, the total error E has been computed as a performance index. It is the sum of two error terms, the fraction *Eoo* of pixels of the image belonging to the object (perimysium) which are falsely attributed to the background and the fraction *Eob* of pixels of the image belonging to the background, which are falsely attributed to the objects. As *Eob* decreases and *Eoo* increases as function of threshold, *E* exhibits a local minimum, which is the optimal value of t (t*), that is a compromise between *Eoo* and *Eob* (figure 3). The t* value is obtained by minimizing the total error E which is the fraction of pixels which have been segmented improperly and it is determined for an a priori fixed confidence level.
Using SAS software (Statistical Analysis Systems Institute, 1995), a stepwise multiple linear regression (GLM procedure) was performed between t* values and histogram features for each reference image (n= 10) at different confidence levels (from 30% to 80%). Thus models were obtained that could predict the thresholding level of all the images taken in the same conditions as our test images, for each confidence level.
Validation of the method The validation was based on a comparison of our method with a reference method already optimized and proposed by Rosin (2001) for the segmentation of this type of images showing a unimodal histogram (RM). It considered a dominant population in the image that produces one main peak relative to the secondary population. This latter class may or may not produce a discernible peak, but needs to be reasonably well separated from the bulk peak to avoid being swamped by it.
Eight sections from 4 muscles of Charolais cows were used. They were stained, digitalized and then the collagen amount was determined as the amount of stain fixed by the collagen fibers and then eluted. (Lopez De Leon and Rojkind, 1985).

Results and discussion

The reference maps Each pixel of the reference map had a value corresponding to a grey level from 0 (black) to 255 (white). A black pixel (0) had a nil probability to belong to the perimysium (none of the judges designated it). A white pixel (255) had the maximal probability (all judges designated it). So the maps were fuzzy because a pixel was attributed a probability of belonging to the perimysium and not a cut decision. The decision happened when a confidence level was chosen. The pixels of the maps can only take a discrete number of intensities equal to the expert number. The maps give information on the location of the connective tissue and also on the error (confidence level, ε) made by keeping this pixel in the "perimysium" class of objects. In this way, the maps give different patterns according to ε. We observed that there was a natural correspondence between ε and the thickness of the thresholded segments.
Thresholding rules For each image the optimal threshold was determined at different confidence levels as shown in table 1. The optimal threshold increased when ε decreased. It segmented lighter and thinner objects, because low ε values accept pixels with a lower belonging probability.
The results of the multiple linear regressions are shown in table 1. They are all linear combinations of the mean and standard deviation or skewness. Models are very informative for each ε value from 30% to 80%. At high confidence levels (70%, 75% and 80%) which correspond to the thick network, the standard error was superior to 3 grey levels. Indeed the segmentation quality was less sensitive to the threshold choice because of the high contrast between myofibers and IMCT. The effective error had fewer consequences than at a small ε values, because it was far from the over segmentation zone.
Comparison with Rosin's method The performance index decreased when the confidence level increased and the quality of segmentation improved (figures 4 and 5). Indeed for high ε values, there were fewer pixels to detect and a higher contrast. RM performance index varies more than with our method (29.6% and 22.2% respectively). This was due to the fact that our method (FCT) adapts the threshold to the confidence level, while RM is adaptative to image context and gives a unique threshold whatever the value of ε. RM *Eoo* (segmentation on thin segments), was almost 2 fold higher at low ε, which indicated that it did not take into account thin segments. With FCT, *Eoo* and *Eob* decreased when ε increased and the indexes were lower than for RM. A better segmentation was obtained with FCT which could be adapted to ε. Indeed when ε decreased, the thinner frame had to be segmented, so the threshold needed to go towards the lighter grey

levels. *Eob* is higher but the performance index stayed inferior to RM. In fact, *Eob* is inherent to the quality of the image, and in particular to its background homogeneity.
The segmentation performance did not actually seem to be largely improved by decreasing ε. However, a slight difference in the performance index (E) could imply a big difference in the segmentation. Only few pixels belonging to the thinnest connective network were detected, but they may provide valuable information on the hierarchy of the network (figure 5). However ε was limited to 55% for this experiment.
Comparison with collagen amount determination
R^2 was the highest at high confidence levels and it decreased markedly with ε (table 2). Thick networks seemed to have more influence on collagen content, since they represented a large number of pixels. Moreover, detection of parasite pixels increased because of the error of the thresholding rules, and it depreciated the correlation when ε decreased.
Discriminating parameters between muscles Three variables were calculated: the area of the segmented network, its length and its average thickness, considered as the ratio between the area and the length (table 3). For ε = 80% (thickest segmentation), the thickness of the segments showed differences between muscles which present visually a thick network (BF, PP), as shown in figure 1, whereas there was no difference in thickness between muscles with thin connective tissue (IS, LT). In contrast, for ε =55% (segmentation of the whole network), IS and LT were discriminated. The values of thickness were in the same range as those published by Geesink et al (1995). Thickness seemed to be related with tenderness because BF and PP (tough muscles) have the thickest networks. The area of the network could not discriminate BF and PP, as the collagen amount did. BF had the largest area of IMCT at 80%, and the smallest at 55%. It had a thick network and few thin segments. In contrast, IS had the largest area of IMCT at 55%. The total length of the network, which was homogeneous to the network branching, segregated the 4 muscles at high confidence levels. PP and IS which showed the greatest degree of branching had longer networks than LT and BF.

Conclusions

We have developed an automatic and validated approach for global thresholding of unimodal images of the intramuscular connective tissue network in muscle and meat. Our method gives objective variables for characterization of the IMCT, and gives further insights into those factors which determine the tenderness of meat by studying the IMCT. It is intended to extend its application to the characterization of the fascicles delimited by the IMCT. As a generic method, it will be adapted to magnetic resonance images at a higher scale on the same samples.

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Figures and tables

Figure 1. Transversal sections of 4 bovine muscles *(Biceps femoris* (BF), *Infraspinatus* (IS), *Longissimus dorsi* (LD), *Pectoralis profundus* (PP)) stained with red Sirius

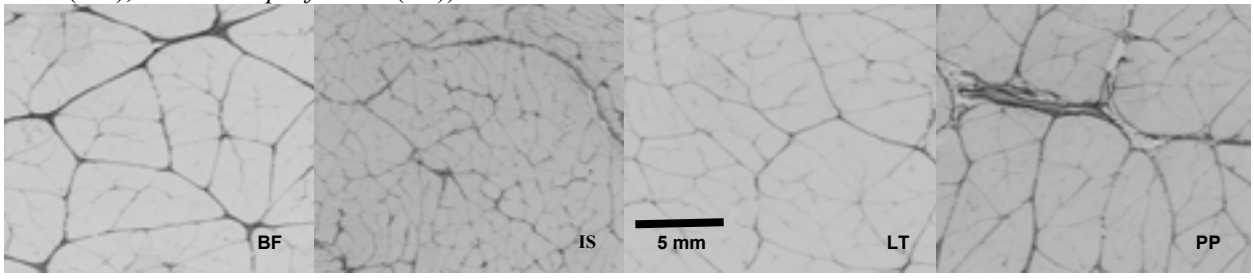


Figure 2. Reference maps of a transversal muscular section. a) original image, b) drawing of one judge, c) reference card which gives the probability of belonging to the class « perimysial network » d) segmentation of a fuzzy map for confidence level $\varepsilon = 80\%$ and e) $\varepsilon = 30\%$

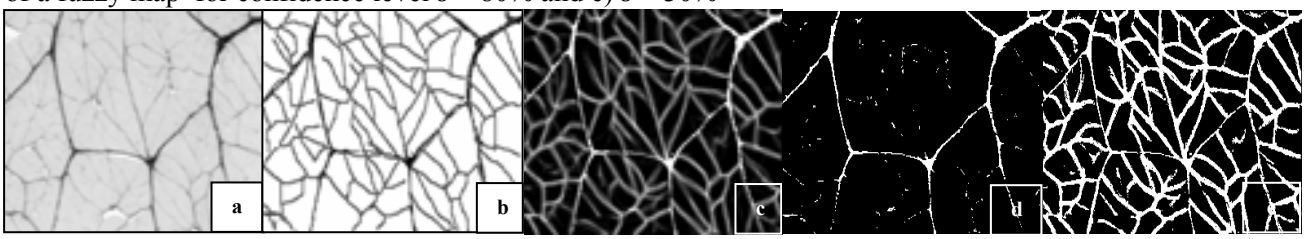


Table 1. Percentages of explained variance (R^2) and standard error of the model given by linear regression of histograms parameters of original image to predict the optimal threshold at different confidence levels

	30%	40%	50%	55%	60%	65%	70%	75%	80%
R^2 of the model	0.995	0.994	0.996	0.989	0.992	0.996	0.975	0.864	0.939
Standard error (grey level)	1.29	1.25	1.19	1.70	1.40	1.16	3.05	6.40	4.56

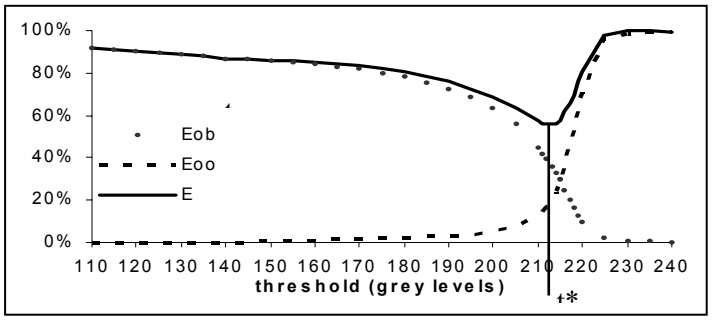
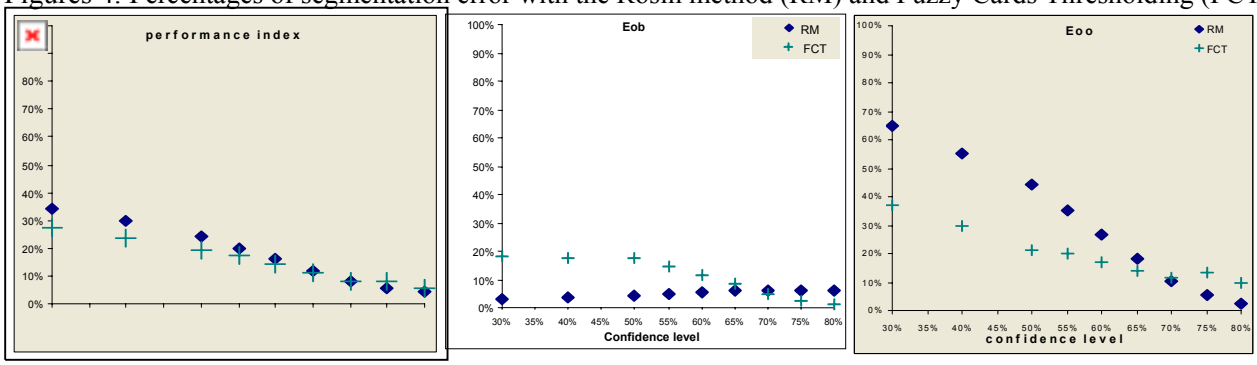
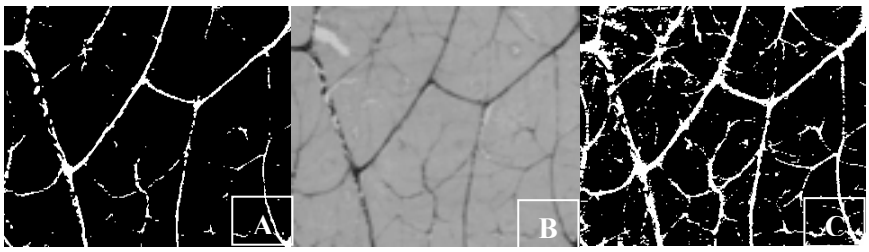


Figure 3. Evolution of the performance indexes for one image at a given confidence level. E is the sum of Eoo and Eob. t* corresponds to the optimal threshold is the local minima of E

Figures 4. Percentages of segmentation error with the Rosin method (RM) and Fuzzy Cards Thresholding (FCT)





Figures 5. Comparison of the segmentation performance between Rosin's algorithm and reference cards method. A) Rosin's segmentation, B) original image, C) segmentation with a confidence level = 60%

Table 2. Correlation coefficients between the segmented area obtained by FCT and the collagen amount determined on the muscular sections for different confidence levels (ε)

ε (%)	55	60	65	70	75	80
(R^2)	0,10	0,44	0,60	0,71	0,76	0,80

Table 3. Connective network measures calculated from the segmentation of images of bovine muscles. Different letters mean significant differences at *p=0.05* per column.
Mean thickness =area/length.

	Collagen amount	Mean thickness (µm)		Area (% total image)		Length (mm)	
Confidence level (ε)	(µg collagen /µg protein)	*55%*	*80%*	*55%*	*80%*	*55%*	*80%*
BF	0.022 a	82 ^{a}	82 ^{a}	13,8 ^{b}	4,9 ^{a}	330 ^{b}	118 ^{c}
IS	0.019 b	69 ^{c}	58 ^{c}	16,6 ^{a}	4,0 ^{b}	469 ^{a}	135 ^{b}
LT	0.012 c	74 ^{b}	58 ^{c}	14,0 ^{b}	3,1 ^{c}	374 ^{b}	104 ^{d}
PP	0.022 a	80 ^{a}	67 ^{b}	14,8 ^{ab}	4,9 ^{a}	363 ^{b}	144 ^{a}

EFFECTS OF PELVIC SUSPENSION ON THE TENDERNESS OF MEAT FROM FALLOW DEER (*DAMA DAMA*)

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Background

Texture, flavour and tenderness are attributes valued by consumers as very important in relation to the eating quality of meat. Different populations of consumers have different preferences for these quality attributes, something that affects the market for all types of meat. However, regardless of the consumer group, the consistency of meat quality is very important, and the product should be of the same quality every time it is purchased. In the Australian beef grading system Meat Standards Australia (MSA) these consumer important sensory quality attributes have been weighted in an overall score where tenderness represents 40%, flavour 20%, juiciness 10% and overall liking 30% (MSA, 2001).

Variation in meat tenderness and techniques developed to minimise this variation, have been investigated in several animal species over a long time period. However, there are very few studies performed on sensory quality attributes of deer meat (venison). Stevenson-Barry *et al* (1999) found a similar relationship between meat ultimate pH and tenderness of red deer (*Cervus elaphus*) venison (*M. longissimus*) to that reported for beef (Smulders *et al*., 1990; Barnier, *et al*., 1992). In contrast, reindeer (*Rangifer tarandus tarandus*) *M. longissimus* has been found to be extremely tender regardless of ultimate pH (Wiklund *et al*., 1997). This phenomenon has been related to the speed of post mortem proteolysis (Wiklund *et al*., 1997) and the small muscle fibre size (Taylor *et al*., 2002) in reindeer.

It is well known that the conditions during rigor development (*e.g.* muscle pH decline, temperature/pH relationship and carcass treatment) are very important in controlling meat tenderisation (Dransfield, 1994). Therefore, carcass suspension techniques have been studied for beef (Hostetler *et al*., 1970; Lundesjö Ahnström *et al*., 2003) where the variation in tenderness is considered to be the main reason for consumer dissatisfaction (Koohmaraie, 1996). To our knowledge, the effect of pelvic suspension on tenderness in deer meat has not been previously studied.

Objectives

The objective of this project was to study the effect of pelvic suspension on tenderness in fallow deer meat.

Materials and methods

Eight fallow deer bucks (18 months old, average live weight 42 kg, body condition score (BCS) 2-3 (Flesch, 2000), 7 fallow deer bucks (≥ 36 months old, average live weight 57 kg, BCS 2-3) and 10 fallow deer does (24 months old, average live weight 38 kg, BCS 2-4) raised at the University of Western Sydney, were included in the study. The animals were fasted for 16 h prior to slaughter, stunned with a captive bolt and bled using thoracic stick exsanguination within 10 s of the stun (ethics approval UWS 00.09). The dressed carcasses were split along the mid ventral axis approx. 45 – 75 min post slaughter. The left side of each carcass was assigned to Achilles tendon suspension (control treatment) and the right side of each carcass was assigned to pelvic suspension. At 2 days post slaughter, 9 selected muscles were collected from each carcass-half (*Mm. semimembranosus, adductor femoris, biceps femoris, semitendinosus, vastus lateralis, rectus femoris, psoas major, longissimus* and *supra spinatus*). The meat samples were vacuum packaged, frozen and stored at -20° C until analysis. Meat samples were cooked on a Silex grill for 4-7 min at 240° C and then wrapped in aluminium foil and allowed to rest for 5 min. Internal temperature was measured during and after cooking to 60-65° C, which is equivalent to medium doneness according to the method described by Shaw (2000). Meat samples were cut to a 1 cm^2 core, with a minimum of 5 replicate sub-samples taken from each muscle for analysis. Meat tenderness was measured using a Warner Bratzler Shear force attachment on a

Stable Micro System TAXT2. Texture analysis was measured by means of force versus time in compression with a crosshead speed of 0.8 mm/s and a trigger force of 10 g with a contact area of 1 mm and contact force of 5 g to determine peak force. The data for each experiment was analysed statistically by residual maximum likelihood (Patterson & Thompson, 1971), with the random effects given by reading within muscle within animal, and the fixed effects by hanging treatment, muscle and their interaction, using the statistical package GenStat (2002).

Results and discussion

The present results suggest that pelvic suspension of the carcasses had the greatest impact on meat tenderness in venison from the young male fallow deer (18 months old, Fig. 1), some impact on tenderness in venison from the older male deer (≥ 36 months old, Fig. 2) but no significant impact at all on tenderness in venison from the female deer (24 months old, Fig 3). In studies of beef, similar differences in effect of pelvic suspension on meat tenderness for bulls and heifers have been reported. The tenderness in meat from bulls was more improved as an effect of pelvic suspension compared with meat from the heifers (Fisher, 1994; Lundesjö Ahnström *et al*., 2003).

In the carcasses from the young fallow deer bucks, the tenderness of the following muscles was significantly improved ($p \leq 0.05$) as a result of pelvic suspension; *Mm. longissimus, biceps femoris, semimebranosus, adductor femoris* and *vastus lateralis*. These results are in good agreement with earlier studies on beef, where the tenderness of *Mm. longissimus, semimembranosus* and *adductor femoris* was positively affected by pelvic suspension (Hostetler *et al*., 1970; Bouton *et al*., 1973). For the older fallow deer bucks, significant effects of pelvic suspension on meat tenderness were found in *Mm. biceps femoris* and *semimembranosus*. The muscles that improved in tenderness as a result of pelvic suspension in the present study are all part of the most valuable cuts in a deer carcass; *M. longissimus* (striploin), *Mm. semimembranosus* and *adductor femoris* (topside), *M. biceps femoris* (silverside) and *M. vastus lateralis* (knuckle).

Conclusions

The positive effect of pelvic suspension on tenderness in venison from the young male fallow deer is important information to consider for the Australian deer industry. This type of animal represents the deer most likely to be supplied for commercial slaughter in Australia. In addition, the important commercial cuts from female deer were generally more tender than the same cuts from males. The slaughter of female deer therefore provides a good option for farmers wishing to supply chilled venison year-round, especially at times of the year when the quality of venison from male deer is negatively affected by the breeding season.

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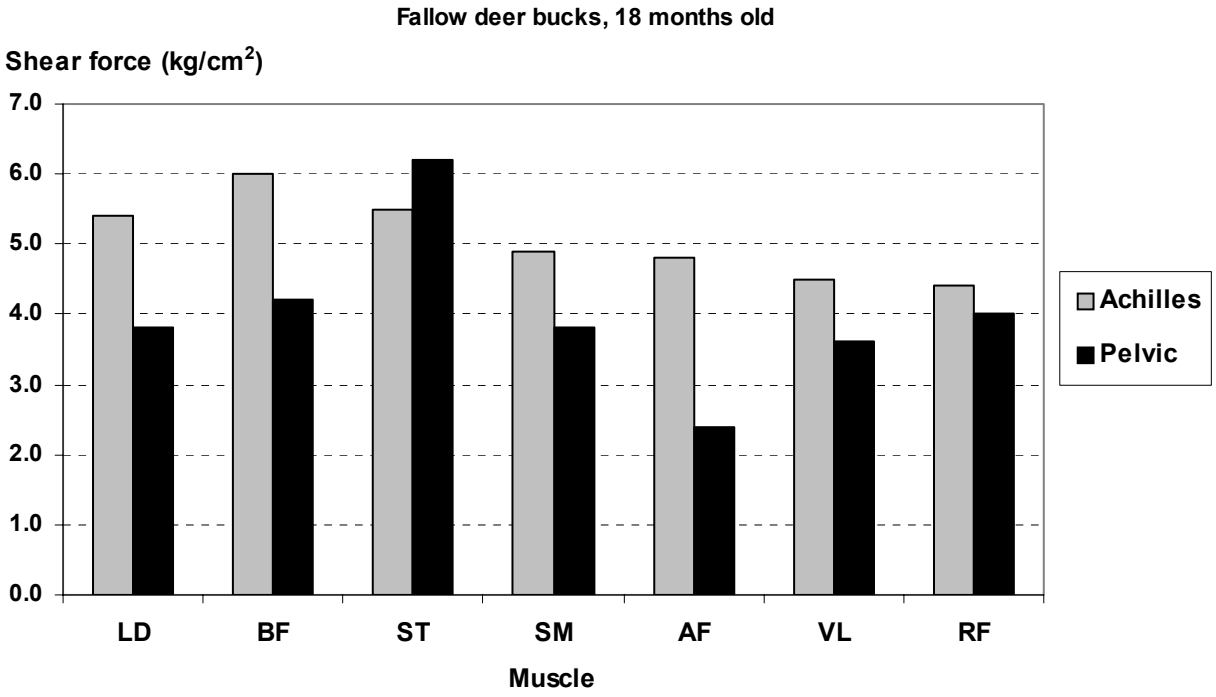

Figure 1. Shear force mean values in 7 muscles (LD = *M. longissimus*, BF = *M. biceps femoris*, ST = *M. semitendinosus*, SM = *M. semimembranosus*, AF = *M. adductor femoris*, VL = *M. vastus lateralis* and RF = *M. rectus femoris*) from fallow deer bucks (18 months old, *n*=8).

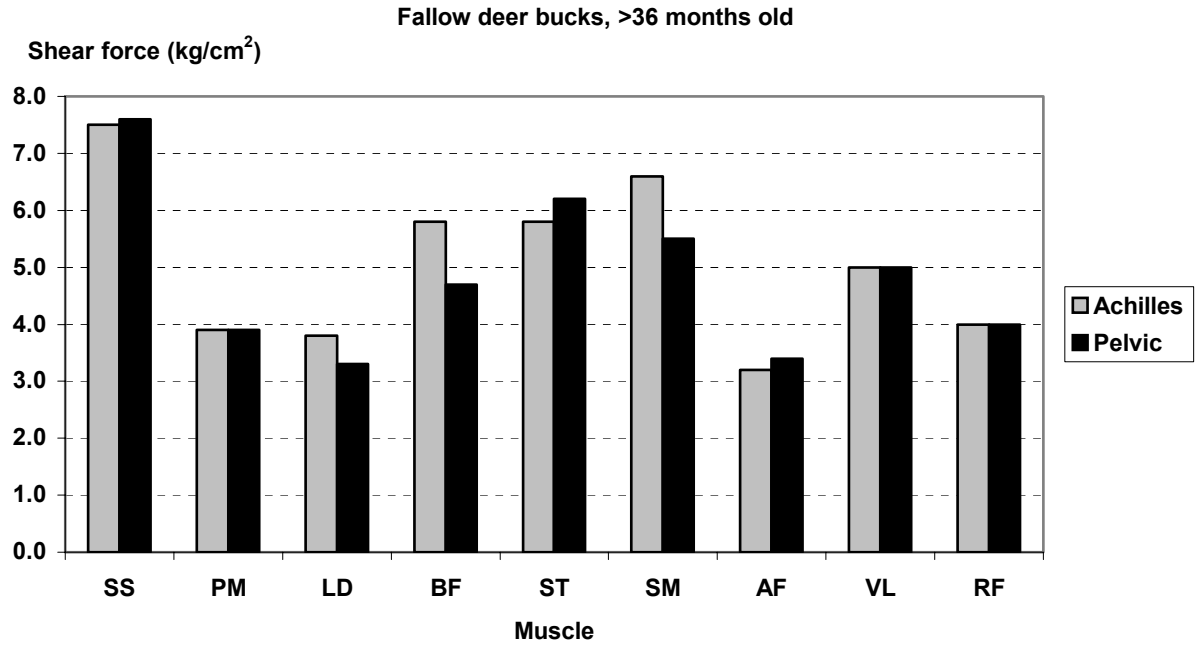

Figure 2. Shear force mean values in 9 muscles (SS = *M. supraspinatus*, PS = *M. psoas major*, LD = *M. longissimus*, BF = *M. biceps femoris*, ST = *M. semitendinosus*, SM = *M. semimembranosus*, AF = *M. adductor femoris*, VL = *M. vastus lateralis* and RF = *M. rectus femoris*) from fallow deer bucks (≥36 months old, *n*=7).

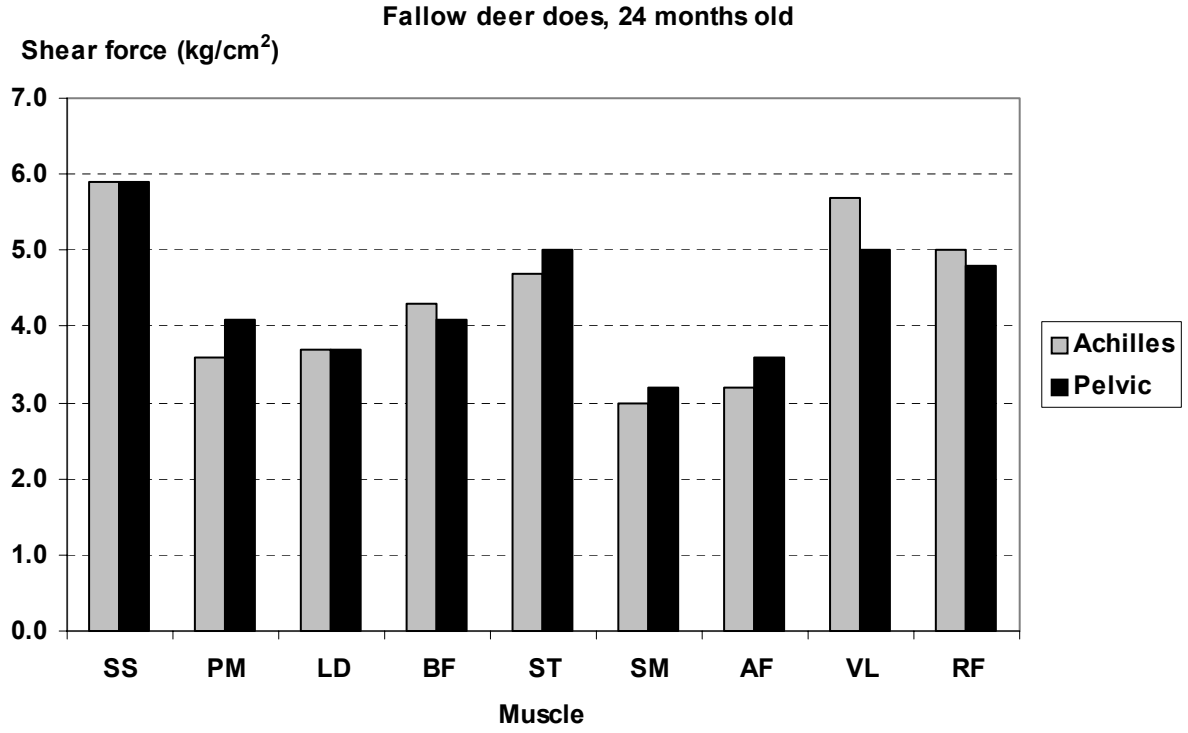

Figure 3. Shear force mean values in 9 muscles (SS = *M. supraspinatus*, PM = *M. psoas major*, LD = *M. longissimus*, BF = *M. biceps femoris*, ST = *M. semitendinosus*, SM = *M. semimembranosus*, AF = *M. adductor femoris*, VL = *M. vastus lateralis* and RF = *M. rectus femoris*) from fallow deer does (24 months old, *n*=10).

EFFECTS OF HIGH OXYGEN PACKAGING ON TENDERNESS AND QUALITY CHARACTERISTICS OF BEEF *LONGISSIMUS* MUSCLES

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Background

High oxygen atmospheres, usually consisting of 70 - 80 % oxygen (O_2) and 20 - 30 % carbon dioxide (CO_2) are commonly used for retail meat packaging. The formation of bright red oxymyoglobin is beneficial, but pigment and lipid oxidation, premature browning of cooked meat, as well as microbiological spoilage, are more prone in high O_2 than anaerobic environments (Hunt *et al*., 1999; Sørheim *et al*., 1999; Jakobsen and Bertelsen, 2000; Tørngren 2003). Recent findings indicate that display of beef in high O_2 atmospheres negatively affects the development of tenderness of the meat (Tørngren, 2003). Little information exists on the extent and causes for the detrimental effect of high O_2 packaging on tenderness. Aitch bone suspension from the *obturator foramen* is a well-known method for stretching pre-rigor muscles in beef carcass sides, thereby reducing muscle contraction and toughening (Hostetler *et al*., 1972). If a detrimental effect of high O_2 display on tenderness development is confirmed, it may be desirable to combine this packaging technology with a method like aitch bone suspension to ensure a satisfactory tenderness and quality of the meat.

Objectives

To study the effects of aitch bone suspension and high O_2 packaging on tenderness and other quality characteristics of beef *longissimus dorsi* (LD) muscles.

Materials and methods

The experiment consisted of LD`s from 4 treatments.
Nine bulls were assigned to treatments A and B:
A – aitch bone suspension + 14 days of high O_2 display of steaks
B – aitch bone suspension + 14 days of vacuum display of steaks
Nine other bulls were assigned to treatment C and D:
C – aitch bone suspension + 14 days of high O_2 display of steaks (equal to A)
D – traditional Achilles tendon suspension + 7 days of vacuum storage of whole muscles + 7 days of high O_2 display of steaks.

The eighteen bulls were of different breeds and were sampled randomly at a commercial abattoir. The average carcass weight was 349 kg within a range of 264-426 kg. The carcasses were electrically stimulated with low voltage. The sides of the carcasses were assigned to two treatments with alternate left and right sides for each treatment. Aitch bone suspension was performed within 60 minutes p.m. The sides were chilled at an air temperature of appr. 4 °C to an average LD core temperature of 11 °C at 10 hours p.m. Two days after slaughter the sides were deboned, and the LD`s were cut in steaks 15 mm thick, except 35 mm for Warner – Bratzler (WB) analysis. Steaks were packaged either in 70 % O_2/ 30 % CO_2 in trays on a Ross Cryovac tray top-seal machine (Ross Cryovac) with film and tray oxygen transmission rates of appr. 15 $cm^3/m^2/24$ h. at 23 °C and 0 % RH, or in vacuum on an Intevac IN30 chamber machine (Intevac Verpackungsmaschinen, Wallenhorst, Germany) with polyamide bags with oxygen transmission rate of 40 $cm^3/m^2/24$ h. at 23 °C and 75 % RH. Meat for all analyses was packaged, except unpackaged meat for the first WB and sensory analyses at 3 days p.m. The meat was displayed in darkness at 4 °C for 15 days.

The following analyses were performed at 3, 10 and 17 days p.m.:

- Warner-Bratzler peak shear force was measured on samples heated in a circulating water bath at 70 °C for 50 min. to a core temperature of 69.5 °C and cut in strips of 10 x 10 x 35 mm with 10 replicates per sample (not at 10 days p.m.)
- Sensory analysis was performed with 11 trained assessors with a quality descriptive test (ISO 6564) for tenderness, hardness, juiciness, rancid taste and rancid odour on samples heated at 70 °C for 40 min. in a water bath to 70 °C core temperature with evaluation in red light for masking possible differences in premature browning (Hunt *et al*., 1999)
- L*a*b* (lightness, redness, yellowness) was analysed with a Minolta Chroma Meter CR-300 (Minolta Camera Co., Osaka, Japan) with 8 mm viewing port and illuminant D_{65} with 3 replicate measurements per sample (treatment D was not analysed at 3 days p.m.)

In the statistical methods, the emphasis was on comparing the effect of display time separately for each treatment, as opposed to comparing the treatments. Analysis of variance (ANOVA) was used for the analyses, with Tukey`s Multiple Comparison Test to determine in detail which groups or display times that were different (SAS System Release 8.2, SAS Institute Inc., Cary, NC, USA).

Results and discussion

Table 1.Warner-Bratzler peak shear force (N/cm^2) and standard deviation (+/-) of LD steaks during display at 4 °C.

Time,	Treatment							
days p.m.	A		B		C		D	
3	59.9 a	+/- 11.7	59.9 a	+/- 11.7	63.0 a	+/- 17.9	72.7 a	+/- 25.6
17	54.7 a	+/- 9.6	47.7 b	+/- 7.1	57.5 a	+/- 7.1	65.2 a	+/- 15.8

For description of treatments, see materials and methods

a,b. Means in the same column with different letters are significantly different ($p < 0.05$).

Table 2. Sensory analysis of tenderness, hardness and juiciness of LD steaks during display at 4 °C. Scale: 1 = none, 9 = very much.

Time,	Tenderness				Hardness			
days p.m.	Treatment				Treatment			
	A	B	C	D	A	B	C	D
3	4.2 a	4.3 b	4.1 b	3.5 b	5.5 a	5.4 a	5.5 a	6.1 a
10	4.7 a	5.8 a	4.7 ab	5.2 a	5.1 a	4.2 b	5.0 a	4.8 b
17	4.9 a	5.8 a	5.0 a	4.8 a	4.9 a	4.1 b	4.8 a	4.9 b

	Juiciness Treatment			
	A	B	C	D
3	4.8 a	4.8 a	5.2 a	5.3 a
10	3.9 b	4.8 a	4.1 b	4.9 a
17	3.9 b	4.2 a	4.2 b	4.2 b

For description of treatments, see materials and methods

a,b. Means in the same column with different letters are significantly different ($p < 0.05$).

Warner-Bratzler shear force (Table 1) and sensory tenderness and hardness values (Table 2) showed that treatment B with 14 days of vacuum display improved the tenderness of the meat ($p < 0.05$), but particularly during the first 7 days, as demonstrated by the sensory analysis. Over all, treatment B with a combination of aitch bone suspension and vacuum display gave the most tender meat. Meat of treatment A, with 14 days of high O_2 display, did not significantly change in shear force, tenderness and hardness during the display period ($p > 0.05$); neither did meat of treatment C change in shear force and hardness ($p > 0.05$). Meat of treatment D with 7 days of vacuum, followed by 7 days of high O_2 display, significantly increased in tenderness and decreased in hardness during the first 7 days of vacuum ($p < 0.05$), but did not change in tenderness or hardness during the subsequent high O_2 display ($p > 0.05$). Steaks of treatments A and C in high O_2 lost juiciness after 7 days display ($p < 0.05$), as well as steaks from treatment D between days 10 and 17 p.m. Vacuum packaging did not alter juiciness during display, either for treatment B or D ($p > 0.05$). The lack of tenderisation by high O_2 treatments is in support to a previous study by Tørngren (2003), who found that beef LD steaks displayed for 16 days in 80 % O_2 / 20 % CO_2 were less tender than steaks displayed in

vacuum or 50 % CO_2/ 50 % N_2. In a comparison of 100 % CO_2 and vacuum storage of beef, no differences in tenderness were found during storage (Bell *et al*., 1996), making it likely that O_2, and not CO_2, is the gas responsible for inadequate tenderisation.

Before display, meat assigned to all treatments was relatively tender, due to relatively high carcass weights, use of electrical stimulation and relatively slow chilling. Although meat treated with aitch bone suspension tended to be slightly more tender than meat from traditional Achilles tendon suspension at start of packaging, no significant differences were found in average peak force between treatments C and D ($p > 0.05$). However, aitch bone suspension reduced the standard deviation of the WB shear force within the group by both day 3 and 17 p.m. compared to Achilles tendon suspension, particularly by lower force of LD`s from the toughest carcasses. Previous studies showed that aitch bone suspension of beef and veal carcasses was significantly beneficial to tenderness at conditions inducing cold shortening, but to various degrees at slow chilling rates (Sørheim *et al*., 2001; Wahlgren and Kalbakk, 2002; Wahlgren *et al*., 2002). In these studies, aitch bone suspension also reduced the variation in WB shear force by improving tenderness of the toughest meat.

Table 3. Sensory analysis of rancid taste and odour of LD steaks during display at 4 °C. Scale: 1 = none, 9 = very much.

Time, days p.m.	Rancid taste Treatment				Rancid odour Treatment			
	A	B	C	D	A	B	C	D
3	1.1 b	1.1 b	1.1 b	1.0 b	1.1 b	1.1 b	1.1 b	1.1 b
10	2.9 a	1.1 b	2.3 a	1.1 b	1.8 ab	1.3 b	1.9 a	1.3 ab
17	3.5 a	2.5 a	2.4 a	2.4 a	2.4 a	2.5 a	2.0 a	2.1 a

For description of treatments, see materials and methods.
a,b. Means in the same column with different letters are significantly different ($p < 0.05$)

High O_2 display increased rate of development of rancid taste in the steaks (Table 3), as shown for treatment A and C as early as 7 days display, and for treatment D at 14 days of display ($p < 0.05$). An increased rancid odour developed in meat of treatment C after 7 days display ($p < 0.05$). The findings are in agreement with Jakobsen and Bertelsen (2000), where 55 – 80 % O_2 storage of beef LD steaks at 4 - 8 °C caused lipid oxidation.

Table 4. a* (redness) and b* (yellowness) values of LD steaks during display at 4 °C.

Time, days p.m.	a* Treatment				b* Treatment			
	A	B	C	D	A	B	C	D
3	25.9 a	19.4 a	26.2 a	-----	14.3 a	5.6 a	14.0 a	-----
10	22.4 b	19.0 a	21.9 b	26.3 a	12.9 b	5.4 a	12.0 b	13.6 a
17	19.7 c	18.5 a	20.0 c	23.1 b	12.3 b	5.4 a	11.8 b	12.4 a

For description of treatments, see materials and methods.
a,b,c. Means in the same coloum with different letters are significantly different ($p < 0.05$)

Packaging conditions affected meat colour (Table 4). a* redness values of the high O_2 treatments A, C and D decreased during display, but not below the a* value of the vacuum treatment B at 14 days. b* yellowness values were considerably higher for all O_2 treatments than the vacuum treatment at all sampling times ($p < 0.05$). L* lightness values were higher for treatment A than B at all sampling times ($p < 0.05$), but treatment C and D did not differ in L* values ($p > 0.05$) (results not shown). Although some redness was lost from high O_2 samples during the 14 days display, the meat was still fairly red and not discoloured at end of display. Vacuum samples had stable L*, a* and b* values at 3, 10 and 17 days p.m.

Conclusions

The study confirmed previous indications of reduced tenderness development in beef LD steaks displayed in high O_2 atmospheres. In addition, steaks displayed in high O_2 were less juicy. Steaks in vacuum improved in tenderness during the display period, and these steaks were ranked highest in final tenderness. The extent and

causes of the negative effect of high O_2 packaging on tenderness, as well as precautionary measures to reduce or avoid this effect, need to be addressed further. Aitch bone suspension did not significantly improve tenderness, probably because the meat was relatively tender by other tenderizing measures in the experiment, but the variation in tenderness was lower with aitch bone than Achilles tendon suspension. High O_2 display increased rancidity of the meat. The initial bright red colour decreased during high O_2 display, but not to the extent of discolouration.

Acknowledgements

The assistance of Gilde Felleslakteriet in Sem in slaughtering and high O_2 packaging is greatly acknowledged. The authors are grateful to the technical assistance of Grethe Enersen, Inger-Johanne Fjøsne, Håvard Johansen, Asgeir Nilsen and Karin Solgaard at Matforsk. Dr. Kjell Ivar Hildrum at Matforsk is thanked for valuable advice to the article.

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EFFECT OF GENETICS ON MEAT QUALITY AND SENSORY PROPERTIES OF PORK

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Background

The swine industry has achieved tremendous progress in genetic gains related to growth and feed efficiency as well as carcass fat reduction. As a consequence of these improvements there has been an increasing perception that the eating quality of pork has deteriorated over time. Some of these concerns are related to the reduction in fat content of muscle, i.e. intra-muscular fat, other concerns are related to the post-mortem biochemistry of pork, particularly the rate and extent of pH decline and the extent of myofibrillar proteolysis.

Objectives

Traits related to meat quality were investigated as part of a larger study, where overall carcass composition was studied in different PIC lines. Physico-chemical traits were compared between the different lines and related to sensory evaluations as well as correlated with each other. The literature provides us with many reports on the relationships between these predictive measurements and organoleptic parameters. However, this data set is quite unique in that it combines 10 different PIC lines of various phenotypic ranges.

Materials and methods

Animals came from the PIC genetic nucleus in Kipling Saskatchewan, Canada and was comprised of 30 market weight gilts from each of 10 lines for a total of 300 animals. The lines included the following basic genotypes: Landrace (Land), Large White (LW), Duroc (Dur), White Duroc (WDur), Berkshire (Berk), Hampshire (Hamp), Synthetic line (Syn), Crossbred Berkshire x Hampshire (BH), Pietrain hal negative (Piet-), Pietrain hal positive (Piet+).

Carcass Traits. Fat thickness (mm) and lean tissue depth (mm) were measured with a Hennessey Grading Probe at the 3rd/4th last ribs, 70 mm from the carcass mid-line approximately 40 minutes after harvest. Dissectible lean was determined as the weight of dissectible lean in the picnic, butt, loin, ham primal cuts plus the weight of the skinned trimmed belly and ribs as a percentage of cold side weight.

Muscle Quality Traits. The pH at 45 minutes (pH-45min) and 48 hours (pH-48h) post-slaughter, CIE L*, intramuscular fat (IMF), drip loss and shear value of the LT (*longissimus thoracis*) muscle were measured as described by Murray et al. (2001).

Sensory Traits. Perceived juiciness, flavor intensity, overall tenderness and overall palatability were assessed using a trained taste panel as described by Jeremiah et al. (1995).

Biochemical Measurements. LT muscle fiber types were determined by the combined SDH and myosin-ATPase method as described by Aalhus et al (1997). Samples, removed from LT muscles at 24 h post-harvest, were used to determine glycogen, glucose and lactate using a YSI Glucose-Lactate Analyzer and glucose-6-phosphate (Lang and Michal 1974). Glycolytic potential (GP) was calculated as (2*[glycogen + glucose + glucose-6-phosphate] + lactate) and is presented as μmoles lactate-equivalents per gram of muscle.

Statistical Analyses. Data were analyzed using the GLM procedure of SAS (2001) with statistical model slaughter day and line as classification variables. Least square means were compared by t-test. Correlation analyses made use of the CORR procedure (SAS 2001).

Results and discussion

Least squares means for meat and carcass traits are presented in Table 1. For most of the traits, Berk and Piet are at the two extremes. A number of line comparisons are highlighted below.

Carcass Traits. Based on both dissectible lean and backfat thickness, Berk carcasses are the fattest and Piet carcasses are the leanest. The genotypes used as sire lines, arranged in order of dissectible lean, are Dur, Hamp and Syn, with the Syn yielding significantly more dissectible lean than the Dur and having a much greater muscle depth than all lines except the Pietrain lines.

LT Quality Traits. Muscle traits differ significantly among genotypes, particularly for the Piet+, a halothane carrier animal that differentiates itself from the other genotypes for all of the muscle traits. Indeed this genotype shows the lowest pH-45min and pH-48h, the highest L* value, the greatest drip loss, lowest IMF and highest shear value. This is typical of what is expected for animals homozygous for the hal gene (Murray and Jones 1994). The Berk and the BH are at the other end of the spectrum for all of the traits. Interestingly the Dur shows intermediate pH values but has IMF levels as high as the Berk and the BH. It is also similar to the Berk in terms of shear value. This differs somewhat from the findings of Suzuki et al (2003) that Berkshire pigs had less IMF and lower drip than the Duroc pigs. Shear values are significantly higher for the Piet+, while the BH is the lowest, although not significantly different from the Berk or Hamp.

LT Sensory Traits. Sensory evaluation results indicate that the Piet has the lowest juiciness and palatability scores. Of interest is the fact that the Berk, BH and Duroc display sensory scores that are quite similar. The Hamp closely follows the Duroc. Similar studies (Brewer et al, 2002) support these observations.

LT Biochemical Measurements: Genotypes differ in their biochemical properties. The extremes in these measurements are depicted by the Piet+ and the Hamp, the latter having a lower proportion of red fiber and higher proportion of white fibers than the Piet+, the Berk being closer to the Piet+ for these traits. Klont et al. (1998) provides a good review of fiber type implications on meat quality. Residual glycogen is included to show that at 24 hours post-mortem metabolizable glycogen is present in all genotypes. GP is lowest for the Berk and highest for the Piet- which is closely followed by the Dur.

Correlation coefficients (Table 2) do not demonstrate a very high degree of association between the carcass, muscle quality, biochemical and sensory traits. The drip loss followed by pH-45min and IMF values seem to be the most related to juiciness, overall tenderness and overall palatability, although they explain at best approximately only 22% of the variation for a given trait (i.e. r=0.47 for pH-45min vs juiciness). Van Laack et al. (2001) found r values between IMF and shear value of –0.21 after 7 days of aging. Muscle quality traits are interrelated. The pH-48h is related to L* (r=-0.39) and drip (r=-0.33), as found by Huff-Lonergan et al. (2002). The pH-45min also show strong relationships with L* (r=-0.52) and drip loss (r=-0.68). The correlation between pH-48h and GP is quite high (r=-0.63) compared to the value of 0.39 reported by Huff-Lonergan et al. (2002).

Conclusions

Results from this study position the Duroc sire as potentially able to deliver fresh meat quality equivalent to the Berk, particularly with respect to marbling or intramuscular fat, in a carcass that has higher dissectible lean content. If IMF is not an issue the Syn genotype has the potential to deliver high meat quality at dissectible lean contents close to the Piet genotypes. Comparison of the ten PIC genetic lines of swine clearly demonstrates great diversity in carcass leanness, and muscle physical, sensory and biochemical traits. Appropriate combinations of these lines, along with a suitable nutritional regime, should produce the types of market pigs required to satisfy the diversity in current markets.

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Table 1. Carcass, muscle, sensory and biochemical traits.

LINE [z]	Lean (%)	Backfat	Lean Depth	pH-45min	pH-48h	L*	Drip	IMF	Shear
LW	59.6^{de}	14.1^{c}	51.2^{fg}	6.37^{bc}	5.55^{cd}	50.1^{e}	2.50^{c}	1.24^{d}	5.48^{b}
Land	59.5^{e}	13.9^{c}	53.2^{ef}	6.40^{abc}	5.57^{bcd}	50.4^{de}	2.49^{c}	1.36^{cd}	5.45^{b}
Dur	60.0^{cde}	15.1^{c}	54.0^{de}	6.34^{c}	5.55^{cd}	52.0^{c}	2.28^{cd}	2.57^{a}	5.15^{bcd}
WDur	61.4^{bcd}	14.7^{c}	54.0^{de}	6.36^{c}	5.58^{bc}	50.3^{e}	2.30^{cd}	2.35^{a}	5.42^{bc}
Hamp	61.6^{bc}	14.3^{c}	56.0^{d}	6.40^{abc}	5.56^{cd}	51.4^{cde}	2.33^{cd}	1.79^{b}	5.20^{bcd}
Berk	52.0^{g}	25.6^{a}	49.7^{g}	6.49^{a}	5.67^{a}	51.2^{cde}	1.84^{d}	2.68^{a}	5.12^{bcd}
BH	56.8^{f}	19.6^{b}	52.5^{ef}	6.47^{ab}	5.61^{b}	51.9^{cd}	2.07^{cd}	2.70^{a}	4.89^{d}
Piet-	62.9^{b}	14.2^{c}	66.5^{b}	6.23^{d}	5.53^{d}	53.6^{b}	3.32^{b}	1.66^{bc}	5.01^{cd}
Piet+	67.0^{a}	10.6^{d}	72.9^{a}	5.80^{e}	5.54^{cd}	57.7^{a}	5.68^{a}	1.25^{d}	6.20^{a}
Syn	63.1^{b}	14.6^{c}	63.0^{c}	6.31^{cd}	5.58^{bc}	50.8^{cde}	2.68^{bc}	1.64^{bc}	5.36^{bc}
SE	*0.66*	*0.53*	*1.0*	*0.03*	*0.02*	*0.55*	*0.25*	*0.14*	*0.17*

LINE [z]	Juiciness	Flavor	Tenderness	Palatability	Red [x]	Intermediate [x]	White [x]	Glycogen	GP
LW	5.01^{bcd}	6.14^{ab}	6.61^{b}	5.20^{cde}	7.2^{abc}	11.7^{abc}	81.1^{bc}	18.5^{b}	178.8^{bc}
Land	4.85^{cd}	5.93^{c}	6.06^{c}	4.88^{e}	7.5^{abc}	9.8^{c}	82.6^{ab}	17.3^{b}	172.1^{bcd}
Dur	5.46^{ab}	6.29^{a}	6.78^{ab}	5.46^{cd}	7.8^{abc}	11.1^{bc}	81.1^{bc}	22.4^{a}	189.6^{bc}
WDur	5.27^{abc}	6.22^{ab}	6.60^{b}	5.32^{cd}	7.8^{ab}	11.3^{bc}	80.8^{bc}	18.8^{b}	171.7^{bcd}
Hamp	5.28^{abc}	6.14^{ab}	6.75^{ab}	5.50^{bc}	6.3^{bc}	9.8^{c}	83.9^{a}	16.7^{bc}	169.1^{cd}
Berk	5.71^{a}	6.21^{ab}	7.04^{a}	5.83^{ab}	8.2^{a}	12.2^{ab}	79.6^{cd}	14.3^{c}	147.6^{e}
BH	5.72^{a}	6.25^{ab}	6.99^{a}	5.96^{a}	7.6^{abc}	10.9^{bc}	81.4^{abc}	17.0^{bc}	164.0^{d}
Piet-	4.59^{de}	6.17^{ab}	6.54^{b}	4.95^{e}	6.2^{c}	12.3^{ab}	81.5^{abc}	23.1^{a}	193.9^{a}
Piet+	4.36^{e}	6.09^{bc}	5.24^{d}	4.23^{f}	8.5^{a}	13.5^{a}	78.0^{d}	18.6^{b}	182.1^{b}
Syn	4.66^{de}	6.12^{ab}	6.44^{b}	5.11^{de}	8.3^{a}	10.6^{bc}	81.1^{bc}	17.1^{bc}	169.3^{cd}
SE	*0.17*	*0.07*	*0.14*	*0.14*	*0.6*	*0.8*	*1.0*	*1.1*	*4.0*

[z] Large White (LW), Landrace (Land), Duroc (Dur), White duroc (Wdur), Hampshire (Hamp), Berkshire (Berk), Berkshire x Hampshire (BH), Pietrain Hal negative (Piet-), Pietrain Hal positive (Piet+), Synthetic (Syn).

[x] % Area of specified fiber type.

[abcde] Means within column bearing different superscripts differ (P<0.05).

Table 2. Correlations between several of the measured traits.

	Dissectible Lean	Backfat Depth	Lean Depth	pH, 45 min	pH, 48 hour	L*, 48 hour	Drip Loss	Intramuscular Fat	Shear Value	Juiciness	Overall Tenderness	Overall Palatability
Backfat Depth	-0.73											
Lean Depth	0.52	-0.36										
pH, 45 min	-0.43	0.37	-0.51									
pH, 48 hour	-0.23	0.32	-0.17	0.23								
L*, 48 hour	0.18	-0.07	0.44	-0.52	-0.39							
Drip Loss	0.35	-0.22	0.52	-0.68	-0.33	0.71						
IMF	-0.45	0.52	-0.30	0.24	0.22	0.02	-0.19					
Shear Value	0.16	-0.21	0.21	-0.28	-0.04	0.13	0.32	-0.21				
Juiciness	-0.31	0.25	-0.36	0.35	0.29	-0.25	-0.46	0.32	-0.25			
OA Tenderness	-0.30	0.29	-0.40	0.47	0.15	-0.27	-0.46	0.34	-0.40	0.60		
OA Palatability	-0.33	0.35	-0.39	0.45	0.24	-0.22	-0.36	0.36	-0.27	0.73	0.73	
GP	0.29	-0.32	0.26	-0.21	-0.63	0.34	0.35	-0.20	0.07	-0.25	-0.07	-0.13

THE EFFECT OF INTRAMUSCULAR FAT ON EATING QUALITY OF PORK DEPENDING ON END POINT TEMPERATURE

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Background

Intramuscular fat (IMF) has for long been known to increase the tenderness of meat. However, the extent of the effect of the IMF has been different in different experiments which might indicate that the effect depends on factors like aging time, end point temperature etc. This leads to a theory that the IMF fat increases the robustness of the meat towards a rougher treatment.

Objectives

The objective of this study was to investigate the effect of IMF on the eating quality of pork, *longissimus dorsi* (LD) and *biceps femoris* (BF), cooked to two end point temperatures to find out whether the IMF would increase the robustness of the meat. In LD the effect of fatty acid composition was also investigated.

Materials and methods

Biceps femoris: BF from two groups of slaughter pigs (DLY), one group fed with roughage (IMF 2.3%, std. 0.6) and one with feed without roughage (IMF 3.3%, std 0.8) (n=20 per group), was excised from one side the day after slaughter and frozen at -18°C without further aging. The meat was cooked as whole roasts in the oven at a temperature of 140°C until the meat had an end point temperature of 65°C or 75°C respectively. A trained sensory panel conducted a sensory profile with the following attributes: Hardness at first bite, tenderness, chewing time, sour flavour, piggy flavour, colour (light to dark grey), uneven colour. On another piece of meat the juiciness was assessed as juiciness after 3 chews (juiciness 1), juiciness after 10 chews (juiciness 2) and juiciness when the sample is ready for swallowing (juiciness 3). The attributes were assessed on a 15-cm line scale from nothing (zero) to very much.

Longissimus dorsi: LD was excised from both sides of slaughter pigs selected at random at a Danish abbatoir. The loins were vacuum packed and frozen without further aging (-18°C). They were divided into three groups (n=20) of low (0.8-1.3%), medium (1.6%-2.0%) and high (2.3%-4.1%) IMF. The fatty acid composition of the intramuscular fat was determined by GC-FID after extraction. The meat was pan-fried as 2-cm thick chops until an end point temperature of 65°C or 80°C. A trained sensory panel conducted a sensory profile with the following attributes: Hardness while cutting, hardness at first bite, tenderness, fibrous, crumbliness, chewing time, chewing rest, roasted meat flavour, sour flavour, piggy flavour. On a separate piece of meat the juiciness was assessed after 3 chews and after 20 chews. The attributes were assessed on a 15-cm line-scale from nothing (zero) to very much.

Results and discussion

Biceps femoris: There were no interactions between IMF content and end point temperature meaning that the effects of IMF was the same for both temperatures. The content of IMF caused a significant decrease in hardness at first bite, chewing time and the piggy flavour as well as increased the tenderness (Table 1). The IMF had no effect on the juiciness, sour flavour or appearance at either end point temperatures. Higher end point temperatures increased the darkness (6.9 to 8.0) and the uneven colour of the meat (5.0 to 5.6) as well as decreased the juiciness (both 1, 2 and 3 from 6.7/6.8/6.8 to 4.6/4.6/4.5) and the sour flavour (3.9 to 3.6) irrespective of the IMF.

Table 1. Effect of intramuscular fat on the sensory profile of *biceps femoris*

	2.3%	3.3%
Hardness at first bite	7.8	6.3
Tenderness	7.0	8.7
Chewing time	10.4	8.9
Piggy flavour	3.5	2.6

IMF increased the tenderness of BF even though it is a rather tough muscle in which the tenderness to a large degree is determined by the amount of connective tissue. As the tenderness did not decrease when cooked to a higher end point temperature the effect of the IMF on robustness was not seen. IMF did not affect the juiciness and even though the juiciness decreased by an increase of the end point temperature, the IMF had no effect on robustness with respect to this attribute. IMF reduced the piggy flavour irrespective of the end point temperature.

Longissimus dorsi: There was no interaction between IMF and the end point temperature except for juiciness. For the other attributes the effect of IMF was the same irrespective of the temperature (Table 2).

Table 2. Effect of intramuscular fat and end point temperature on the sensory profile of *longissimus dorsi.*

	IMF			End point temperature	
	Low (0.8-1.3%)	Medium (1.6-2.0%)	High (2.3-4.1%)	65°C	80°C
Hardness while cutting	7.7^{a}	6.9^{b}	6.6^{b}	6.6^{a}	7.5^{b}
Hardness at first bite	6.6^{a}	6.3^{b}	6.0^{c}	5.9^{a}	6.8^{b}
Tenderness	5.7^{a}	6.7^{b}	7.3^{c}	7.2^{a}	5.9^{b}
Fibrous	7.8^{a}	7.2^{b}	7.1^{b}	7.1^{a}	7.6^{b}
Crumbliness	0.4	0.3	0.3	0.3	0.4
Chewing time	9.7^{a}	8.9^{b}	8.4^{c}	8.5^{a}	9.5^{b}
Chewing rest	8.7^{a}	7.9^{b}	7.4^{c}	7.6^{a}	8.4^{b}
Roasted meat flavour	7.2^{a}	7.4^{b}	7.6^{b}	7.2^{a}	7.6^{b}
Sour flavour	5.4^{a}	5.2^{b}	5.0^{b}	5.8^{a}	4.6^{b}
Piggy flavour	0.9	0.9	0.9	1.1^{a}	0.7^{b}

Different letters in the same line for IMF respectively end point temperature show significant differences.

IMF increases the tenderness and decreases the hardness, chewing time and chewing rest irrespective of the end point temperature. The tenderness increased over the entire interval of IMF even though the difference was largest from low to medium IMF.

As for the BF an increasing IMF content will decrease the sourness of the meat. As the intensity of piggy flavour was very low, no effect of IMF was seen on this attribute but the roasted meat flavour slightly increased from low to medium IMF whereas a further increase in IMF did not further increase the roasted meat flavour.

There was a significant interaction between IMF and the end point temperature for juiciness both after 3 and after 20 chews (see Table 3). At the low end point temperature there was no difference between IMF groups whereas the group with low IMF had a lower juiciness compared to the other groups at 80°C.

Table 3. Effect of IMF in LD on juiciness depending on end point temperature.

End point temperature	65°C			80°C		
IMF-group	Low (0.8-1.3%)	Medium (1.6-2.0%)	High (2.3-4.1%)	Low (0.8-1.3%)	Medium (1.6-2.0%)	High (2.3-4.1%)
Juiciness, 3 chews	4.7[a]	4.7[a]	4.6[a]	2.7[b*]	3.1[c]	3.0[c]
Juiciness 20 chews	8.6[a]	8.4[a]	8.5[a]	5.5[b]	6.0[c]	5.9[c]

Different letters in same line shows significant differences *: significance only P=0.06

The fatty acid composition was analysed and given as percentage of the total fatty acid content. The phospholipids are more unsaturated compared to the triglycerides. As the amount of phospholipids are almost the same for all samples the amount of triglycerides will vary with increasing amount of intramuscular fat. The degree of saturation will therefore be larger in the samples with a high amount of IMF compared to those with a low amount of IMF. To investigate the effect of fatty acid composition on the texture we did a PLS-regression with the absolute content of fatty acids (% fatty acid*content of IMF) as X-matrix and the texture attribute as Y-matrix for each of the three IMF-groups.

The composition of fatty acids did only explain 26% of the variation in the group with low intramuscular fat but it explained 44% of the variation in the other two groups. The fatty acid composition seems therefore to have a larger influence on the texture in the triglycerides compared to the phospholipids. Table 4 shows the correlation between measured and predicted value of the texture attributes predicted from the fatty acid composition for each of the three IMF-groups.

Table 4. Correlation (calibrated) between measured and predicted value of the sensory attributes predicted from the fatty acid composition.

	Hardness at cutting	Hardness at first bite	Tenderness	Chewing time	Chewing rest	Fibrous	Crumbleness
Low IMF 0.8-1.3%	0.45	0.47	0.44	0.46	0.50	0.49	0.46
Medium IMF 1.6-2.0%	0.67	0.72	0.85	0.88	0.88	0.82	0.81
High IMF 2.3-4.1%	0.75	0.75	0.80	0.81	0.82	0.85	0.83

If the amount of intramuscular fat is above a certain level, the fatty acid composition can predict around 80% of the variation in texture.

The regression coefficients showed that it was not a single or a few fatty acids, which influenced the texture. The picture was that the saturated fatty acids had a positive influence on the tenderness whereas the unsaturated fatty acids decreased the tenderness.

Conclusions

Intramuscular fat increases the tenderness in both LD and BF irrespective of end point temperature. The juiciness was only affected in the LD where there was an interaction as there was no difference in juiciness at 65°C whereas the meat with a low content of IMF was less juicy at the low end point temperature. The IMF increases the roasted meat flavour (in LD) and decreases the piggy flavour (BF) and the sour flavour (LD and BF). However, the difference was between the low and medium IMF group as no further increases in IMF did not further affect/change the flavour. The fatty acid composition influenced the texture especially in the medium and high IMF groups. The meat in this experiment w as not aged and the influence of IMF might therefore be smaller had the meat been aged for a longer time.

It can therefore be concluded that the IMF increases the general eating quality of meat irrespective of the cooking temperature.

HOW DIFFERENT OPERATING CONDITIONS OF FILTER PAPER PRESS METHOD AFFECT MEAT WATER HOLDING CAPACITY MEASUREMENT

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Background

Water Holding Capacity (WHC) is the ability of meat to hold fully or in part its own water (Honikel, 1987) and it is influenced by the change in volume of myofibril (Offer and Knight, 1988). This qualitative parameter - affecting technological traits, sensory attributes and nutritional constituents - is of primary importance for the Meat Industry which has considerable pressing problems in this area and needs answers to assure firm quality. It is known that meat WHC is a result of metabolic events prior to harvest and slaughter and following the immediate *post mortem* conversion of the muscle to meat in relation to different factors.
Present methods available to measure the WHC of meat and its products (Honikel, 1987; Trout, 1988; Barton-Gade *et al*., 1993), despite many efforts over the years, do not have a sufficient standardization, essential for comparison (Honikel, 1998). It has been particularly observed that the application of Filter Paper Press Method (Grau and Hamm, 1957) - which requires the compression of a little amount of meat on filter paper, the subsequent determination of the surfaces formed by meat and juice and the estimation of the difference between the areas - has been suffering a lot of interpretations and adaptations. Some Authors (Irie *et al*., 1996; Onega *et al.*, 2000; Fiems *et al*., 2003) utilized different filter paper types and amounts of meat; when considering the load, they put it under different pressures and compression times; sometimes they measured surfaces with the aid of a planimeter, at times outlining areas on the original by pencil, or by the use of an optical electronic system (Video Image Analyzer). Finally, Authors even applied different formulae to measure WHC (Wierbicki and Deatherage, 1958; Hofmann *et al.*, 1982; Van Oeckel *et al.*, 1999).
It was necessary to verify if investigations carried out according to different adaptations of Filter Paper Press Method could be directly comparable.

Objectives

The purpose of this study is to verify if different operating conditions (applied load, filter paper type and length of compression) could influence the results in the Water Holding Capacity measurements by Filter Paper Press Method thus hindering the comparing of data coming from different researches.

Materials and methods

The Filter Paper Press Method of Grau and Hamm (1957) for measuring WHC was adopted, but the procedure (load, time and surface measurement) was automated by using an instrument (BT-WHCi) that applies Video Image Analysis (VIA) to area measurement (Barbera, 2003). Water Holding Capacity was investigated on samples of different meats (beef, chicken and pork) obtainable on the market. Experimental data was gathered from the analysis of beef *M. longissimus thoracis et lumborum*, chicken *M. pectoralis major* and pork *M. longissimus dorsi*. The meat, chilled at 4°C and freed from external fat and connective tissue, was rapidly homogenised (Braun Multiquicksystem ZK100) for 10 seconds. A sample of 250 mg of homogenised meat was placed on a filter paper and compressed between two plexiglass sheets. Filter paper was dried in the oven at 105°C and maintained in a dryer until the analysis started. The meat and the liquid areas (mm^2) were measured by VIA, every minute for ten minutes.
Water release was measured in different conditions, referring to the following factors: load applied to compress the meat (294.2, 490.3 and 588.4 N); filter paper type - Whatman 1 (W1) and 42 (W42); animal type to test effectiveness of BT-WHCi, using three animals for each type.
The data were analysed using the SAS 8.2 package. Factorial analysis of covariance for each minute was performed on the three factors and meat sample weight as covariate. The dependent variables obtained were:

- **TAn** (mm^2) — Total Area, n=0 to 10 min;
- **M/Tn** (%) = Meat Area / TAn*100 — Ratio, n=1 to 10 min (Hoffman *et al.*, 1982);
- **LAn** (mm^2) = TAn - Meat Area — Liquid Area, n=1 to 10 min;

- **FWn** (mg of H_2O) = (LAn / 9.48) – 8 Free Water, n=1 to 10 min (Grau and Hamm, 1957).

These dependent variables are an example of a few different ways to express the Water Holding Capacity found in the quoted bibliography.

Results and discussion

A three-factor main effects model (load, filter paper and animal type) and meat sample weight as covariate, was used because interactions among main factors were not statistically significant. The problem to measure the particularly pale meat of pork and the even paler chicken meat was overcome changing the BT-WHCi light threshold (Fig. 1). The covariate (meat sample weight: 250.6±2.9 mg) was not significant, indicating a homogeneous sample preparation. The compression time effect was not submitted to statistic analysis, since the measurement is usually taken at a fixed time, but the increasing trend is clearly evident (Table 1). The loads were respected and variability was kept low: 294.4±2.7, 491.8±2.0 and 584.7±6.2 N. The dependent variable mean data are reported in Table 1, while animal type data were only partially reported in the text.

Total Area

The load effect was always significant and a higher load corresponded to a higher TA. No paper filter effect appeared until the 7th min; TA was significantly higher in W42 between 8th and 10th min. The increasing trend in time observed in TA indicated still available free water beyond the 10th min at 588.4 N load. Significant differences among animal types appeared at all minutes. Values were lower in pork and higher in beef. At the 5th min, Total Areas were: 1234, 1319 and 1397 mm^2 respectively for pork, chicken and beef. A different order was found for the Meat Area (Time 0') that was: beef, pork and chicken (678, 723 and 865 mm^2).

Meat Area / Total Area

The load effect started at the 5th min; M/T grew as the load increased. No effect was ascribed to the filter paper. Decreasing trend in time confirmed the presence of free water still available beyond the 10th min. M/T was significantly different among animal types throughout the ten minutes. Values were lower in beef and higher in chicken meat. The percentages at the 5th min were: 48.4, 59.2 and 65.6% respectively for beef, pork and chicken.

Liquid Area

The load effect appeared at the first min until the 7th min; values were higher with higher load. After the 7th min, no significant differences were seen in LA, though always positively correlated with load. The filter paper effect started at the 7th min with values significantly higher in W42. LA was significantly different among animal types throughout the total elapsed time. Chicken meat had the lowest values and beef the highest ones. The values at the 5th min were: 454, 511 and 720 mm^2 respectively for pork, chicken and beef.

Free Water

FW is a formula based on LA, consequently the effects of the load, the filter paper and the time were the same already observed in Liquid Area. Also FW showed significant differences among animal types throughout the ten minutes. In this case chicken had the lowest values and beef the highest ones. The values at the 5th min were: 39.9, 45.9 and 67.9 mg H_2O for chicken, pork ad beef, equivalent respectively to 16, 18 and 27% of Free Water.

These results confirm that load and paper filter significantly influence WHC values. Different applications of Grau and Hamm's method do not allow to ensure comparable data from researches. Moreover the way of the area measurement must be considered as an important source of variability (planimeter, outlining or not, VIA) which has been solved in this research. Finally, diverse formulae to express WHC complicate comparison, do not highlight considered factor effects and lead to different conclusions, such as evinced by M/T, TA, LA and FW.

Conclusions

The Filter Paper Press Method has the advantage to be rapid and simple but a proper standardisation is necessary. The obtained results clearly show that WHC values depend on: applied load, filter paper type, compression time, area measurement method and the dependent variable used to express it.

In this research all these factors have been kept under control so that it will be possible to define and standardize a set of working conditions and to automate it in a reliable way.

The application to different animal types, in particular chicken, shows that BT-WHCi also works under very difficult light conditions and last but not least, results can be directly comparable.

Many methods and different operating conditions of Filter Paper Press Method have been published in literature causing confusion to interpret data in this field. Now it will be possible to reassess the Filter Paper Press Method and to define the most useful operating conditions for it.
In this way an instrumental technique for rapid screening can be used to measure the meat Water Holding Capacity and improve meat quality control which is of great importance to the Industry and the Consumers.

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Beef Pork Chicken

Figure 1. Different hues in beef, pork and chicken on filter paper (Whatman 1).

Table 1. Dependent variable mean values (EDF=155).

Time	Load (N)			Filter paper		EMS
(min)	294.2	490.3	588.4	W1	W42	
Total Area (mm^2)						
0	686^{A}	758^{B}	822^{C}	755	754	2854
1	946^{A}	1049^{B}	1119^{C}	1052	1024	10899
2	1049^{A}	1155^{B}	1223^{C}	1151	1134	10684
3	1119^{A}	1229^{B}	1303^{C}	1219	1215	10202
4	1174^{A}	1285^{B}	1360^{C}	1271	1275	9695
5	1220^{A}	1329^{B}	1401^{C}	1309	1324	9551
6	1257^{A}	1365^{B}	1437^{C}	1344	1363	8969
7	1287^{A}	1393^{B}	1462^{C}	1368	1393	9087
8	1318^{A}	1417^{B}	1486^{C}	1393^{a}	1422^{b}	8204
9	1342^{A}	1437^{B}	1503^{C}	1411^{a}	1444^{b}	7924
10	1362^{A}	1453^{B}	1517^{C}	1426^{a}	1462^{b}	7622
Meat Area / Total Area (%)						
1	73.8	73.4	74.4	72.8	75.0	66
2	66.3	66.4	68.0	66.2	67.6	49
3	62.0	62.2	63.6	62.4	62.9	37
4	59.0	59.4	60.9	59.7	59.8	30
5	56.7^{A}	57.4	59.1^{B}	58.0	57.5	26
6	55.0^{A}	55.8	57.5^{B}	56.4	55.9	23
7	53.7^{A}	54.7^{a}	56.6^{Bb}	55.4	54.6	22
8	52.3^{A}	53.7^{a}	55.6^{Aa}	54.3	53.5	20
9	51.4^{aA}	53.0^{b}	55.0^{cB}	53.6	52.6	19
10	50.6^{aA}	52.3^{b}	54.5^{cB}	53.0	52.0	18
Liquid Area (mm^2)						
1	260^{a}	291	297^{b}	296	269	9706
2	363^{a}	398	401^{b}	395	379	10019
3	433^{a}	471^{b}	482^{b}	464	460	9474
4	488^{aA}	527^{b}	538^{B}	515	521	9011
5	534^{a}	571^{b}	579^{b}	553	570	9000
6	572^{a}	607^{b}	616^{b}	589	608	8587
7	601^{a}	636^{b}	640^{b}	613^{a}	639^{b}	8797
8	632	659	664	637^{a}	667^{b}	8207
9	657	679	682	656^{a}	689^{b}	8129
10	677	696	695	671^{a}	707^{b}	8017
Free Water (mg of H_2O)						
1	19.4^{a}	22.7	23.3^{b}	23.3	20.4	108.0
2	30.3^{a}	33.9^{b}	34.4^{b}	33.7	32.0	111.5
3	37.7^{a}	41.7^{b}	42.8^{b}	40.9	40.5	105.4
4	43.5^{aA}	47.6^{b}	48.8^{B}	46.3	47.0	100.3
5	48.4^{a}	52.2^{b}	53.1^{b}	50.4	52.1	100.1
6	52.3^{a}	56.1^{b}	57.0^{b}	54.1	56.1	95.5
7	55.4^{a}	59.1^{b}	59.6^{b}	56.6^{a}	59.4^{b}	97.9
8	58.7^{a}	61.5	62.1^{b}	59.2^{a}	62.4^{b}	91.3
9	61.3	63.7	63.9	61.2^{a}	64.7^{b}	90.4
10	63.4	65.4	65.3	62.8^{a}	66.6^{b}	89.2

A B C = $P<0.01$; a b c = $P<0.05$

EFFECTS OF INTRAMUSCULAR FAT DEPOSITION ON THE BEEF TRAITS OF JAPANESE BLACK STEERS

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Background

Marbling score is the most important factor for the carcass classification in Japanese market. Japanese Black cattle, known as "WAGYU", are characterized for their unique ability to deposit a large amount of intramuscular fat in muscles during the fatting period (Zembayashi et al.,1988). Matsuishi et al. (2001) reported that one of the main reasons why Japanese people preferred Wagyu beef was the preferable, sweet and fatty aroma that comes only from high marbling WAGYU. A higher price is therefore paid in Japan for carcasses with more marbling than for those with less marbling. Recently the production of beef containing significant marbling has been more strongly encouraged than previously, which seems to have occurred after the liberalization of beef imports in 1990 because of the need to distinguish domestic beef from imported beef.

Objectives

Beef quality traits as seen in the market place appear to have changed in the last decade due to this trend. Little is known about the effects of increased marbling on beef quality such as chemical composition and free amino acid content. The purpose of this study was to clarify the relationship between fat content and meat quality, and to determine any new trends or characteristics of recent Wagyu meat.

Materials and methods

Twenty-one Japanese Black steers were slaughtered, and a portion of *M. Longissmus thoracicus* (*LT* muscle) was taken from the carcasses and aged at 0°C for 15 days. The sample of *LT* muscle was minced. The moisture content was calculated by the weight difference between before and after freeze-drying. Crude protein was determined by Kjeldahl crude nitrogen analysis and crude fat by Soxhlet extraction. To evaluate the cooking loss and toughness, samples were cut into cubes (ca. 100g each). These samples were weighed before and after cooking, the difference being the rate of cooking loss. Then, the Warner-Bratzler shear force value of cooked sample was measured. Approximately 10 g of fresh muscle was homogenized with an equivalent weight of 5% sulfosalicylic acid and the solution was then used for the analysis of free amino acids by an auto amino acid analyzer.

Results and discussion

Moisture, protein and fat content

As the fat content increased from 4.8% to 39.0%, the moisture decreased linearly from 72.9% to 45.6%, and the coefficient of this correlation was 0.97 ($p<0.01$, Figure 1). In contrast, the protein level was relatively constant with ca. 18% to low fat content. A marked decrease was observed in the region of higher fat content (Figure 2). A linear, piecewise regression model was used to describe the relationship between fat content (x) and protein content (y) and equations were expressed by $y = -0.048x + 19.082$ and $y = -0.288x + 24.578$. The intersection point was $x = 22.867$ ($r^2 = 0.91$). It is generally accepted that the moisture content of bovine muscle has a negative correlation with fat content, and protein is relatively constant irrespective of fat content. Our result of the protein content with lower fat agreed with this statement, but markedly decreased protein has not been reported before. Gotoh et al. (1999) suggested that type I fibers were replaced by type IIB fibers in *LT* muscles during the fatting period. Type I fibers had the ability to accumulate intramuscular fat (Suzuki et al., 1978) and the diameter of type I fibers was shorter than that of type IIB fibers (Calkins et al., 1981). From these reports, *LT* muscles containing higher fat in this study would have a higher percentage of type I fibers, and fat would be present where fibers (protein) should be. The protein content

per unit mass consequently decreased and fat developed in the endomysium. This finding is applicable to beef containing 23% or more fat.

Cooking loss

Cooking loss was negatively correlated with fat content and the slope of the relation was weak in the area of low fat content, but a steep slope was observed in the high fat meat. This tendency was similar to the relation between fat and protein. A piecewise linear regression model was also applied and the relationship was expressed by $y = -0.116x + 25.247$ and $y = -0.662x + 40.555$ and the intercept of the two line segments was 28.02 ($r^2 = 0.97$). This indicates that the cooking loss remarkably decreases in meat including 28% of fat or more. A not significant but negative correlation ($r = -0.32$) was reported between fat content and cooking loss using beef with fat below 20% (Mitsumoto et al., 1995). Our result of a weak correlation in the region of low fat agreed with their report. However, a strong correlation was observed with fat of 28% or above in this study. Cooking loss mainly occurs by keeping the meat juice out of the contracted muscle fibers by heating, and the loss of holding capacity by protein denaturation. Therefore, cooking loss is lower in meat with lower moisture, and in addition, moisture release is lower with lower protein content. Markedly decreased cooking loss was consequently observed in meat with 28% fat content or above.

Tenderness

The results of SFV ranged from 2.08 to 4.56 kg/cm^2 and a significant negative correlation ($r=-0.83$, $p<0.05$) was observed between the SFV and fat level (Figure 4). This observation was different from the relation of protein content or cooking loss to fat content which was expressed by piecewise regression model (Figures 2 and 3). Adipose tissues in *LT* muscle appeared to disrupt the structure of intramuscular connective tissue (Nishimura et al., 1999). And matrix metalloproteinases (MMPs) which increased during adipocyte differentiation (Bouloumine et al., 2001) could improve the meat tenderness (Phillips et al., 2000). In addition to the above factors, the decreased diameter of muscle fibers would lead to the production of unique tender beef, due to the replacement of type IIB with typeI in beef with significant marbling (>23% of fat) in the present study.

Free amino acid content

Table 1 shows the correlation coefficient between fat content and each free amino acid and peptide, expressed as both wet meat and protein base. In the wet meat base, almost all free amino acids and Ans and Car content except Glutamine (Gln) had a significantly negative correlation with fat content ($p < 0.001$ - 0.05). In the protein base, although there was no significant relationship in some free amino acids, a significant negative correlation was still observed in several amino acids (Threonin, Serine, Alanin, Valine, Methionin, IsoLeucine, Leucine, Arginin). The same trend was found in Car. It can be seen that these negative correlations were due to a lower protein content to higher fat beef as mentioned above (Figure 2). However, this does not apply to meat with low fat (<23%), because of a relatively constant protein content (Figure 2). Lower levels of free amino acids were observed in muscles at 35 months than 25 months in cattle (Watanabe et al., 2004), and this suggested that the results of lower concentration would be due to a lower muscle growth rate. Therefore, the reason for the negative correlation between the fat content and most free amino acids can be explained by not only protein content but also animal maturity. On the other hand, Gln has a positive relationship to fat content in both wet and protein bases. Gln has the ability to stimulate the formation of lipids (Yatzidis, 2002) or lipogenesis (Lavoinne et al., 1987). Cornet and Bouset (1998) reported that red oxidative muscle had higher levels of Gln, and increased fat content lead to increased type I fibers (red oxidative muscle) as mentioned above. These reports could explain why Gln increases as fat increases.

Conclusions

The effects of intramuscular fat deposition on the chemical composition, tenderness, and free amino acid concentration in beef were studied using various classified carcasses of 21 Japanese Black (Wagyu) steers. Fat content ranged from 4.8 to 39.0% in *M. Longissmus thoracicus,* respectively. The protein content was relatively constant until there was ca. 23% of fat, and then decreased as the fat increased. Cooking loss was also constant until there was ca. 28% of fat, and markedly decreased with the fat increase. A weak correlation was found between the shear-force value and the fat content ($r=-0.83$, $P<0.05$). Most of the amino acids had a negative correlation to the fat content except glutamine, and this observation continued even when calculated by protein base. It is concluded that too much amount of fat in beef would be a possibility of leading to the protein content decrease. And consequently, it would influence the level of cooking loss and free amino acid content.

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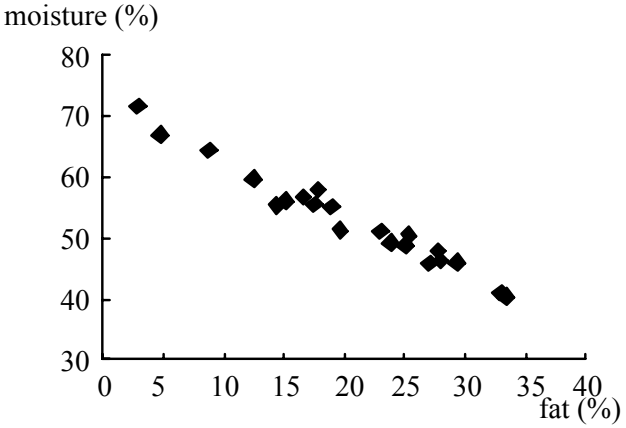

Figure 1. Correlation between fat content and moisture content in *M.Longissimus thoracicus* from Japanese Black steer.
$y = -0.743x + 74.933$ ($p<0.01$, $r = -0.97$)

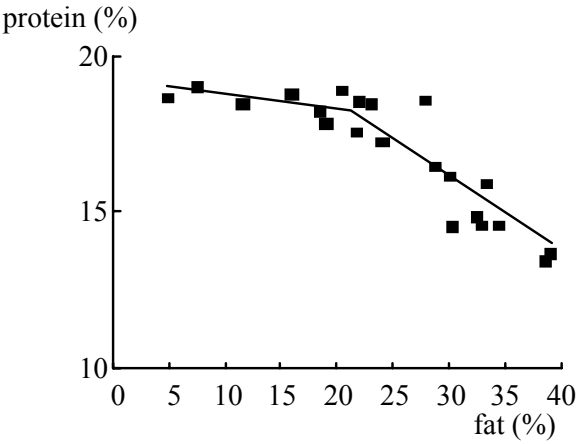

Figure 2. Correlation between fat content and protein content in *M.Longissimus thoracicus* from Japanese Black steer

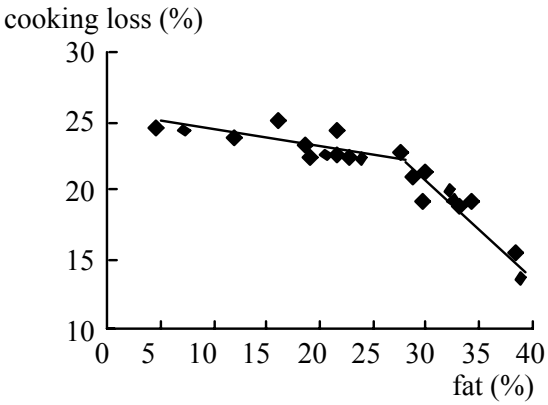

Figure 3. Correlation between fat content and cooking loss in *M.Longissimus thoracicus* from Japanese Black steer

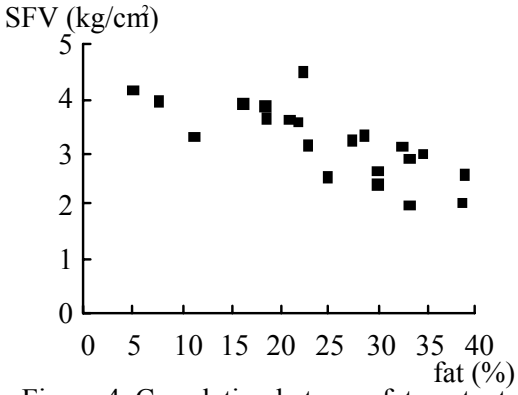

Figure 4. Correlation between fat content and SFV in *Longissimus thoracicus* from Japanese Black cattle.
$y = -0.053x + 4.519$ ($p<0.05$, $r = -0.83$)

Table 1. Correlation coefficients between fat content and free amino acids

	wet meat base	protein base
Asp	-0.76 *	-0.60
Thr	-0.78 **	-0.72 *
Ser	-0.78 **	-0.72 *
Glu	-0.70 *	-0.64
Gln	0.69 *	0.67 *
Gly	-0.81 ***	-0.72
Ala	-0.88 ***	-0.74 *
Val	-0.77 **	-0.72 *
Met	-0.81 **	-0.75 *
Ile	-0.78 **	-0.73 *
Leu	-0.79 **	-0.73 *
Tyr	-0.78 **	-0.72
Phe	-0.78 **	-0.72
His	-0.78 ***	-0.70
Lys	-0.74 *	-0.69
Ans	-0.46	-0.08
Car	-0.87 ***	-0.61
Arg	-0.76 **	-0.69 *

*;$p<0.05$, **;$p<0.01$, ***;$p<0.001$

EFFECT OF CARCASS SUSPENSION METHOD ON WATER HOLDING CAPACITY OF *M. LONGISSIMUS* FROM FALLOW DEER (*DAMA DAMA*) AND LAMB

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Background

Quality assurance of deer meat (venison) is essential to long-term product marketability and has been identified as a key challenge for the Australian deer industry. Consumer attitudes and preferences are increasingly important for all meat industries, and pasture based production systems (as used for deer and lamb) are often valued by consumers as more animal friendly and ethical compared with the intensive production of beef in feed-lots, pork and chicken. Technological meat quality attributes like pH and water holding capacity are related to the functional properties of meat during storage and processing. These quality parameters also have an indirect influence on the sensory quality of meat, such as tenderness, juiciness and flavour, valued by consumers as the most important in relation to the eating quality of meat.

Techniques for hanging carcasses (Achilles tendon suspension and pelvic suspension) have been studied for beef (Hostetler *et al*., 1970; Lundesjö Ahnström *et al*., 2003) where the variation in tenderness is considered to be the main reason for consumer dissatisfaction (Koohmaraie, 1996). The tenderness in meat from bulls was more improved as an effect of pelvic suspension compared with meat from heifers (Fisher, 1994; Lundesjö Ahnström *et al*., 2003). Similar results have also been reported for fallow deer (*Dama dama*), where pelvic suspension of the carcasses had the greatest impact on meat tenderness in venison from young male fallow deer (Sims *et al*., 2004). In addition, previous and recent research has demonstrated quality differences in meat from ruminants (beef, reindeer (*Rangifer tarandus tarandus*) and red deer (*Cervus elaphus*) that have been grazing pasture or fed grain-based feed mixtures (Daly *et al*., 1999; Wiklund *et al*., 2001a; 2003a; 2003b; Wood *et al*., 2003; Bruce *et al*., 2004). However, research in the area of venison quality has been very limited and further studies of the relationship between production system, slaughter handling techniques and consumer acceptance of the final products are essential.

Objectives

The objective of this study was to compare the effects of two different techniques for hanging carcasses (Achilles tendon suspension and pelvic suspension) on the water-holding properties of deer and lamb meat. In addition, the water holding capacity of long term chilled (up to 6 weeks post slaughter) deer and lamb meat was measured.

Materials and methods

Ten female lambs (F1 Merino/Border Leicester x Texel, 5½ months old) and 10 female fallow deer (15 months old) raised at the University of Western Sydney, were included in the study. The animals were fasted for 16 h prior to slaughter, stunned with a captive bolt and bled using thoracic stick exsanguination within 10 s of the stun (ethics approval UWS 04.01). The dressed carcasses were split along the mid ventral axis approx. 45 – 75 min post slaughter, and each carcass side was randomly assigned to either Achilles tendon suspension (control treatment) or pelvic suspension. At 1 day *post mortem* (*pm*), both *M. longissimus* (striploins) from each carcass were collected, cut in 5 pieces and randomly assigned to a treatment of 1, 3 or 6 weeks of refrigerated storage (+2° C), freezing (-20° C) or to be analysed as fresh meat. Drip loss was measured on the fresh meat samples after hanging meat samples in plastic bags for 2 days at + 2° C. All other samples were vacuum packaged. Purge (drip loss in the vacuum bags) was measured after 1, 3 and 6 weeks of refrigerated storage by the following procedure: (1) the weight of meat and vacuum bag was recorded before opening; (2) at opening, any surplus drip on the meat was removed using a paper towel and

the drip-free weight of the meat recorded. Thaw loss was measured using the same technique as described for the purge measurements after the meat samples had been removed from the freezer and thawed overnight at +2° C. Cooking loss was measured for all treatments (fresh, frozen/thawed and chilled-stored meat) after the vacuum packed meat samples had been heated to 70° C in a water bath. The samples were weighed in the same way as described for the purge measurements. The data was analysed statistically by analysis of variance, fitting species for the slaughter data, and species, hanging treatment and their interaction with animal as a blocking factor for the meat quality data, using the statistical package GenStat (2002).

Results and discussion

The lambs had higher live weights compared with the deer ($p \leq 0.01$), but lower carcass weights and dressing percentages ($p \leq 0.001$) (Table 1). pH decline registered 1-24 hours *pm* did not differ ($p > 0.05$) between the two carcass treatments or between species (Fig. 1), and the mean ultimate pH values recorded at 24 h *pm* in *M. longissimus* (LD) were 5.69 (lamb) and 5.68 (deer). However, after 1 week of refrigerated storage pH dropped below 5.60, and was significantly lower in the lamb than in the deer samples (Table 1). After 3 and 6 weeks of storage pH was not significantly different from ultimate pH (Table 1). These results are in good agreement with an earlier study of red deer meat, where the same pattern for pH variation over storage time was observed (Wiklund *et al*., 2001b). Mean temperature was higher in lamb than in deer LD at 1 hour ($p \leq 0.05$) and lower at 24 hours ($p \leq 0.05$) *pm*, but was not significantly different between species during the main intermediate period of decline.

Among the measured water-holding traits, the carcass suspension technique had an effect on drip loss and purge. Drip loss in fresh meat was significantly lower with Achilles tendon suspension, while purge in the vacuum bags during storage was lower with pelvic suspension, significantly so for 3 weeks storage (Fig. 2). The only significant difference between species was found in drip loss where the deer meat had better water-holding capacity than the lamb meat (1.67% versus 2.24% of drip loss). Cooking loss and freeze/thaw loss was not affected by carcass treatment ($p \geq 0.05$), although a significantly higher cooking loss in the deer samples compared with the lamb samples was registered in fresh meat (Fig. 3). The present results on the positive effects of pelvic suspension on purge in deer and lamb support earlier findings in beef (Lundesjö Ahnström *et al*., 2003), though in the beef study positive effects of pelvic suspension on cooking loss and freeze/thaw loss were also reported. The present values measured for drip loss and cooking loss in lamb samples were in good agreement with previously reported values for lamb LD (Hoffman *et al*., 2003).

Conclusions

Knowledge about the quality attributes of fresh chilled venison is of strategic importance to the Australian deer industry. Today most of the venison produced is sold frozen, but the demand for fresh meat is expected to increase in the future. Pelvic suspension of carcasses has been demonstrated to improve tenderness in meat from young fallow deer bucks, the type of animals most likely to be supplied for commercial slaughter in Australia. The present results indicate that pelvic suspension also could be positive for water-holding properties of fresh chill-stored fallow deer venison. Pelvic suspension is suggested to be a method that can be used to enhance consumer important quality attributes of venison. Sensory analysis (using a trained expert panel and consumer preference tests) of venison from the two different carcass treatments as well as similar studies on red deer venison are subjects recommended for further investigation.

Acknowledgements

The authors wish to thank Dr Jason Flesch for his professional assistance during slaughter and the collection of samples. Financial support for this work was provided by OECD (Organisation for Economic Co-operation and Development, Paris, France) within the programme ”Biological Resource Management for Sustainable Agricultural Systems”.

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Table 1. Mean live weight, carcass parameters and pH values in *M. longissimus* after 1, 3 and 6 weeks of refrigerated storage (+2° C) of the fallow deer and lamb included in the study, with standard errors of the difference (S.E.D.)

Trait	Deer (*n*=10)	Lamb (*n*=10)	S.E.D.	Degree of sign.[1]
Live weight, kg	36.7	39.9	1.08	**
Carcass weight, kg	21.2	19.1	0.58	***
Dressing percentage	57.9	47.8	0.72	***
pH values in *M. longissimus*				
1 week	5.59	5.55	0.010	***
3 weeks	5.67	5.67	0.015	n.s.
6 weeks	5.68	5.66	0.009	*

[1] n.s. = p>0.05; * = p≤0.05; ** = p≤0.01; *** = p≤0.001.

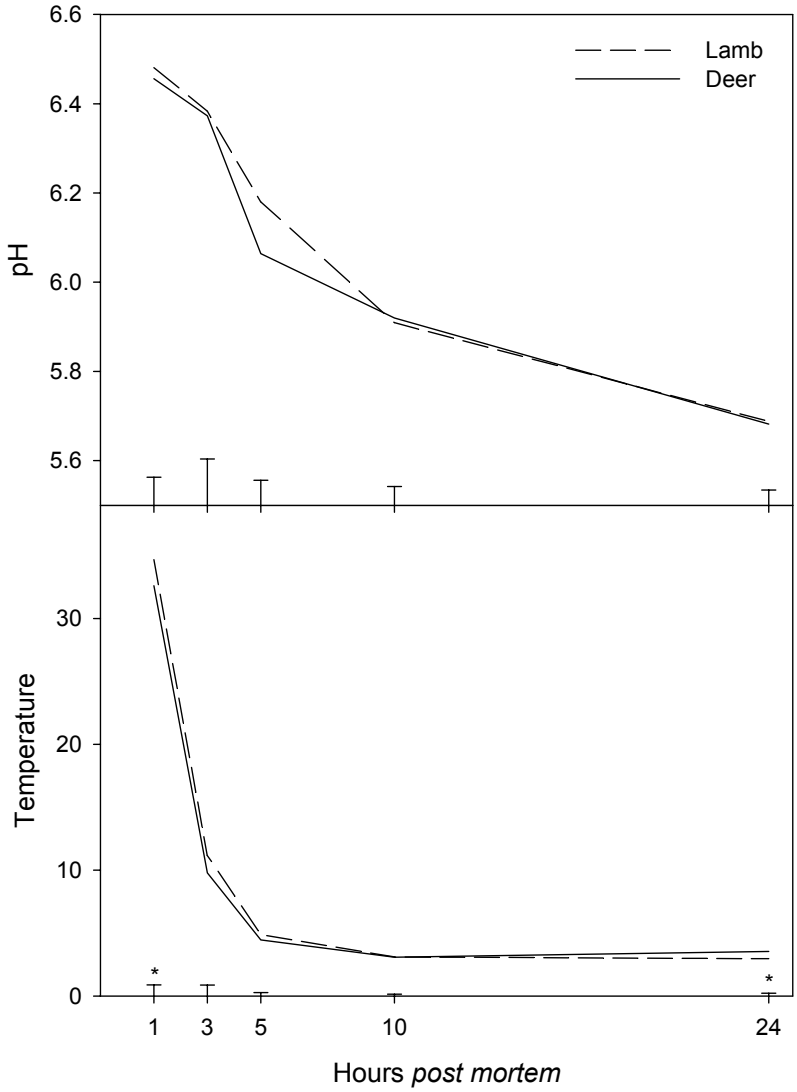

Figure 1. Mean temperature and pH (with standard errors of difference) measured at 1, 3, 5, 10 and 24 hours *post mortem* in *M. longissimus* from lamb (*n*=10) and fallow deer (*n*=10) included in the study.

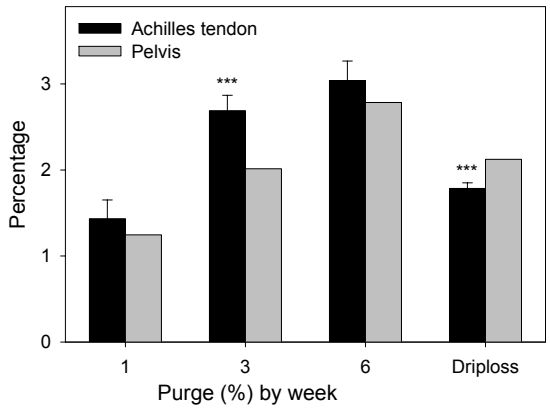

Figure 2. Purge (measured after 1, 3 and 6 weeks of refrigerated storage at +2° C) and drip loss in *M. longissimus* from lamb (*n*=10) and fallow deer (*n*=10) carcasses subjected to two different treatments; Achilles tendon suspension and pelvic suspension.

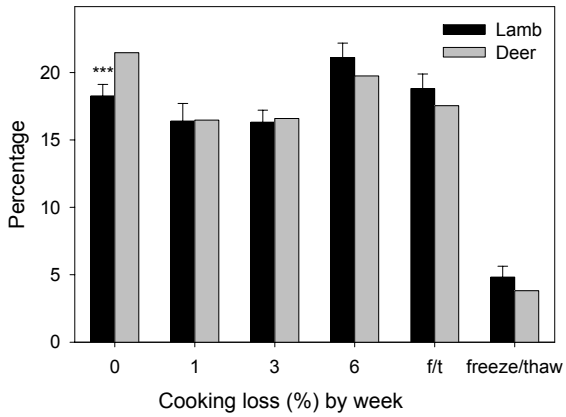

Figure 3. Cooking loss (in fresh meat (0), frozen/ thawed meat (f/t) and meat stored for 1, 3 and 6 weeks at +2° C) and freeze/thaw loss in *M. longissimus* from lamb (*n*=10) and fallow deer (*n*=10) included in the study.

50th International Congress of Meat Science and Technology

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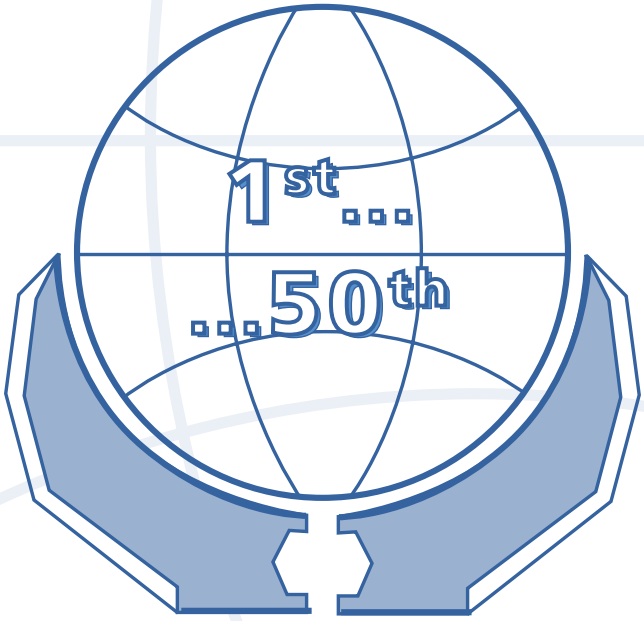

THE SAFETY OF PASTEURISED IN-PACK CHILLED MEAT PRODUCTS WITH RESPECT TO THE FOODBORNE BOTULISM HAZARD

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Abstract

There has been a substantial increase in sales of pasteurised in-pack chilled products over the last decade. It is anticipated that this trend will continue. These foods address consumer demand in being of high quality and requiring little preparation time. The microbiological safety of these foods commonly depends on a combination of a minimal heat treatment, refrigerated storage and a restricted shelf-life. The principal microbiological safety hazard for pasteurised in-pack meat products is foodborne botulism, as presented by non-proteolytic *Clostridium botulinum*. This review provides a summary of research that has contributed to the safe development of these foods without incidence of botulism.

Key words:
Clostridium botulinum*, botulism, pasteurised, sous-vide, meat, chilled*

* corresponding author

Introduction to pasteurised in-pack chilled products

Consumer demand for foods that require minimal preparation time compared with conventional meals, are of high quality, contain low levels of preservatives, and are only minimally processed has led to the development of foods that are pasteurised in-pack. These foods are also known as sous-vide foods, cook-chill ready-to-eat foods, and refrigerated processed foods of extended durability (REPFEDs). These foods are processed at a lower temperature (maximum generally within the range 70°-95°C) than, for example, canned foods. This heat process is intended to minimise loss of sensory and organoleptic quality. After heating, the food is cooled rapidly, and stored at refrigeration temperatures (1° - 8°C). These foods are not sterile, and product shelf-life is dependent on the heat treatment applied and storage temperature. In some circumstances intrinsic properties of the food (e.g. pH, water activity) may also contribute to an extended shelf-life. Typically, the shelf-life can be up to 42 days. Sales of pasteurised in-pack products and other chilled foods are currently increasing at a tremendous rate in many European countries. The European prepared chilled food sector showed a 50% increase in the period 1991-1996, to an estimated € 9,100 million (ECFF, 1998). In the UK alone, sales of prepared chilled foods increased by 45% between 1999 and 2003, to reach an estimated € 7,400 (Chilled Food Association, 2004).

Assessment of microbiological safety hazards associated with pasteurised in-pack chilled products

Pasteurised in-pack products are not sterile, and safety and quality is dependent on a combination of the minimal heat process, storage temperature, shelf-life and perhaps also intrinsic properties of the food (Peck,

1997). The mild heat treatment applied should eliminate cells of vegetative bacteria, but not bacterial or fungal spores. Thus, non-spore-forming pathogens such as *Listeria monocytogenes*, *Yersinia enterocolitica*, diarrheagenic *Escherichia coli*, *Campylobacter jejuni*, and salmonellae should be eliminated. By heating in-pack, post process contamination is prevented. Pasteurised in-pack products are frequently packed under vacuum or an anaerobic atmosphere. This restricts growth of microorganisms that require oxygen for growth, such as molds, but favours growth of microorganisms that can grow in the absence of oxygen. The storage of these foods at refrigeration temperatures prevents growth of mesophilic spore-forming bacteria, although growth may be possible if temperature abuse occurs. Overall, this minimal process favours spore-forming microorganisms that grow in the absence of oxygen at refrigeration temperatures. In particular, concern exists about the potential for growth and neurotoxin production by non-proteolytic *Clostridium botulinum* in the absence of a competing microflora, and the associated foodborne botulism hazard (Peck, 1997; Lund and Peck, 2000). It is essential that pasteurised in-pack products be developed without an associated increase in the incidence of foodborne botulism. Additionally, concern also exists about the hazard presented by psychrotrophic strains of *Bacillus cereus* (Carlin *et al.,* 2000). While food poisoning caused by *B. cereus* is serious, it is generally viewed as a less severe disease than foodborne botulism.

Recommendations and guidelines to ensure the safe production of pasteurised in-pack chilled products with respect to *C. botulinum*

Guidelines and a code of practice have been targeted at ensuring the safe production of these foods by preventing growth and toxin production by non-proteolytic *C. botulinum* (ACMSF, 1992, 1995; ECFF, 1996; Betts, 1996; Gould, 1996; Martens, 1997; Gould, 1999). Growth and toxin production by proteolytic *C. botulinum* is prevented by ensuring storage is below 10°C. Recommendations produced by the UK Advisory Committee on the Microbiological Safety of Food (ACMSF) on procedures to ensure safety with respect to non-proteolytic *C. botulinum* are summarised in Table 1. It is recommended that the heat treatments or combination processes deliver a safety a factor of 10^6 (a 6D [six decimal] process) with regard to spores of non-proteolytic *C. botulinum* (ACMSF, 1992; ECFF, 1996). Possible limitations of these recommendations are described in later sections. It is likely that for a majority of these pasteurised foods, safety will rely on either option (4) or (8), as detailed in Table 1.

Table 1 Recommended procedures to ensure the safety of pasteurised in-pack chilled products with respect to non-proteolytic *C. botulinum* (ACMSF 1992, 1995)

(1) storage at <3.0°C*
(2) storage at ≤5°C and a shelf-life of ≤10 days
(3) storage at 5°-10°C and a shelf-life of ≤5 days
(4) storage at chill temperature** combined with heat treatment of 90°C for 10 min or equivalent lethality (e.g. 70°C for 1675 min, 75°C for 464 min, 80°C for 129 min, 85°C for 36 min (ACMSF, 1992) (note that the European Chilled Food Federation (ECFF, 1996) recommended 80°C for 270 min, 85°C for 52 min)).
(5) storage at chill temperature combined with ≤pH 5.0 throughout the food
(6) storage at chill temperature combined with a salt concentration ≥3.5% throughout the food
(7) storage at chill temperature combined with $\leq a_w$ 0.97 throughout the food
(8) storage at chill temperature combined with a combinations of heat treatment and other preservative factors which can be shown consistently to prevent growth and toxin production by *C. botulinum.*

Notes:
* originally 3.3°C, but growth has now been demonstrated at 3.0°C (Graham *et al*., 1997)
** chill temperature is specified as 8°C in UK

Clostridium botulinum and foodborne botulism

Characteristics of Clostridium botulinum and other neurotoxin producing clostridia

Six physiologically and phylogenetically distinct Gram-positive spore-forming anaerobic bacteria can produce botulinum neurotoxin (Table 2), although the name of *C. botulinum* is retained to emphasise the importance of neurotoxin production (Lund and Peck, 2000). Some strains of *C. baratii* and *C. butyricum* also produce neurotoxin. For each of the six organisms, a non-neurotoxigenic phylogenetically equivalent organism is known (Hatheway, 1992). The different physiology of the six organisms is reflected in the circumstances in which they present a hazard. For example, proteolytic *C. botulinum* and non-proteolytic *C. botulinum* are responsible for most cases of foodborne botulism (Lund and Peck, 2000). Differences in the physiology of these two organisms are summarised in Table 3, and because of these differences they present a hazard in different types of foods. Proteolytic *C. botulinum* is a mesophile, and produces spores of high heat resistance. The canning process for low-acid foods is designed to inactivate spores of this organism, and botulism outbreaks have occurred when the full heat treatment has not been appropriately delivered. Spores of non-proteolytic *C. botulinum* are of moderate heat resistance, but this organism can multiply and form neurotoxin at temperatures as low as 3.0°C. Botulism outbreaks associated non-proteolytic *C. botulinum* have occurred most frequently with processed fish, with for example outbreaks involving vacuum packed smoked fish reported in Sweden and Germany. This organism is the principal concern for the safety of pasteurised in-pack chilled meat products.

Table 2. Characteristics of the six phylogenetically distinct clostridia that can produce the botulinum neurotoxin

Neurotoxigenic organism	Neurotoxins formed	Non-neurotoxigenic equivalent organism	Associated with foodborne botulism
C. botulinum Group I (proteolytic)	A, B, F	*C. sporogenes*	++
C. botulinum Group II (non-proteolytic)	B, E, F	no name given	++
C. botulinum Group III	C, D	*C. novyi*	-
C. botulinum Group IV (*C. argentinense*)	G	*C. subterminale*	-
C. baratii	F	all typical strains	+
C. butyricum	E	all typical strains	+

++ commonly associated, + rarely associated, - not known to be associated

Table 3 Characteristics of the two physiologically distinct clostridia most commonly responsible for foodborne botulism

	Proteolytic *C. botulinum* (*C. botulinum* Group I)	non-proteolytic *C. botulinum* (*C. botulinum* Group II)
neurotoxins formed	A, B, F	B, E, F
minimum growth pH	4.6	5.0
minimum growth temperature	10-12°C	3.0°C
maximum growth NaCl	10%	5%
spore heat resistance ($D_{100°C}$)	>15 min	<0.1 min
foods involved in botulism outbreaks	home canned foods, faulty commercial processing	fermented marine products, dried fish, vacuum packed fish
potential food problems	canned foods	pasteurised in-pack chilled products

Neurotoxins and characteristics of botulism

Foodborne botulism is a severe but rare disease. It is an intoxication resulting from consumption of pre-formed botulinum neurotoxin in food, with as little as 30 ng of neurotoxin sufficient to cause illness and even death. The consumption of as little as 0.1g of food in which a neurotoxin-producing clostridia has grown can result in botulism (Lund and Peck, 2000). There are seven botulinum neurotoxins (A to G), with the type of neurotoxin formed dependent on the producing organism (Table 2). The neurotoxins were originally distinguished on the basis of antigenic response. More recently, however, the amino acid sequence and mode of action of all the neurotoxins have been established (Dodds and Austin 1997; Lund and Peck, 2000). All seven botulinum neurotoxins comprise a heavy and light chain, and are often associated with other proteins (e.g. haemagglutinin, non-toxin non-haemagglutinin). The heavy chains deliver the light chains to the cytosol of the motor neuron, their site of action. The light chains possess zinc endopeptidase activity, and cleave protein components of the acetylcholine-containing synaptic vesicle docking/fusion complex. Each light chain cleaves a specific protein in this complex at a specific site. This cleavage prevents binding of acetylcholine-containing synaptic vesicles, preventing neurotransmitter release and leads to flaccid paralysis of the muscle. Typically symptoms of botulism are neurological and include, blurred vision, dysphagia (difficulty swallowing), generalised weakness, nausea/vomiting, dysphonia (difficulty speaking), dizziness/vertigo, and muscle weakness. Flaccid paralysis of the respiratory muscles can result in death if not treated. In many countries, equine antitoxin is administered in cases of foodborne botulism. Rapid treatment with equine antitoxin and supportive therapy has led to a reduction in the fatality rate to approximately 10% of cases, although full recovery may take many months or even longer. A fatality rate of 10% of cases is very high for a foodborne illness.

Incidence of spores of C. botulinum in foods and the environment.

Spores of proteolytic *C. botulinum* and of non-proteolytic *C. botulinum* are found in soils, sediments and the gastrointestinal tract of animals, and their distribution has been reviewed previously (Dodds, 1992a, 1992b; Dodds and Austin, 1997; Lund and Peck, 2000). Recently, information has been published on the incidence of spores in the environment and in foods in France (Fach *et al*., 2002; Carlin *et al*., 2004). Although often present in low numbers, their ubiquitous nature ensures that raw products cannot be guaranteed free of spores. Foods which are, or can become, anaerobic may allow growth of *C. botulinum* and must therefore be subjected to treatments that destroy spores, or stored under conditions that prevent growth and toxin formation.

Outbreaks of foodborne botulism

The name “botulism” was given to a disease reported in central Europe several hundred years ago that was frequently associated with consumption of blood sausage (“botulism” is derived from the Latin *botulus* meaning sausage). At the end of the nineteenth century, Emile van Ermengem, first isolated a causative organism from home made raw salted ham and the spleen of a man who later died of botulism. In the early part of the twentieth century, many outbreaks of botulism were identified across the world, and were frequently associated with the increased use of canning processes to extend shelf-life. By implementing effective control measures, the incidence of botulism is now lower than a century ago. Foodborne botulism involving commercial processing is uncommon, but on the rare occasions when commercial foods are involved, the medical and economic consequences can be high. It has been estimated that in the USA the cost per case of botulism is approximately $30 million, compared with $10,000-12,000 for each case of illness associated with *Listeria monocytogenes* and *Salmonella* (Setlow and Johnson, 1997). Today, most outbreaks of foodborne botulism are associated with home-prepared foods, where known control measures have not been implemented. For example, in Poland, 1301 outbreaks were reported between 1984-1987, and in Russia, 542 outbreaks were described between January 1998 and September 1999. These high incidences were associated with an increased reliance on the home bottling/canning of foods, reflecting difficult economic conditions. The incidence in Poland has fallen considerably in recent years. Many other countries have lower, but significant rates of foodborne botulism. For example, over the past 20 years, approximately 20-40 cases have been reported annually in Italy, Germany, France, and USA. Examples of recent outbreaks of foodborne botulism are given in Table 4 (see end of the paper). A recent finding has been the association of neurotoxigenic strains of *C. baratii* and *C. butyricum* with foodborne botulism. Outbreaks involving neurotoxigenic *C. butyricum* type E have been reported in China, India and Italy (Anniballi *et al.,* 2002). A suspected outbreak involving neurotoxigenic *C. baratii* type F, was reported in USA in 2001, and was associated with consumption of spaghetti noodles and meat sauce by a 41 year old

woman. A full recovery was eventually made, although 12 weeks were spent on a life support machine (Harvey *et al*., 2002).

Many cases of foodborne botulism have been associated with meat products (Hauschild, 1992; Lucke and Roberts, 1992). In France, Germany and Poland most cases of foodborne botulism are associated with consumption of meat dishes, while in Spain and Italy other foods are more frequently implicated (Lund and Peck, 2000). A large proportion of outbreaks in Germany and France are associated with home or farm salted ham. A majority of meatborne cases in Poland are associated with the home preparation of pork in glass jars (Lucke and Roberts, 1992). Several examples of recent outbreaks of meatborne botulism are included in Table 4. Commercial pasteurised in-pack chilled products have a good safety record with respect to foodborne botulism, although outbreaks involving clam chowder (USA, 1994) and vacuum packed fish (Germany, 1997) have occurred following temperature abuse. It is essential, however, that as new types of food are developed, they are developed safely with respect to the foodborne botulism hazard. Outbreaks involving home prepared vacuum packed fish are also reported (Table 4; see end of the paper).

Control of foodborne botulism hazard presented by proteolytic *C. botulinum* in pasteurised in-pack chilled meat products

Factors influencing growth and toxin formation

Providing that pasteurised in-pack chilled products are stored at an appropriate temperature, growth and toxin production by proteolytic *C. botulinum* is prevented. There is, however, concern that proteolytic *C. botulinum* may grow and form toxin in products that are temperature abused. The effect of individual environmental factors on growth of proteolytic *C. botulinum*, under otherwise optimum conditions, has been established. The minimum temperature at which growth and toxin production occurs is within the range of 10°-12°C. Growth and toxin formation have been described at 12°C in 3-4 weeks (Smelt and Haas, 1978; Peck, 1999), and Ohye and Scott (1953) determined growth rates at 12.5°C. Attempts to establish growth at 10°C or below have been generally unsuccessful (e.g. Ohye and Scott, 1953; Smelt and Hass, 1978; Peck, 1999). Although Tanner and Oglesby (1936) reported growth from vegetative inocula at 10°C, this observation was marred by a temporary rise in the incubator temperature to 20°C, and in the words of the authors "the results of this test may not be very significant". Growth from a spore inoculum was not observed at 10°C in 10 weeks (Tanner and Oglesby, 1936). It is generally accepted that growth is prevented at pH 4.6 or below, that 10% NaCl will prevent growth, and that the minimum water activity permitting growth is 0.93 and 0.96 with glycerol and NaCl, respectively, as the humectants (Hauschild, 1989; Lund and Peck, 2000). The use of other factors to control or prevent growth of proteolytic *C. botulinum* has been reviewed (Hauschild, 1989; Lund and Peck, 2000). The safety of some foods with respect to proteolytic *C. botulinum* may rely on a combination of factors rather than on one single factor. The effect of combinations of pH and NaCl concentration/water activity on time to growth has been determined in laboratory medium (Baird-Parker and Freame, 1967), vacuum-packed potatoes (Dodds, 1989) and in lumpfish caviar (Hauschild and Hilsheimer, 1979). Predictive models that quantify the effect of combinations of environmental conditions on the growth response of proteolytic *C. botulinum* have been developed (Baker and Genigeorgis, 1992; Lund, 1993; Dodds, 1993; Lund and Peck, 2000). These models provide information on interactions between two or more factors, allow predictions to be made for sets of conditions not tested (within the interpolation zone), and are of use in the targeting of challenge testing.

Heat resistance of spores of proteolytic C. botulinum

The relatively high heat resistance of spores of proteolytic *C. botulinum* makes their survival in low acid canned foods a subject of considerable importance. In response to outbreaks of botulism associated with the consumption of inadequately heat-treated foods, studies on the heat resistance of spores of proteolytic *C. botulinum* began in the early part of the twentieth century. From extensive research, it is generally accepted that a heat treatment at 121.1°C for 3 min (or heat treatments of equivalent lethality at other temperatures) will reduce the number of spores of proteolytic *C. botulinum* by a factor of 10^{12} (a 12D process), and this was adopted as the minimum standard for a "botulinum cook" for low acid canned foods (Stumbo *et al*., 1975). The heat resistance of spores of proteolytic *C. botulinum* is generally taken as $D_{121°C}$ = 0.2 min and z = 10°C (Stumbo *et al*., 1975), thus by extrapolation, at 91°C the D-value would be 200 min. The minimal heat treatments applied to pasteurised in-pack and related foods would, therefore, be unlikely to have any significant effect on spores of proteolytic *C. botulinum.*

Combinations of heat treatment, storage temperature and other factors that prevent growth and toxin production from spores of proteolytic C. botulinum

Studies on proteolytic *C. botulinum* have focused on assessing the hazard following temperature abuse. In one study, spores of a mixture of strains of proteolytic *C. botulinum* were inoculated into meat slurry (used as a model food) prepared under N_2, heated at 95°C for 23 min, cooled and subsequently incubated at refrigeration and abuse temperatures (Table 5). The heat treatment had only a marginal effect on time to growth. Growth and toxin production did not occur at 8°C, and at 12°C growth was observed within 21 days and toxin production confirmed (although time to growth was rather variable). At 16°C growth was first observed in 4-7 days, and at 25°C growth was first observed in 2 days (Table 5). These results indicate that if pasteurised in-pack products are abused at 16°C or higher (and possibly even at 12°C), then proteolytic *C. botulinum* is a serious concern.

Evaluation of time to growth and toxin formation in foods

Tests on time to toxin formation have been carried out with pasteurised in-pack pasta products subject to temperature abuse (Table 6). Also, while Del Torre *et al*. (1998) detected toxin formation in salmon-filled tortelli stored at 20°C for 30 days, toxin formation was not detected when this product was stored at 12°C for 50 days. Simpson *et al.* (1995) detected toxin formation after storage at 15°C for 14 days, when a spaghetti and meat-sauce was inoculated with 10^3 spores/g of proteolytic *C. botulinum*. These data emphasise the danger presented by proteolytic *C. botulinum* if these food products are temperature abused.

Table 5 Effect of heat treatment, subsequent incubation temperature and inoculum concentration on time to growth from spores of proteolytic *C. botulinum* types A, B and F in meat slurry (Plowman and Peck, unpublished data)

Incubation temperature	Heat treatment	Incubation time (days) to first observation of growth for each inoculum concentration	
		10^3 spores/tube	10^6 spores/tube
8°C	None	not tested	>60
12°C	None	49	21
	95°C/23min	56	28
16°C	None	6	4
	95°C/23min	7	4
25°C	None	2	2
	95°C/23min	2	2

Table 6 Examples of reported time to toxin formation in pasteurised in-pack chilled products inoculated with a low concentration of spores of proteolytic *C. botulinum* (Glass and Doyle, 1991; Del Torre *et al.,* 1998)

Pasta filling	Product details			Time (days) to toxin formation at specified temperature[a]	
	a_w	pH	atmosphere (CO_2:N_2:O_2)	20°C	30°C
Cheese	0.94	5.7	80:20:0	-[b]	70-NT
Meat	0.95	6.2	0:100:0	-	35-42
ricotta/spinach	0.95	5.9	15:83:2	30-50	-
Salmon	0.95	6.1	15:83:2	15-30	-
Meat	0.95	6.2	15:83:2	30-50	-
Artichoke	0.96	5.6	15:83:2	50-NT	-

[a] - last negative test and first positive test are given (NT = no toxin detected), inoculum spore concentration 1-7 x10^2 spores/g

[b] - not determined

Control of infant botulism hazard presented by proteolytic *C. botulinum* in pasteurised in-pack chilled meat products

Infant botulism is an infection. An immature intestinal flora in infants is unable to prevent colonisation by proteolytic *C. botulinum* (and also neurotoxigenic strains of *C. baratii* and *C. butyricum*), allowing ingested spores to germinate leading to cell multiplication and neurotoxin production. Infants aged between two weeks and six months are most susceptible. Symptoms typically include extended constipation and flaccid paralysis. Infant botulism is rarely fatal. Two sources of spores have been identified, honey and general environmental contamination (e.g. soil, dust). It is estimated that between 10 and 100 spores are sufficient to bring about infection. The first clinical cases of infant botulism were described in USA in 1976, although subsequent investigations revealed earlier cases. Infant botulism has now been reported in twelve European countries, and about half of these were associated with a history of honey consumption (Aureli *et al*., 2002). In view of this association, there are recommendations in several countries that jars of honey should carry a warning indicating that the product is not suitable for infants less than twelve months of age. A disease similar to infant botulism also very rarely affects adults, and occurs when competing bacteria in the normal intestinal flora have been suppressed (e.g. by antibiotic treatment).

It is anticipated that the heat treatment delivered to pasteurised in-pack chilled products will bring about little reduction in numbers of spores of proteolytic *C. botulinum*. While the foodborne hazard is controlled by storage at chilled temperature, the infant botulism hazard is not controlled (since spores will remain in the food, but will be unable to germinate and lead to growth in the food). Thus, there is a concern that these minimally processed foods may represent a risk of infant botulism (and possibly also infectious botulism in susceptible adults). This concern is particularly great for pasteurised in-pack products that are specifically targeted at infants less than twelve months of age.

Control of foodborne botulism hazard presented by non-proteolytic *C. botulinum* in pasteurised in-pack chilled meat products

Factors influencing growth and toxin formation

The minimum temperature at which growth and toxin production have been described is 3.0°C (Graham *et al*., 1997). In this study, vials containing 10 ml of PYGS (peptone, yeast extract, glucose, starch) medium were inoculated at 10^4 spores/ml, and growth was observed at 3.0°C after seven weeks, 3.1°C after six weeks, and 3.2°C after five weeks. The presence of toxin was confirmed. Earlier studies had demonstrated growth and toxin production at 3.3°C within five weeks (Schmidt *et al*., 1961; Eklund *et al*., 1967a, b). Growth and toxin production have not been detected during incubation at 2.1-2.5°C for 90 days (Ohye and Scott, 1957; Schmidt *et al*., 1961; Eklund *et al*., 1967a, b; Graham *et al*., 1997). Maintaining pasteurised in-pack products at a temperature of <3.0°C might be possible in some circumstances (e.g. institutions, catering establishments), but there is doubt as to whether temperatures in this range can always be maintained throughout the distribution chain, particularly in products intended for domestic use. Indeed, regulations require foods in this group to be held at 0°-3°C in Spain, 0°-4°C in France, ≤7°C in Belgium and ≤8°C in the UK (Martens, 1997). Thus, retail products should contain additional hurdles. It is generally recognised that growth and toxin production do not occur below pH 5.0, at a NaCl concentration above 5%, and that the minimum water activity permitting growth is 0.94 and 0.97 with glycerol and NaCl, respectively, as the humectants (Baird-Parker and Freame, 1967; Hauschild, 1989; Graham *et al*., 1996b; Lund and Peck, 2000). The effect of other preservative factors on growth of non-proteolytic *C. botulinum* has been reviewed elsewhere (e.g. Hauschild, 1989; Lund and Peck, 2000). The effect of redox potential and oxygen concentration on growth from unheated spores has been quantified (Lund, 1993). Furthermore, in studies with a model food system (meat slurry), an initial aerobic atmosphere (20% oxygen) did not restrict growth compared to a control atmosphere of nitrogen (Peck, 1999). In these circumstances, although the atmosphere was aerobic, the model food was sufficiently reduced so as to support growth and toxin production by non-proteolytic *C. botulinum*. The use of oxygen as a preservative factor is therefore cautioned, since despite the presence of oxygen, the food itself may be sufficiently reduced and support growth and toxin production by non-proteolytic *C. botulinum* (Snyder, 1996).

In some foods, safety with respect to non-proteolytic *C. botulinum* may be dependent upon more than one preservative factor. For example, although a sub-optimal pH or NaCl concentration might not prevent growth individually, they might in combination. The effect of combinations of pH and NaCl concentration on time to growth at chilled temperatures has been reported (Graham *et al*., 1997). An important development in describing the effect of combinations of preservative factors has been that of predictive models. Predictive models for non-proteolytic *C. botulinum* have been developed in foods and whilst they may predict well for some food groups, they may be of limited use in other types of food. Other models have been developed in laboratory media and may be rather more generic in application. These models provide information on interactions between two or more factors, and can be used to reduce the amount of challenge testing required to ensure product safety. Models have been developed that deliver growth curves, describe the effect of single and multiple factors on the probability of growth, or on time to toxin production at a single inoculum level (Baker *et al*., 1990; Baker and Genigeorgis, 1992; Dodds, 1993; Meng and Genigeorgis, 1993; Lund, 1993; McClure *et al*., 1994; Graham *et al*., 1996b; Whiting and Oriente, 1997; Lund and Peck, 2000; Fernandez *et al*., 2001). Where tested, predictions from these models generally compare well with observed growth and toxin production in independent datasets, giving the user confidence that these models can be used to target, more effectively, challenge testing. Some of these models are freely available through predictive modelling packages [Growth Predictor (www.ifr.ac.uk/Safety/GrowthPredictor/default.html), Pathogen Modeling Program (www.arserrc.gov/mfs/PMP6_Start.htm)]. Published (and in some cases also unpublished) original growth and death curves are compiled and also available free of charge in ComBase (www.combase.cc).

Heat resistance of spores of non-proteolytic C. botulinum

Spores of non-proteolytic *C. botulinum* are of moderate heat resistance, and are considerably less heat resistant than those of proteolytic *C. botulinum*. For example, heating spores for one min in phosphate buffer (pH 7.0) at 85°C, followed by enumeration of survivors on a nutrient medium gave in excess of a 5D kill (Peck *et al*., 1992a). Studies of spore heat resistance by thermal death time methods and by enumeration of survivors have led to estimates of D-values in phosphate buffer, the highest of which have generally been within the range of $D_{82.2°C}$ = 0.5-2.4 min (Lund and Peck, 2000). The measured heat resistance of spores of non-proteolytic *C. botulinum* can be influenced by the menstruum in which the spores are heated and the nutrient medium used for enumeration of survivors. When spores were heated in a model food (meat slurry, pH 6.5), heat treatments at 70°C for >2545 min, 75°C for 1793 min, 80°C for >363 min, 85°C for 36 min, and 90°C for 10 min were necessary to prevent growth from an inoculum of 10^6 spores of non-proteolytic *C. botulinum* (Fernandez and Peck, 1997). Thus, the heat treatments advocated by the ACMSF and the ECFF at 70°-80°C (Table 1) failed to reduce the number of spores of non-proteolytic *C. botulinum* by a factor of 10^6 (a 6D process). The advocated heat treatments were, however, adequate when combined with refrigerated storage and a restricted shelf-life (Fernandez and Peck, 1997).

The heat treatments described in the previous paragraph result in sub-lethal injury to spores of non-proteolytic *C. botulinum*. In particular, the heat treatments inactivate the spore germination system (Peck *et al*., 1992b; Lund and Peck, 1994). A proportion of these heat-damaged spores are permeable to lysozyme, and are able to germinate and give growth in/on media containing lysozyme, giving biphasic survival curves (Sebald and Ionesco, 1972; Alderton *et al*., 1974; Smelt, 1980; Peck *et al*., 1992a, 1992b, 1993; Lund and Peck, 1994; Stringer and Peck; 1996; Peck, 1997). Lysozyme can diffuse through the coat of a proportion (for most strains 0.1 - 1%) of heat-damaged spores, inducing germination by hydrolysing peptidoglycan in the spore cortex (Gould, 1989). Treatment of the heated spores with sodium thioglycollate disrupts the coat of a majority of the spores allowing lysozyme access to the cortex, inducing germination and subsequent growth of vegetative bacteria from a majority of sub-lethally heat-damaged spores. The straight line obtained with thioglycollate-treated spores provides evidence of the mode of action of lysozyme (Peck *et al*., 1992b, 1993; Lund and Peck, 1994; Peck, 1997). Following heat treatment in phosphate buffer, D-values for lysozyme-permeable spores were: $D_{85°C}$ = 100 min, $D_{90°C}$ = 18.7 min, $D_{95°C}$ = 4.4 min (Peck *et al*., 1993). These D-values are approximately two orders of magnitude greater than those obtained when spores were recovered in the absence of lysozyme. Lysozymes from several sources increase the measured heat resistance of spores of non-proteolytic *C. botulinum*, including; hen egg white lysozyme, other type c lysozymes (e.g. from other bird's eggs), other enzymes (chitinase, papain), fruit and vegetable extracts, egg yolk emulsion and horse blood (Peck and Stringer, 1996; Stringer and Peck, 1996; Stringer *et al*., 1999). Lysozyme activity has also been detected in many raw foods, and in most cases the activity is higher than that required to

increase the measured heat resistance of spores of non-proteolytic *C. botulinum* (Peck *et al*., 1992a; Lund and Peck, 1994; Peck and Stringer, 1996). Proposals have also been made to add hen egg white lysozyme and other lytic enzymes to foods as a preservative factor (Nielsen, 1991; Fuglsang, 1995), and enzymes capable of initiating germination may also be produced by other microorganisms (Lund and Peck, 1994). Because of this widespread occurrence of enzymes with lysozyme activity and the relatively high heat stability of lysozyme (Proctor and Cunningham, 1988; Lund and Peck, 1994; Peck and Fernandez, 1995), the effect of lysozyme on sub-lethally heat damaged spores is potentially important in foods that rely on heat-inactivation of spores of non-proteolytic *C. botulinum* for safety. In studies with a model food (meat slurry) with no added lysozyme, heat treatments of 85°C for 36 min, 90°C for 10 min and 95°C for 15 min each prevented an inoculum of 10^6 spores of non-proteolytic *C. botulinum* leading to growth and toxin formation at 25°C in 60 days (Peck *et al*., 1995; Peck and Fernandez, 1995; Graham *et al*., 1996a; Fernandez and Peck, 1997). When hen egg white lysozyme was added to this model food prior to heating (at 625-2400 units/ml), and heat treatments of 85°C for 84 min, 90°C for 34 min or 95°C for 15 min were applied, growth was observed at 25°C after 13, 14 and 32 days, respectively (Peck *et al*., 1995; Fernandez and Peck, 1999). A similar effect may also have been noted with endogenous lysozyme in crabmeat. Enzymes with lysozyme activity have been found in crabmeat, and it is estimated that the concentration is approximately 200 µg/g prior to heating (Lund and Peck, 1994). Heat treatments at 88.9°C for 65 min, 90.6°C for 65 min, 92.2°C for 35 min, or 94.4°C for 15 min were required to prevent growth and toxin formation from 10^6 spores of non-proteolytic *C. botulinum* when the heat treatment was followed by enrichment at 27°C for 150 days (Peterson *et al*., 1997). These heat treatments are substantially greater than those advocated by the ACMSF (1992) and ECFF (1996). The effect of intrinsic properties of foods and other environmental factors on the thermal inactivation of spores of non-proteolytic *C. botulinum* has been studied, and predictive models of thermal inactivation have been produced (Juneja and Eblen, 1995; Juneja *et al*., 1995a, 1995b; Peck and Stringer, 1996; Lindstrom *et al*., 2003).

Combinations of heat treatment, storage temperature and other factors that prevent growth and toxin production from spores of non-proteolytic C. botulinum

It is important to define combinations of preservative factors (e.g. heat treatment and subsequent storage at refrigeration temperatures) that provide an appropriate degree of protection against growth and toxin production by non-proteolytic *C. botulinum*, since the microbiological safety of most pasteurised in-pack products relies on such a process. In studies in which meat slurry was used as a model food, the effect of heat treatment at 65°-95°C combined with subsequent storage at 5°-25°C on time to growth from an inoculum of 10^6 spores of a mixture of strains of non-proteolytic *C. botulinum* was determined (Table 7). Growth was confirmed by the presence of *C. botulinum* neurotoxin. In tests where lysozyme was not added, heat treatments of 70°C for 2545 min, 75°C for 464 min, 80°C for 70 min, 85°C for 23 min, and 90°C for 10 min each prevented growth within 42 days when combined with storage at 8°C (Table 7). The heat treatment at 70°C is greater than that in current recommendations, while the heat treatments at 80°C and 85°C are lower (ACMSF, 1992; ECFF, 1996). A predictive model was developed that described the effect of heat treatment and storage temperature on time to growth (Fernandez and Peck, 1997). The model provided a good description of the data used to generate it, and predictions from the model compared well with observations of time to growth/toxin production in independent experiments (Fernandez and Peck, 1997).

Table 7 Combined effect of lysozyme, heat treatment and subsequent storage temperature on time to visible growth from 10^6 spores of non-proteolytic *C. botulinum* types B, E and F in meat slurry (Peck *et al*., 1995; Peck and Fernandez, 1995; Fernandez and Peck, 1997; Fernandez and Peck, 1999)

Heat treatment	Added lysozyme (units/ml)	Time (days) to growth at specified storage temperature					
		5°C	6°C	8°C	12°C	16°C	25°C
none	0	14	-[a]	7	4	2	1
70°C/529 min	0	35	-	9	7	4	1
70°C/1000 min	0	57	-	21	8	5	2
70°C/1596 min	0	NG[b]	-	35	14	8	3
70°C/2545 min	0	NG	-	50	22	8	3
75°C/285 min	0	NG	-	33	13	10	4
75°C/464 min	0	NG	-	48	38	23	8
75°C/734 min	0	NG	-	NG	18	15	5
75°C/1072 min	0	NG	-	NG	NG	NG	9
80°C/11 min	0	58	-	24	12	7	4
80°C/70 min	0	NG	-	44	19	8	5
80°C/184 min	0	NG	-	NG	37	21	11
80°C/295 min	0	NG	-	NG	NG	NG	33
85°C/23 min	0	NG	-	NG	30	38	15
85°C/36 min	0	NG	-	NG	NG	NG	NG
85°C/52 min	0	NG	-	NG	NG	NG	NG
90°C/10 min	0	NG	-	NG	NG	NG	NG
none	625	-	7	4	2	-	1
65°C/364 min	625	-	11	4	2	-	1
70°C/8 min	625	-	8	6	4	-	1
75°C/27 min	625	-	13	9	5	-	1
70°C/529 min	2400	39	-	9	7	4	1
70°C/1000 min	2400	48	-	17	8	3	2
70°C/1596 min	2400	NG	-	38	16	9	3
70°C/2545 min	2400	NG	-	NG	27	9	4
75°C/285 min	2400	NG	-	33	13	11	4
75°C/464 min	2400	NG	-	NG	23	15	6
75°C/734 min	2400	NG	-	NG	21	11	7
75°C/1072 min	2400	NG	-	NG	NG	NG	10
80°C/11 min	2400	46	-	24	10	8	4
80°C/23 min	625	-	40	23	12	-	3
80°C/184 min	2400	NG	-	NG	NG	19	11
80°C/295 min	2400	NG	-	NG	NG	NG	16
85°C/19 min	625	-	53	53	42	-	6
85°C/23 min	2400	NG	-	NG	23	15	6
85°C/36 min	2400	NG	-	48	26	15	7
85°C/52 min	2400	NG	-	NG	46	23	8
90°C/10 min	2400	NG	-	54	30	18	6
90°C/20 min	480	-	-	NG	51	29	20
95°C/15 min	625	-	NG	NG	NG	-	32

[a] not tested, [b] no growth at day 60

In view of previous comments, it is also appropriate to consider foods that contain lysozyme (Table 7). When relatively gentle heat treatments (e.g. heating at 70°C or 75°C) were applied, a large number of spores survived the heat treatment as measured by recovery in the presence and absence of lysozyme (Fernandez and Peck, 1999). In these circumstances, addition of lysozyme prior to heating had little effect on time to growth in a model food system (Fernandez and Peck, 1999). When more severe heat treatments were applied (e.g. heating at 85°C to 95°C), few or no spores were recovered in the absence of lysozyme, while a substantial number of spores were able to germinate and give growth in the presence of lysozyme (Fernandez and Peck, 1999). Following these heat treatments growth was observed more rapidly and over a wider range of conditions when hen egg white lysozyme was added prior to heating, than when no addition of lysozyme was made (Fernandez and Peck, 1999). From these results combinations of heat treatment and storage temperature required for a specific shelf-life can be estimated for a product suspected to contain lysozyme. These data have been used to develop a predictive model that describes the effect of heating temperature (70°-90°C), heating time (10-2545 min), and storage temperature (5°-25°C) on time to growth with lysozyme added prior to heating (Fernandez and Peck, 1999). Predictions from this model also compared well with the experimental results used to produce it, and with independent reports of time to growth/toxin production (Fernandez and Peck, 1999).

In some pasteurised in-pack products, factors other than heat treatment and subsequent refrigerated storage may also contribute to ensure safety with respect to non-proteolytic *C. botulinum*. The combined effects of heat treatment, pH, NaCl concentration, lysozyme addition, and subsequent storage temperature on time to growth from 10^6 spores of non-proteolytic *C. botulinum* in meat slurry have been described (Figure 1; see the end of the paper). In these tests, meat slurry was prepared at different pH values (pH 5.6-6.5) and different NaCl concentrations (0.6% - 4.9%, salt-on-water). Hen egg white lysozyme was either not added (Figure 1a; see the end of the paper), or was added at 1,200 units/ml prior to heat treatment (Figure 1b; see the end of the paper). Spores from eight strains of non-proteolytic *C. botulinum* types B, E and F were then added in equal numbers to give a final concentration of 10^6 spores per tube of meat slurry. The heat treatment applied were the equivalent of approximately 20 min at each of 80°C, 85°C, 90°C and 95°C (see Graham *et al*., 1996b). After rapid cooling, the tubes were incubated at 5° - 16°C for 90 days. Tubes were observed for growth, and the presence of toxin production confirmed. In the absence of added lysozyme, growth and toxin were observed in tubes that were not heated and tubes heated at 80°C for 20 mins, but not in tubes heated at 85°C or higher (Figure 1a; see the end of the paper). For tubes heated at 80°C for 20 mins, combinations of pH, NaCl concentration and shelf-life were identified that prevented growth from 10^6 spores of non-proteolytic *C. botulinum* (Figure 1a; see the end of the paper). In the presence of added lysozyme, growth and toxin were observed in tubes that were not heated and tubes heated at 80°C for 20 mins, 85°C for 20 mins and 90°C for 20 mins, but not in tubes heated at 95°C for 20 mins (Figure 1b; see the end of the paper). Again combinations of pH, NaCl concentration and shelf-life were identified that prevented growth from 10^6 spores of non-proteolytic *C. botulinum* (Figure 1a; see the end of the paper).

It is important to recognise that the heat treatments advocated by the ACMSF (1992) and ECFF (1996) do not, in themselves deliver a 6D process. For many pasteurised in-pack products, it is necessary to identify a combination process that achieves a 6D process. This will probably include a consideration of storage temperature and shelf-life, and possibly also intrinsic properties of the food or mild preservatives that may be added. It should be noted that in some circumstances, the recommendations of the ACMSF (1992) and ECFF (1996) appear to leave only a small margin of safety, but in other circumstances might lead to unnecessary overprocessing. By adopting effective combination processes it should be possible to avoid potentially dangerous situations and maintain an organoleptically acceptable product. The identification of appropriate combinations of mild preservative factors is an important step forward in the development of rational processes for pasteurised in-pack meat products.

Table 8 Examples of reported time to toxin formation in foods inoculated with a low concentration of spores of non-proteolytic *C. botulinum* (Lund and Peck, 2000)

Food[a]	Inoculum (spores/g)	Time (days) to toxin formation at specified temperature					
		4°C	5°C	8°C	10°C	12°C	16°C
cod[1]	5x10[1]	18	-	8	-	6	-
turkey[2]	10^2	-[b]	-	8	-	5	2
salmon[3]	10^2	21	-	6	-	3	-
catfish[4]	10^2	46	-	6	-	-	3
red snapper[5]	10^3	21	-	9	-	3	3
cooked cauliflower[6]	10^3	-	19	17	13	-	4
cooked mushroom[6]	10^3	-	20	10	10	-	3

[a] references: 1, Post *et al.* (1985); 2, Meng and Genigeorgis (1993); 3, Garcia and Genigeorgis (1987); 4, Reddy *et al*. (1997); 5, Baker *et al.* (1990) Lindroth and Genigeorgis (1986); 6, Carlin and Peck (1996).
[b] tests not carried out at this temperature

6 days at 8°C (Table 8). Other studies have evaluated the safety of pasteurised in-pack chilled meat products. In one study, it was found that the heat treatments applied by industry failed to eliminate small or large numbers of spores of non-proteolytic *C. botulinum* (Table 9). Two of these heat processes (applied to beef cubes and beef liver cubes) followed recommendations made by the ACMSF (1992) and ECFF (1996), but failed to deliver a 6D process. Neurotoxin was formed when two of these products (pork cubes and ground beef) were stored at 8°C, but not at 4°C (Hyytia-Trees *et al*., 2000). During growth, non-proteolytic *C. botulinum* does not produce off odours to the same extent as some other bacteria (e.g. proteolytic clostridia). Comparisons of time to spoilage with time to toxin production by non-proteolytic *C. botulinum* have shown that toxin production often occurs in the absence of spoilage (Peck, 1997).

Process risk modelling for safety of pasteurised in-pack products

A relatively recent development has been that of process risk models for these foods (Barker *et al*., 1999; Carlin *et al.,* 2000; Barker *et al*., 2002, 2004). These process risk models have used a probabilistic modelling approach to assess the risk of foodborne botulism presented by non-proteolytic *C. botulinum* by considering the entire food chain. In process risk modelling, recent developments in mathematics and computing are utilised to assess the magnitude of the risk, and to identify hazard scenarios for each operation in the process. Probabilistic modelling approaches used have included Markov Chain Monte Carlo methods and Bayesian Belief Networks. This information can then be used to identify and prioritise steps to minimize detrimental events and to maximize awareness and process control. This includes the development of a probabilistic framework that accounts for complex combinations of information that relate to a single hazard and which incorporates an intrinsic representation of uncertainties.

Acknowledgements

The authors are grateful to the competitive strategic grant of the BBSRC for funding.

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Table 4. Examples of recent outbreaks of foodborne botulism (modified from Peck, 1997; Peck, 2004)

Year Country	Food associated with outbreak	Cases: Total (fatal)	Toxin type: Neurotoxigenic organism
1989 UK	Commercially produced hazelnut yoghurt	27 (1)	Type B: Proteolytic *C. botulinum*
1991 Egypt	Commercially produced uneviscerated salted fish ("faseikh")	>91 (18)	Type E: Non-proteolytic *C. botulinum*
1993 Italy	Commercially produced aubergine in oil	7 (0)	Type B: Proteolytic *C. botulinum*
1993/4 Switzerland	Commercially prepared, dry-cured ham	12/0	Type B: *C. botulinum*[a]
1994 USA	Commercial vacuum packed clam chowder soup	2/0	Type A: Proteolytic *C. botulinum*
1994 USA	Commercial black bean dip	1/0	Type A: Proteolytic *C. botulinum*
1994 USA	Restaurant, potato dip ("skordalia")	30 (0)	Type A: Proteolytic *C. botulinum*
1996 Italy	Commercially produced mascarpone cheese	8 (1)	Type A: Proteolytic *C. botulinum*
1997 Germany	Commercially vacuum packed smoked fish ("Raucherfisch")	2 (0)	Type E: Non-proteolytic *C. botulinum*
1997 Iran	Traditionally made cheese preserved in oil	27 (1)	Type A: Proteolytic *C. botulinum*
1997 Argentina	Home-cured ham	6 (0)	Type E: Non-proteolytic *C. botulinum*
1997 Germany	Home vacuum packed smoked fish ("Lachsforellen")	4 (0)	Type E: Non-proteolytic *C. botulinum*
1998 Croatia	Ham	20 (0)	[b]
1999 Morocco	Commercially produced Mortadella sausage	78 (20)	Type B: *C. botulinum*[a]
2001 USA	Fermented beaver tail and paw	3 (0)	Type E: Non-proteolytic *C. botulinum*
2001 Canada	Fermented salmon roe (two outbreaks)	4 (0)	Type E: Non-proteolytic *C. botulinum*
2001 USA	Commercially produced chilli dish	16 (0)	Type A: Proteolytic *C. botulinum*
2002 South Africa	Commercially produced tinned pilchards	2 (2)	Type A: Proteolytic *C. botulinum*
2002 USA	Muktuk (from Beluga whale)	8 (0)	Type E: Non-proteolytic *C. botulinum*
2003 France	Halal sausage	4 (0)	Type B: *C. botulinum*[a]
2003 Ukraine	Home prepared canned corn	6 (0)	Type B: Proteolytic *C. botulinum*
2003 South Korea	Commercially produced canned sausage	3 (0)	[b]
2003 Norway	Home prepared "rakfisk"	4 (0)	Type B or E: Non-proteolytic *C. botulinum*[c]
2003 Germany	Home prepared dried fish	3 (0)	Type E: Non-proteolytic *C. botulinum*

[a] only toxin identified - unclear whether proteolytic *C. botulinum* type B or non-proteolytic *C. botulinum* type B
[b] toxin reported as present, but type not indicated
[c] likely toxin type and organism

Table 9 Safety evaluation of pasteurised in-pack meat products with respect to non-proteolytic *C. botulinum* (Hyytia-Trees *et al*., 2000)

product	Properties of the pasteurised product				Detection of non-proteolytic *C. botulinum* by PCR and neurotoxin after storage at 8°C			
	NaCl (% wt/vol)	pH	Processing value (min at 82.2°C)	Recommended shelf-life (days)	SL[a]	SL+7d[a]	SL[b]	SL+7d[b]
Pork cubes	0.7	6.0-6.3	22.9	14	-	-	+	++
Pork cubes	0.7	5.8-6.1	153.5	30	+	-	+	-
Beef cubes	0.2	5.8-6.1	496.7	21	-	+	+	+
Beef cubes	0.7	5.6-6.1	186.7	14	-	+	-	-
Pork fillet	2.0	5.8-6.0	10.6	10	+	-	+	-
Roast beef	1.6	5.7-6.1	19.6	30	-	-	-	-
Roast beef	1.9	5.6-5.8	5.5	9	+	-	+	+
Ground beef	0.2	5.5-5.9	0.0	21	-	+	++	++
Beef liver cubes	0.3	6.1-6.3	450.8	21	+	-	-	-
Broiler fillets, mariande	1.4	5.9-6.1	83.9	21	-	-	-	-
Beef, pork, vegetables	1.3	4.8-5.1	2.5	21	-	-	+	+

SL = at recommended shelf-life, SL+7d = at recommended shelf-life plus 7 days
[a] low inoculum of spores (10^2 spores/1.5kg pack), [b] high inoculum of spores (10^5 spores/1.5kg pack)
- PCR test and neurotoxin test both negative, + PCR test positive and neurotoxin test negative, ++ neurotoxin test positive

Figure 1a

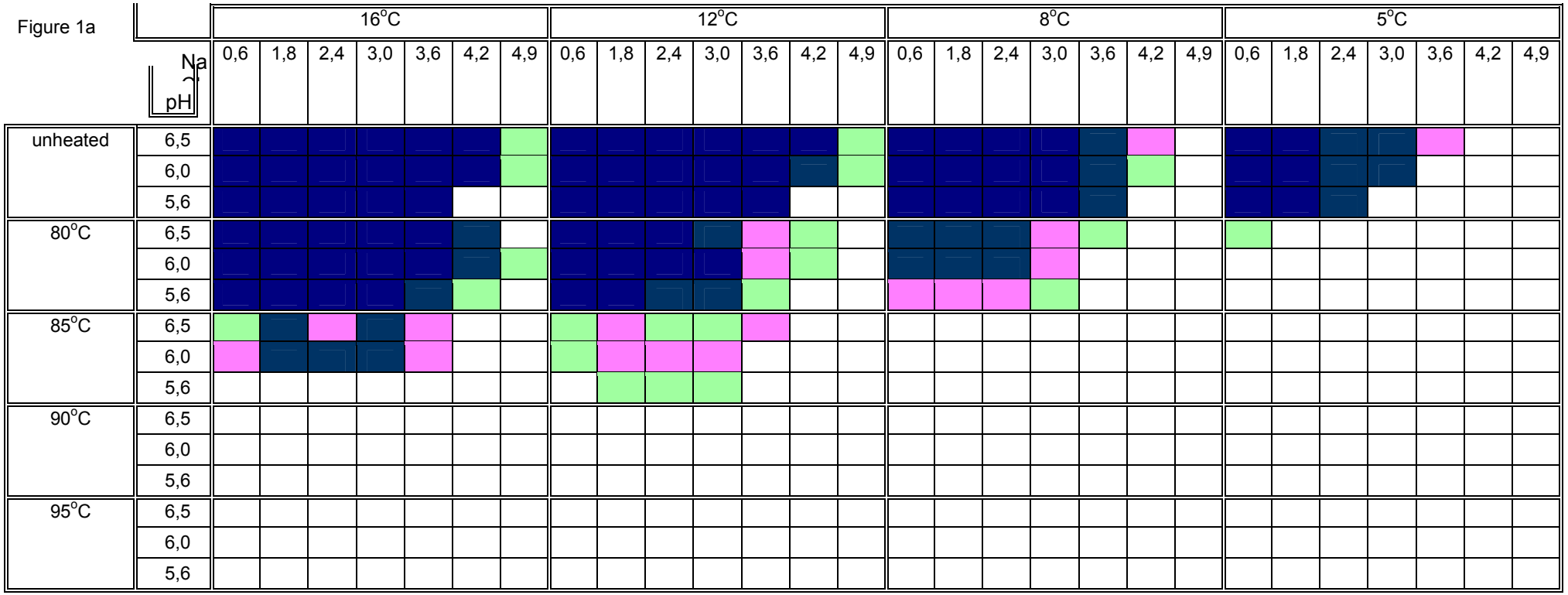

Figure 1 Combined effect of pH, NaCl concentration, lysozyme addition, heat treatment and subsequent storage temperature on time to visible growth from 10^6 spores of non-proteolytic *C. botulinum* types B, E and F in meat slurry (Graham *et al*., 1996a; Peck, Mason and Mitchell, unpublished results). Figure 1a - in absence of added lysozyme.

Figure 1a

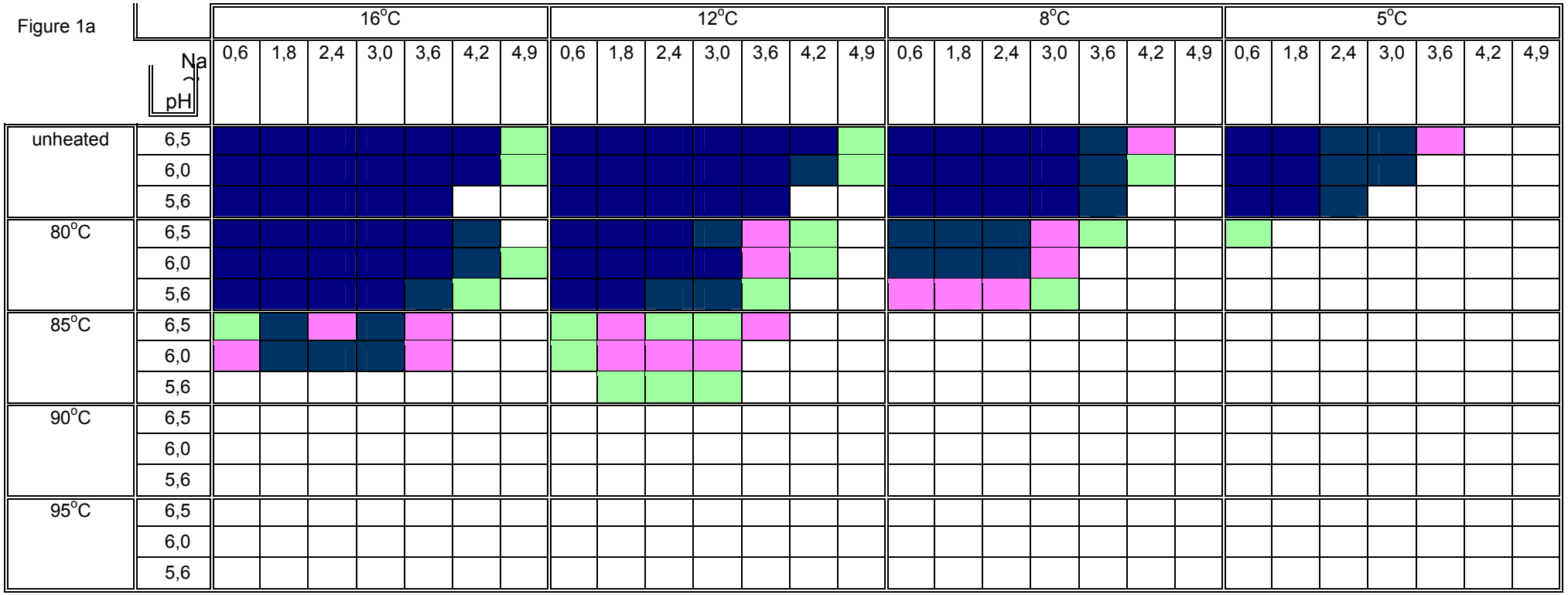

Figure 1 Combined effect of pH, NaCl concentration, lysozyme addition, heat treatment and subsequent storage temperature on time to visible growth from 10^6 spores of non-proteolytic *C. botulinum* types B, E and F in meat slurry (Graham *et al*., 1996a; Peck, Mason and Mitchell, unpublished results). Figure 1a - in absence of added lysozyme.

MICROBIAL ECOLOGY OF MARINATED MEAT PRODUCTS

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Introduction

The consumption of marinated meat has been steadily rising in Finland. Marinating is especially used for poultry products. It has been estimated by the Finnish meat processing industry that about 80% of poultry sold at the retail level is marinated. These products are variable containing skinned meat strips and fillets and skin-on leg and breast fillet cuts. For consumers they are convenient in meal preparing since only heat treatment is need without further handling or spicing of the meat. As an additional advantage of easy handling, although *Salmonella* carriage in Finnish poultry is scarce, reduced risk of food cross contamination with pathogenic bacteria should be mentioned.

The word “marinate” comes probably from the Latin word “marine” to Italian, Spanish and French languages referring to soaking/pickling in salt brine. What is being meant by marinating today varies a great deal between different countries. Sometimes salting, adding phosphates and some spices is considered as marinating. Some marinating procedures have been targeted to tenderize poorer quality meat (Young and Lyon 1997a and b). In Finland, marinades are nowadays complex sauces which have a great effect on product appearance and taste. They are water-oil emulsions typically containing salt, sugar and acids (acetic, citric), rheology-improving additives (like xanthan gum and guar gum), antimicrobial agents (like sorbate and benzoate) spices and aroma strengtheners. The pH of these marinades is usually acidic, less than 5, so sugar is needed to cut the edge of the acidic taste. Basic flavor is often obtained using pepper, onion and tomato base together with other added spices. There is a big selection of different flavors, including curry, Chinese-type, Italian-type, honey and barbecue marinades. The amount of marinade added on meat is variable among product types, 20 to 30% (wt/wt) being quite typical for meat strips.

Aims of packaging and marinating technology

Marinated Finnish meat products are usually packaged under modified atmospheres (MA) to prevent the growth of aerobic spoilage organisms. Modified atmosphere packaging (MAP) of meat usually results in the dominance of psychrotrophic lactic acid bacteria (LAB) if proper cold-storing is applied to control the growth of *Enterobacteriaceae* (Borch et al. 1996, Smolander et al. 2003). In addition to the LAB, *Brochothrix thermosphacta* has also been associated with spoilage in some countries (Borch et al. 1996) but this species has not been detected to form a major part of the spoilage population in Finnish MAP marinated broiler meat strips (Susiluoto et al. 2003) .

The main aims of marinating have been considered to be tenderizing, flavoring and enhancing safety and shelf life of meat products due to inhibition of microbial growth. The growth of microbes has been considered to be suppressed by the acidic pH and the use of sorbates and benzoates in the marinades. In the period 1997 to 2000 the National Technology Agency of Finland supported in the first study program (Uudistuva elintarvike tutkimusohjelma) a project associated with meat marinating technology. During these studies, marinating was surprisingly not found to increase the shelf life of these products and also the effect on tenderizing was not as good as anticipated. It was actually found that there were higher bacterial levels in the marinated products than in the corresponding non-marinated products. In 1997, severe spoilage problems

were noticed in certain marinated poultry products. This prompted our interest to study and identify the specific spoilage organisms in the Finnish products. During the same time, the group of Professor Hänninen (Department of Food and Environmental Hygiene, University of Helsnki) started to analyze the effect of marinating on *Campylobacter*. Since then these studies have been carried out in collaboration with the Finnish meat industry and with support obtained from the Academy of Finland and National Technology Agency. Our work has targeted beef, pork and poultry products. Currently we have the best knowledge of the microbial ecology associated with marinated poultry products. Some of these studies are presented in the following paragraphs in order to elucidate the microbial complexity associated with marinating.

Microbial ecology in marinated poultry products

Specific spoilage LAB in marinated poultry

In our first study (Björkroth et al. 2000), LAB associated with gaseous spoilage of MAP, raw, tomato-marinated broiler meat strips were identified. We use a 16+23S rDNA RFLP (ribotyping) database for LAB identification since the psychrotrophic LAB species cannot be identified using traditional phenotypical tests. A mixed LAB population dominated by a *Leuconostoc* species resembling *L. gelidum* was found to cause the spoilage of the product. *Lactobacillus sakei, Lactobacillus curvatus* and a Gram-positive rod phenotypically similar to heterofermentative *Lactobacillus* species were the other main organisms detected in this spoilage population. Increase in pH together with the extreme bulging of packages was considered to suggest a rare LAB spoilage type called "protein swell". This spoilage is characterized by excessive production of gas due to amino acid decarboxylation and the rise of pH is attributed to the subsequent deamination of amino acids. However, in recent studies (not yet published) we have shown that these LAB are not able to decarboxylate the major meat-associated amino acids. The rise in pH values is likely to result from the buffering capacity of the meat. A polyphasic taxonomy approach, including classical phenotyping, whole-cell protein electrophoresis, 16+23S rDNA RFLP, 16S rDNA sequence analysis and DNA-DNA reassociation analysis, was used for the identification of the dominating *Leuconostoc* species. 16S rDNA gene sequences of two spoilage strains possessed 98.8 and 99.0% sequence similarity with the *L. gelidum* type strain. DNA-DNA reassociation, however, clearly distinguished these two species. These same strains showed only 22% and 34% hybridization with the *L. gelidum* type strain. Based on these results, a separate species status was proposed for these *Leuconostoc* strains, and the name *Leuconostoc gasicomitatum* was given to this spoilage-associated species. Recently also the unidentified Gram-positive rod has been shown to be a new psychrotrophic *Lactobacillus* species, *Lactobacillus oligofermentans* (Koort et al. 2004b).

In another study (Susiluoto et al. 2003) we wanted to evaluate if LAB in retail, MAP, marinated broiler meat strips on sell-by day commonly harbor *L. gasicomitatum.* A total of 32 packages, 3 to 5 packages of 7 differently marinated broiler meat products, were studied at the end of the producer-defined shelf life (at 6ºC, 7 to 9 days depending on the manufacturer). Bacteria were cultured on MRS and Tomato Juice Agar (TJA), Rogosa SL agar (SLA), Plate Count Agar (PCA) and Streptomycin Thallium Acetate Agar (STAA) for the enumeration of LAB, lactobacilli, total bacterial count and *B. thermosphacta*, respectively. The average CFU/g of the 32 packages was 2.3×10^8 on PCA. The highest bacterial average, 3.1×10^8, was recovered on TJA, the corresponding CFU/g averages on MRS and SLA being 2.3×10^8 and 1.3×10^8, respectively. Despite the high LAB numbers detected, radical spoilage changes such as unpleasant odor, slime production and formation of gas were not seen. *B. thermosphacta* did not form a significant part of the bacterial population since none of the levels exceeded the spoilage threshold level of 10^5 CFU/g reported in previous studies for this organism. In order to characterize the dominating LAB population, 85, 85 and 88 colonies from MRS, TJA and SLA, respectively, were randomly picked, culture purified and identified to species level. Fifty-six of the 170 isolates picked from the non-selective LAB media (MRS and TJA) were identified as *L. gasicomitatum,* followed by *Carnobacterium divergens* (41 isolates), *L. sakei* (31 isolates) and *L. curvatus* (20 isolates) species. SLA proved not to be completely selective for lactobacilli because the growth of *Leuconostoc* spp. was not inhibited, *Carnobacterium* spp. were the only species not detected on SLA. This study confirmed that *L. gasicomitatum* commonly occurs in MAP, marinated poultry products.

Initially contaminating and spoilage LAB in the products and processing environment

In order to show which of the initial LAB contaminants are also causing spoilage of a MAP, marinated broiler leg product at 6ºC, LAB were enumerated and identified on the 2nd and 17th day following

manufacture. A total of 8 fresh and 13 spoiled packages were studied for LAB levels. In addition, aerobic mesophilic bacteria and *Enterobacteriaceae* were determined. The average CFU/g values in the 8 fresh packages were 1.3×10^3, 9.8×10^3 and 2.6×10^2 on MRS, PCA and Violet Red Bile Glucose agars (VRBG), respectively. The commercial shelf life for the product had been set as 12 days and all packages analyzed on the 17th day were deemed unfit for human consumption by the sensory analysis. The corresponding CFU/g averages in the spoiled product were 1.4×10^9, 1.1×10^9 and 3.9×10^7 on MRS, PCA and VRBG agars, respectively. For characterization of the LAB population, 104 colonies originating from the fresh and 144 colonies from the spoiled packages were randomly picked, purified and identified to species level. The results showed that enterococci (35.7% of the LAB population) were dominating in the fresh product whereas carnobacteria (59.7%) dominated among the spoilage LAB. *Enterococcus faecalis*, *Carnobacterium piscicola* and *Carnobacterium divergens* were the main species detected. In general, when the initial LAB population is compared to the spoilage LAB, a shift from homofermentative cocci towards carnobacteria, *L. sakei/curvatus* and heterofermentative rods (*L. oligofermentans*) was seen in this marinated product. A novel *Enterococcus* species, *E. hermannienesis* (Koort et al. 2004a), was also described from the enterococci present in the initial population.

After the three studies showing many novel LAB species in these marinated products we wanted to study what is the role of the broiler chicken as a source of these spoilage LAB. We made four slaughterhouse visits during which carcasses and air of the slaughterhouse and adjacent processing facilities were sampled. These results showed that the broiler skin or mucous membranes were not major sources of the spoilage LAB but they are commonly found in the air in the processing rooms suggesting environmental contamination of the processing facilities (Vihavainen et al. 2004). We think that the cold processing facilities favour the adaptation of the psychrotrophic strains in the processing facilities because the marinades themselves have not been found to harbour spoilage LAB.

Survival of Campylobacter spp. in marinated poultry products

Marinating has been considered to increase safety of poultry products. Perko-Mäkelä et al. (2000) studied the survival of *Campylobacter jejuni* in plain marinade and in both marinated and non-marinated drumsticks and sliced breast meat strips during storage at 4ºC. The marinade had a pH of 4.5 and NaCl content of 5.9% (wt/wt). Inocula consisted of a cocktail of 7 *C. jejuni* strains. In the plain marinade, the *C. jejuni* inocolum level was initially log 5.4 CFU/ml and within 24 hours a log 2.4 CFU/ml decrease in *C. jejuni* level was noticed. After 48 hours no *Campylobacter* were detectable in the marinade. However, the fate of *Campylobacter* in both marinated and non-marinated meat products was quite different in comparison to the situation in the plain marinade. Surprisingly there was no difference between the marinated and non-marinated products. When an inoculum of log4 to log5 CFU/ml was added to 300g product, the organisms were detectable at least for 9 days. With a smaller inoculum level of log1 to log2 CFU/ml, *Campylobacter* were detected for at least five days. These results show that marinating may not necessarily have an effect on the survival of enteric pathogens, like *Campylobacter*. This may also be due to the buffering capability of meat quickly neutralizing the pH of the acidic marinade. The change in the acidic pH towards neutrality results in dissociation of the lipophilic acids making their antimicrobial effect nonexistent.

Conclusions and further studies

Marinating is changing the spoilage LAB population initially present in the product. We have detected many novel species in these products. Currently we think that addition of marinades is favoring the growth of some psychrotrophic LAB which are rarely detected in non-marinated meat products. *L. gasicomitatum* has also been detected from carrots and a spoiled fish product (Lyhs et al. 2003) but not from the skin or membranes of broiler chicken. As a psychrotrophic organism it does not survive in gastro intestinal tract and we currently consider this species as an environmental bacterium well-adapted in the cold processing facilities. The use of MAP and marinating is favoring the growth of this bacterium and to lesser extent also *L. oligofermentans*. In order to control *L. gasicomitatum* and *L. oligofermentans* growth in these products we should understand the metabolism of these species better.

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EVALUATION OF PORK HYGIENE QUALITY USING BIODETECTING METHODS

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Background

Bacterial or other microbial contamination is the major cause of most incidents of food-borne illness. Among food groups, animal origin products are the most likely sources of pathogens threatening human health. To develop more accurate and non-time-consuming methods for evaluating hygiene quality of meat products is an important issue for securing day-to-day health of the consumers.

Objectives

In the present study, we compare the activities of succinate dehydrogenase and hexosaminidase in raw meat samples collected from healthy animals (normal pork) or diseased animals (abnormal meat). We also utilize cultured murine macrophage cell line as biodetecting model for evaluating the microbe contamination in raw pork.

Materials and Methods

Sample collection and preparation: Normal pork samples are obtained from the local meat market immediately after slaughter. Abnormal pork samples are collected from the animal died from illness in a local pig farm. Muscle proteins are extracted from *M. longissimus dorsi* according to the method reported previously by Kuo (1989), and used for enzyme activities and TNF measurements. For immune cell response assay, the extracts are added to the cell culture medium, and the cytokines released from the cell and cell viability are measured after the treatment. Microbiological assay: Microorganism growth is analyzed by FDA method. Immune response assay: Raw 264.7 cells, murine macrophages, are used for immune response assay. The cells are cultured in Dulbecco's modified essential medium (DMEM, GIBCO BRL, Rockville, MD) supplemented with 10 % feta bovine serum and incubated in an incubator at 37℃ with 5% CO_2. To determine the effects of muscle extracts on the immune cells, Raw 264.7 cells are treated with medium containing extract for 48 hours. Cytokine measurement: TNF in the medium is measured by enzyme-linked immunosorbent assay (ELISA) kit (Neogen). Cell viability: Cell viability after treated with muscle extract is determined by MTT assay. Succinate dehydrogenase (SDH) activity and Hexosaminidase activity: SDH activity is measured based on the procedure reported by Colowick *et al*. (1957). Hexosaminidase activity is assessed according to the method reported previously by Ching et al.(2001).

Results and Discussion

The data show that the level of volatile basic nitrogen (VBN), pH value and bacterial number are higher in abnormal pork than in the normal samples (Table 1); however, VBN concentrations in the early stage still remain at normal range. After 24-hour storage at room temperature, the level of VBN accumulation in the abnormal pork are higher than the normal pork but total bacterial counts of the abnormal pork are not higher than the standard values set by FDA, suggesting that total bacterial counts are not only an appropriate indicator for determining the quality of pork.

The results from enzyme activity assay demonstrate the alterations in the levels of cellular enzymes in the abnormal pork. The activity of HSA in abnormal tissue is much higher compared with the one in normal sample. The enzyme activity increases in the abnormal pork during storage, and reaches the level of absorbance 0.729 at 12 hour after storage, while the activity in the normal sample remains constantly at low level. The activity in the abnormal pork is 2 fold higher than the normal sample at every time point tested. The abnormal pork also exhibits significantly higher activity in SDH activity compared with the normal pork

at the early stage (within 6 hours) of storage. However, the activity in the abnormal pork decreases when storage period is prolonged. There is not significant difference in the activity of SDH between two groups of sample after 12 hour storage.

It is well known that microbial infection induces the production of TNF that can serve as an indicator for the infection. The abnormal pork tested in this study is obtained from the animal died from illness, and shows higher level of TNF compared with normal pork sample, indicating the previous infection before slaughter (Table 2). The level in the normal tissue does not change during storage. The TNF level in the abnormal pork is 2 folds higher than the level in the normal tissue at 12 hour after storage. The data from MTT assay further demonstrate the cytotoxicity of the extract from the abnormal pork in the cultured immune cells. Raw 264.7 cells treated with the extract of the abnormal pork shows lower cell viability after activation with LPS compared with the culture treated with the extract from the normal pork, suggesting the application of immune inhibition in Raw 264.7 cell as biodetecting model for evaluation of hygiene quality of pork.

Conclusions

In conclusion, this preliminary study provides evidence for establishing new cellular indicators, including HSA activity, TNF production, and immune cell cytotoxicity, for discriminating abnormal pork from normal pork. This study also suggests viability of Raw 264.7 cells may be an appropriate indicator for biodetecting model for hygiene quality evaluation of pork. These parameters are quite stable within 24 hours, and are suitable for the hygiene quality evaluation in Taiwan. Since the traditional meat markets in Taiwan sell fresh raw meat only in the morning, within 6 hours after animal slaughter. Evaluating the hygiene quality of frozen and refrigerated products by detecting these parameters is an interesting subject for the future studies.

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Table 1. Changes in freshness parameters and enzymatic activity of normal and abnormal pork during storage at 28℃.

Parameters \ Storage time(hrs)		0	6	12	24
pH	Normal	6.13	5.98	5.77	5.98
	Abnormal	6.57	6.29	6.38	6.55
Total bacterial counts (CFU/g)	Normal	4.99	4.44	3.99	5.32
	Abnormal	4.51	5.42	5.83	6.55
VBN (mg/100g)	Normal	8.1	9.8	12.4	11.6
	Abnormal	20.8	19.7	37.6	51.0
Succinate dehydrogenasse (Reduction rate %)	Normal	21.3	31.0	36.9	22.7
	Abnormal	40.2	42.3	31.9	25.3
Hexosaminidase (Absorbance at 400nm)	Normal	0.289	0.258	0.255	0.238
	Abnormal	0.281	0.560	0.729	0.422

Table 2. Changes in TNF concentration and cytotoxicity of normal and abnormal pork during storage at 28℃ .

Items \ Storage time(hrs)		0	12	24
Tumor necrosis factor (TNF) concentration (pg/g)	Normal	27.16	30.25	28.34
	Abnormal	34.55	58.69	38.38
*Absorbance $_{at\ 570nm}$ of MTT cytotoxicity test by RAW 264.7 cells	Normal	1.712	1.917	1.767
	Abnormal	1.947	2.064	1.927
**Absorbance $_{at\ 570nm}$ of MTT cytotoxicity test by RAW 264.7 cells	Normal	1.85	1.90	1.88
	Abnormal	1.69	1.58	1.52

MTT: 3-(4,5-dimethyl thiazol-2-yl)-2,5-diphenyltetrazoilum bromide
*:Treatment without lipopolysaccharide ,**: treatment with lipopolysaccharide

COMPARISON BETWEEN TRADITIONAL AND PCR TECHNIQUES IN THE IDENTIFICATION OF FOODBORNE PATHOGENS IN BLOOD SAUSAGE

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Background

One of the most popular cooked blood sausage in Spain is *Morcilla de Burgos*, which is produced in the region around Burgos in the north of Spain. It consists of a mixture of onion, rice, animal fat, blood, salt and different spices such as black pepper, paprika and cumin. The product is cooked for 45-50 min at 94-95ºC, air cooled to 8-10ºC and finally chilled stored at 4ºC. This product is commercialised as a fresh product with a shelf-life of around 8-10 days, however, the use of vacuum packaging and modified atmosphere packaging has recently increased in order to extend the shelf life and widen the present market. (Santos et al, 2003). The typical way to consume this product is by deep frying in vegetable oils (mainly olive oil), roasted or boiled as part of other traditional dishes.

In a technical study made on *morcilla de Burgos* (Anonymous, 1983), it was underlie the good sanitary quality of this product, since from 67 samples analysed by traditional microbiology tests there were not detected any strain of *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp., *Escherichia coli*, or *Bacillus cereus*.

There are also another previous work that has studied the microbiology of *morcilla de Burgos*, basically the spoilage microbial population and some indicator species of the microbiological quality of the product, such as *S. aureus*, *Clostridium perfringens*, enterobacteria, pseudomonads, enterococi and moulds, without obtaining positive results in these species. (Santos 2001).

The recent advances achieved in molecular biology techniques have developed numerous genetic procedures as PCR and Real-Time PCR, which have been applied successfully to food analysis, principally in the detection of pathogenic microorganisms.

Objectives

Although there is some information about the basic microbiology of *morcilla de Burgos*, as it was mentioned above, little is known about the presence of foodborne pathogens in this product. The aim of this preliminary study is the detection of 5 species of pathogenic bacteria in this blood sausage, comparing traditional microbiology test, *versus* PCR and RT-PCR, in order to obtain a sensitive and reliable method that can assure the food safety of these kinds of products.

Materials and methods

Samples

This initial study was done with 4 *morcilla* producers, 6 samples of each one were analysed. All samples were vacuum-packed, and 3 *morcillas* were stuffed in natural beef casing and the other one in pork casing. The samples were purchased in local supermarkets and were transported to the laboratory in refrigeration conditions in iceboxes.

Microbiology assay

For detection of *Staphylococcus aureus*, *Listeria monocytogenes* and *Yersinia* spp. The pathogenic species studied were*: S. aureus* by means of homogenization of the sample and directly plated in Baird Parker agar (Oxoid LTD, Basingstoke, Hampshire, England) with supplement of egg yolk-tellurite emulsion (Oxoid), incubated at 37ºC for 72 hours; *Yersinia* spp was grown after alkaline treatment of the homogenised sample, in CIN agar (Oxoid) at 30ºC for 48 hours; *L. monocytogenes* enrichment of 20 g of sample in 180 ml of Fraser broth base (Oxoid) with ½ Fraser selective supplement (Oxoid) at 37ºC for 20-24 hours, incubation in Fraser broth base (Oxoid) with Fraser selective supplement (Oxoid) at 37ºC for 24 hours and finally cultivated in Palcam agar (Oxoid) at 37ºC for 48 hours.

PCR assays

For detection of *Salmonella* spp., *S aureus* producing enterotoxins A and D (SEA and SED), *L. monocytogenes* and *Y. enterocolítica*, 20 g of each sample was inoculated into 180 ml TSBYE medium. The samples were incubated at 37ºC ± 1ºC overnight (16 – 20 h), with shaking at 150 rpm. A portion of the sample (1 ml from the upper phase) was mixed with 1 ml of sterile phosphate buffered saline (PBS) and centrifuged at 12000 rpm for 5 min. The pellet was washed three times with PBS, then resuspended in 500 µl of water, incubated in a boiling water bath for 10 min, and immediately cooled in ice water. A 5 µl portion of each sample was added to 45 µl of PCR mixture containing 75 mM Tris HCl (pH 9), 50 mM KCl, 2mM $MgCl_2$, 0.2 mM of each dATP, dTTP, dCTP, dGTP, 0.5 µM each primer, and 1 U of Tth DNA polymerase, (Biotools DNA polimerase, Madrid. Spain.). The PCR was conducted in a Mastercycler gradient, (Eppendorf AG, Hamburg. Germany.) The amplification condition was one cycle of 94ºC for 3 min, then 30 cycles of 94ºC at 50-58ºC depending upon the primers used (see table 1) for 30 s and at 72ºC for 1 min, and finally, one cycle of 72ºC for 90 s. A positive and a negative control were used for each PCR reaction. The PCR products (10 µl of each) were separated by electrophoresis in 2% agarose gels. To stain the gel with ethidium bromide during 10 min, and the result was observed in UV light (Wang et al, 1997, Rosec at al, 2002).

RT-PCR assay

For detection of *L. monocytogenes*, samples were prepared as it was described above for PCR, 5 µl portion of each sample was added to 45 µl of PCR mixture containing 1x TaqMan universal PCR master mix (part number 4304437; Applied Biosystems, Foster City. U.S.A.), 0.3 µM concentrations of each primer, and 0.2 µM concentration of fluorogenic probe. DNA amplification was carried out in MicroAmp optical 96-well reaction plates (part number N801-0560; Applied Biosystems), sealed with MicroAmp optical caps (part number N801-0935; Applied Biosystems.). The cycling program consisted on heating for 2 min at 50°C, and then 10 min at 95°C followed by a two-stage temperature profile of 15 s at 95ºC and 1 min at 60ºC, repeated for 40 cycles. Amplification, data acquisition, and data analysis were carried out with an ABI Prism 7000 sequence detector (Applied Biosystems).

Results and discussion

Table 2 shows the results obtained for all pathogenic species evaluated. No positive samples were found for *S. aureus* enterotoxin D producer, *Salmonella* spp, and *Y. enterocolitica*, with any of the techniques used. In case of *S. aureus* producer of enterotoxin A, 10 positive samples were found by PCR, distributed between all the manufacturers, and in *L. monocytogenes* 9 positive samples were obtained by PCR and 15 positive results with RT-PCR distributed between all the manufacturers as well. Both species did not show positive results with conventional microbiology tests.

The different results obtained between traditional microbiology tests, and those of molecular biology, can be due to the different sensibility of those techniques, the use of different enrichments culture media or the growth of competitive flora. RT-PCR is 100 times more sensitive than the PCR and 1000 times more than the traditional microbiology tests used in this study. These results indicate that pathogenic bacteria detected in this study are in very low quantities. (Llanos et al, 2003).

Despite of the strong thermal treatment of 45-50 minutes at 94 º C, it is surprising, the presence of *S. aureus* producing enterotoxin A that used to be related to bad hygienic practices, and *Listeria* spp. which is an ubiquitous microorganism, and it is very extended in nature. The presence of these microorganisms can be due to a contamination in post cooking steps of cooling and manipulation during packing.

Conclusions

The molecular biology techniques used, are extremely sensitive to discover the presence of pathogenic microorganisms in low quantities *versus* the microbiological conventional technologies.

It could be very interesting to develop a more accurate HACCP system along the production line, especially in the cooling step, in manipulation before packaging, to try to keep the product in refrigeration, without breaking the cold-chain, and consuming the product according to the traditional culinary ways that imply some thermal treatment that warranties the inactivation of *S. aureus* as *L.monocytogenes*. However, other strategies, carried out in previous studies by our group, such as post-packaging short pasteurization could be very useful to obtain an acceptable sanitary quality of these kinds of products. Further studies will be developed in that sense.

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Acknowledgements

We want to express our thanks to the Spanish distributing company Mercadona S.A. for supporting this project, as well to the Area of Nutrition and Bromatology, Department of Hygiene and Food Technology of the University of León, where this study was realised.

Table 1. PCR primers and working temperatures.

Species	Target gene	PCR primers´ sequences (5´-3´)	Product size	Reference	Temperature anneal (ºC)
L. monocytogenes	hemolysin gene	LM1, cggaggttccgcaaaagatg LM2, cctccagagtgatcgatgtt	234 bp	Furrer et al. (1991)	58
Y. enterocolitica	enterotoxin gene	YE1, ctgtcttcatttggagcattc YE2, gcaacatacatcgcagcaatc	159 bp	Ibrahim et al. (1992)	55
Salmonella spp.	invA gene	Sal3, tatcgccacgttcgggcaa Sal4, tcgcaccgtcaaaggaacc	275 bp	Rahn et al. (1992)	55
S. aureus	enterotoxinA gene	ESA1, acgatcaatttttacagc ESA2, tgcatgttttcagagttaatc	544 bp	Betley et al. (1998)	50
S. aureus	enterotoxinD gene	ESD1, ttactagtttggtaatatctcctt ESD2, ccaccataacaattaatgc	334 bp	Bayles et al. (1989)	50

Table 2. Results of the presence in morcilla of foodborne pathogens obtained by different analytical techniques.

Producers	Samples	*L. monocytogenes*			*Y. enterocolitica*		SEA		SED		*Salmonella* spp.
		M.A.	PCR	RT-PCR	M.A.	PCR	M.A.	PCR	M.A.	PCR	PCR
1	1	–	–	–	–	–	–	–	–	–	–
	2	–	+	+	–	–	–	–	–	–	–
	3	–	+	+	–	–	–	+	–	–	–
	4	–	+	+	–	–	–	+	–	–	–
	5	–	–	–	–	–	–	–	–	–	–
	6	–	–	+	–	–	–	–	–	–	–
2	1	–	+	+	–	–	–	–	–	–	–
	2	–	+	+	–	–	–	–	–	–	–
	3	–	+	+	–	–	–	+	–	–	–
	4	–	–	+	–	–	–	+	–	–	–
	5	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	–	–
3	1	–	+	+	–	–	–	+	–	–	–
	2	–	–	+	–	–	–	–	–	–	–
	3	–	–	–	–	–	–	+	–	–	–
	4	–	+	+	–	–	–	+	–	–	–
	5	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	–	–
4	1	–	+	+	–	–	–	+	–	–	–
	2	–	–	+	–	–	–	–	–	–	–
	3	–	–	+	–	–	–	+	–	–	–
	4	–	–	+	–	–	–	+	–	–	–
	5	–	–	–	–	–	–	–	–	–	–
	6	–	–	–	–	–	–	–	–	–	–

M.A.: Microbiology assay. SEA: *S. aureus* producing enterotoxin A, SED *S. aureus* producing enterotoxin D

INFLUENCE OF THE ONION VARIETY ON THE SHELF LIFE OF THE BLOOD SAUSAGE "MORCILLA DE BURGOS"

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Background

Morcilla de Burgos is a popular cooked blood sausage produced in the region around Burgos, in the north of Spain. *Morcilla* is made of a mixture of chopped onion, rice, animal fat, blood, salt and different spices such as black pepper, paprika and cumin. Physicochemical and sensory properties of this traditional product were investigated in order to support the claim for a Protected Geographical Indication.
Three types of *morcilla* could be differentiated according to their sensorial and physical-chemical characteristics, composition and geographical origin (Santos et al., 2003). Due to the high percentage of onion included in this product (between 15% and 70%), especially in two of the types, this ingredient is determinant of their sensory properties (Santos et al., 2002) and probably it will have a great influence on shelf-life of the product.
"Horcal" is a regional variety of onion, grown in river banks. This onion has an elliptical shape and bigger size compared with others kinds of onions. This variety is the most often used as ingredient of *Morcilla de Burgos*, since, according to traditional manufacturers, its less firm texture and mild pungent flavour gives to *Morcilla* a sensibly better sensory quality. However, its crop is seasonal with a short period of storage and it must be substituted by other varieties of onion when *morcilla* manufacturers run short of "Horcal" onion of suitable quality. In this case, the variety most often used is "Grano" or "Valenciana", with globular shape. Besides, it is smaller than "Horcal" variety and its sensory properties also differ, since it is firmer and more pungent than "Horcal" onion.

Objectives

The aim of this study is to know the effect of the variety of onion used in the manufacture of the blood sausage "Morcilla de Burgos" on their sensory characteristics and shelf-life of the product.

Materials and methods

Samples: Two batches of 60 *morcillas* were made with the same formulation with the only difference of the kind of onion added. One batch was made with a local variety of onion *Allium cepa L.* var. horcal and the other batch with *Allium cepa L.* var. grano. Sausages were vacuum-packaged in the factory, after cooking and cooling. *Morcillas* were kept in refrigeration at 4ºC, during storage. Samples for microbiological and physical-chemical analysis were taken after manufacture (day 0) and at days 7, 14, 21, 33 and 63 of storage. At each sampling time two *morcillas* were analysed, randomly chosen. Initially samples for microbiological analysis were taken, under sterile conditions, and later, from the same *morcillas*, samples for physical-chemical analysis were taken. All analysis were made in duplicate.

Microbiological analyses. The microbiological parameters analysed were Total Viable Count (TVC) using pouring-plate method on PCA agar (Oxoid. Basingstoke, UK), incubated at 30ºC for 48h; Lactic Acid Bacteria (LAB) on MRS agar (Biokar Diagnostics. Beavais, France) incubated at 30ºC for 48h in aerobic conditions; *Pseudomonas* spp. (PSD) on Pseudomonad agar (Oxoid) supplemented with CFC (supplement Oxoid), incubated at 30ºC for 48h; *Clostridium perfringens* on TSN agar (Biokar Diagnostics. Beavais, France.), using the pouring method, and incubated at 37ºC for 48h; anaerobic conditions were achieved by pouring an agar sealing tap around 20mm high on solid TSN agar, after sample inoculation. Samples consisted of 25g of *morcilla* slices with casing (Santos, 2001).

Physical-chemical analysis. Along storage time pH was measured with a penetration probe in four different points for each sample.

Organic acids analysis was done after some preliminary steps as desiccation and fat removing. Five grams of *morcilla* were placed on a plate and kept in a desiccator cabinet for 24h at 105ºC. Then, fat were extracted for 6 h by Soxhlet method and finally organic acids were extracted from the dry and defatted sample. The organic acids were extracted by miliQ water (1:3, w/v), heated till boiling, clarified by centrifuge at 13000 rpm for 10 minutes and the supernatant was membrane filtered (0.45 µm) before injection.
Chromatographic analysis was carried out using a Hewlett-Packard Series HP 1100 with diode array detector and Chemstation software. Aliquots from each extract (20 µl) were injected onto a 30 cm x 7.8 mm i.d. Supelcogel C-610H (Bellefonte, PA), which was maintained at 40 ºC with a column heater (Bio-Rad Ca, USA), and eluted using 0.1 M H_3PO_4 at a flow rate of 1ml min^{-1}. The eluate was monitored at 210 and 280 nm.

Sensory analysis comprises two different steps. First, sensory differences between *morcillas* made with different kinds of onions were determined at the beginning of storage by consumer analysis. A triangle test was done by a panel consisting of 40 not trained panellists. Panellists were asked to found overall differences between samples. Additionally, panellists evaluated the intensity of odour and taste, soft texture and presence of onion. *Morcilla* was served to the panellist in slices of 1 cm thickness and cooked in microwave till 70ºC in the core. The second step evaluated the influence of onion on self-life of the *morcilla*. At the 22nd day of storage, 34 sausages of each type were examined by a trained panel of 5 panellists and graded them in three groups: acceptable, medium and rejected. Visual parameters of the sealed packs were evaluated and visual and odour parameters were tested after opening the packs. "Acceptable" corresponds to sausages without defects, "Medium" sausages present some small changes, but they would not be rejected by consumers and "Rejected" corresponds to sausages with remarkable defects. The typical sensory changes occurring in spoilage of "Morcilla de Burgos" are blowing of the packs, development of drip, slime formation and souring of the product (Borek, 2002).

Results and discussion

At the beginning of storage, *morcillas* made with "horcal" and "grano" varieties show differences. Piruvic acid concentration is higher in *morcilla* with "grano" onion (Table 1), in agreement with Llamazares et al. (2003). Piruvic acid is the main responsible for the pungency of onion (Bedford, 1984; Schwimmer and Weston, 1961), and therefore it would have also a significant contribution to pungency in the *morcilla*. Their concentration could be considered an index of hot taste of product.
According to sensory discriminatory tests both kinds of *morcilla* were significantly different ($\alpha = 0{,}01$). If specific parameters are considered the intensity of odour and taste of both kinds of *morcilla* were not significantly different, but differences were found in the presence of onion and texture. With the descriptor "presence of onion" panellists evaluated distinguishable particles of onion and also smell and taste of onion, being more intense in *morcilla* with grano variety than with horcal variety. Concerning texture, *morcilla* with horcal onion was softer than *morcilla* with grano onion. These results suggest the high influence of onion variety on sensory properties of onion since grano variety is firmer and more pungent than horcal variety.
Table 1 shows physical-chemical parameters of both types of *morcilla* along storage period. These results were expected, since the increase of concentration of lactic acid and the decrease of pH can be related to the development of lactic acid bacteria throughout the 63 days of storage. Lactic acid increases along storage, altough until the day 14 it is not detected due to the level of detection of the analytical method used. Differences were found between *morcillas* with the two varieties of onion. A more rapid formation of lactic acid and the fall of pH in *morcillas* with "horcal" onion was observed. Pyruvic acid, present in onion, also decreases, since it can be used as nutrient by LAB. Piruvic acid increases in the first days of storage and later decreases progressively along time in a similar rate in both types of sausages.
Concerning to microbiological data, table 1 shows the results for all parameters analysed, except for *Clostridium perfringens* because no colonies were identified during the study. Total Viable Counts (TVC) and Lactic Acid Bacteria (LAB) present a progressive increase along storage. Some differences were founds between both *morcillas*, since initial number and rate of growth were slightly higher in *morcilla* with horcal onion.
Pseudomonads present a growth until to reach a maximum count, afterwards counts suffer a decrease due to the lack of oxygen because of vacuum packaging. The initial development of these microorganisms owning to the low concentration of retained oxygen in the vacuum package and after 14 days, when oxygen is depleted, their decline comes about. The pattern of PSD development is similar in sausages with both kinds

of onion, but in *morcilla* with "horcal" onion counts are around 1000 ufc/g higher than in sausages with "grano" onion.

The change in the rate of growth of lactic acid bacteria and in the production of lactic acid and fall of piruvic acid concentration happen one week before of sensorial deterioration of *morcilla* and this coincide with pH fall. This evolution is mainly similar for *morcilla* made with both kinds of onions, however, it differs the time when the changes in *morcilla* characteristics come about. In *morcilla* made with "horcal" onion spoilage takes place at the days 14 to 21, as in *morcilla* made with "grano" onion takes place the days between 21 to 33.

Results obtained in sensory evaluation of spoilage parameters are shown in table 2. Panellists evaluated acceptability of *morcillas* at the day 22 of storage. Overall appearance of packages and *morcillas* showed different signals of spoilage as blowing, slime, sour, pungent and acid odour, pink and green colours. These sensory changes came about one week earlier in horcal than in grano onion *morcillas*.

The main deterioration process is the blowing of the package; later pink spots appear, followed by slime production with milky appearance together with the change in the texture of the product and also the modification of odour characteristics. The sausage becomes soft and presents a release of acid odour.

This results agree with other researchers (Mattana et al., 1980, Lin et al., 1995, Llamazares et al., 2002) that found potential preservation of onions depends on variety; usually smaller onions and with higher contents of dry matter have longer shelf-life. These onions are firmer and more pungent than onions of bigger size. Oxygen is necessary for the formation of compounds responsible for pungency. Smaller onions present a low resistance to penetration of oxygen, with easier development of these compounds. The smaller superficial area led to lower initial contamination, and so preservation of these onions is better, in agreement with results obtained in this study for "grano" onion.

Conclusions

The variety of onion used in the formulation of cooked blood sausage *morcilla* has a notable effect on physical-chemical and sensory characteristics of the product. The only difference of the kind of onion used leaves to significantly different its sensory properties.
On the other hand, the type of onion has also a remarkable influence on *morcilla* preservation, since sausages made with "horcal"onion are spoiled around one week earlier than the sausages manufactured with "grano" onion. It could be due to the amount of sugars, fibre, alcohols, aldehydes, aromatic compounds, and the different compounds coming from the onion as potential nutrients of the microorganisms.

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Acknowledgements

We want to express our thanks to *Excma. Diputación Povincial de Burgos* for supporting with a grant this project. We also want to express to gratitude to the Junta de Castilla y León for supporting this research project.

Table 1.- Physical-chemical and microbiological parameters in *morcilla* made with two different varieties of onion. Morcilla A: *Allium cepa L.* var. horcal, Morcilla B: *Allium cepa L.* var. grano.

***Morcilla* A**						
Day	% Piruvic acid	% Lactic acid	pH	LAB (log cfu/g)	TVC (log cfu/g)	PSD (log cfu/g)
0	3.03	0.00	6.43	3.55	4.30	3.48
7	3.79	0.00	6.24	4.76	5.94	4.01
14	2.35	1.77	6.02	7.79	6.03	5.80
21	2.16	3.06	5.12	8.20	8.18	5.63
33	1.47	6.22	4.69	8.20	8.32	4.58
63	1.38	7.74	4.45	8.85	8.42	3.57
***Morcilla* B**						
Day	% Piruvic acid	% Lactic acid	pH	LAB (log cfu/g)	TVC (log cfu/g)	PSD (log cfu/g)
0	3.60	0.00	6.13	2.70	4.60	2.70
7	3.94	0.00	6.26	4.67	5.62	4.30
14	2.80	0.67	6.19	7.67	7.70	4.30
21	2.62	2.42	5.78	7.62	8.34	4.93
33	2.09	5.80	5.14	7.62	7.92	3.10
63	1.41	7.24	4.52	8.44	8.48	2.51

Table 2.- Grading of *morcilla* made with two different varieties of onion according to sensorial signals of spoilage at day 22 of storage (expressed as percentage). Morcilla A: *Allium cepa L.* var. horcal, Morcilla B: *Allium cepa L.* var. grano.

Groups	Percentage of sausages	
	***Morcilla* A**	***Morcilla* B**
Aceptable	31	60
Medium	23	30
Rejected	46	10

AN APPLICATION OF BLEND OF NATURAL ANTIOXIDANTS IN DRY-FERMENTED SAUSAGES

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Background

One of the main reasons for limitation of the quality and acceptability of dry-fermented meat products is the lipid peroxidation. This process causes discoloration, appearance of unusual odour and taste and generation of potential toxic compounds (Morrissey et al., 1994; Gray et al., 1996). The investigators examine this problem a long time ago. In the most contributed form this process has not actuality for scientists, but in the poorly developed form - warmed over flavor (WOF) it exists as a problem for some foods, including meat products (Spainer et al., 1992). The "Lukanka" type dry-fermented sausages, traditional for Bulgaria and Balkan's region, are an interesting model for study of this subject, because they are made by mincing, grinding, and mixing of meat raw materials with salt, additives and spices, and after that filling in casings, fermentation (known as maturation), and drying. The sausages are exhibited on significant impact of prooxidant factors during their processing. As a result of those three types of lipid derivatives are formed: primary products - hydroperoxides, secondary products expressed as free malondialdehyde (free MDA) and oxidized cholesterols. The protection of the sausages against lipid peroxidation must start from the beginning of meat storage and to continue during sausage processing. The oxygen intake, exposure to light, and the high temperature must be restricted during the sausage maturation (Lai et al. 1995). The manners for restriction of lipid and pigment peroxidation showed above are not useful or are insufficient effectively, during processing of "Lukanka" type dry-fermented sausages. This is a reason to add different antioxidants and synergists to the minced meat. Those types of blends have not universal application, because their effect depends on the proximate composition of oxidized substrate (Raharjo et al., 1993; Trout and Dale, 1990). The effective stabilization of the lipids in dry-fermented sausages can be realized only after determination of the impact of endogen pro- and antioxidant factors.

Objectives

The objective of this study is to estimate the efficiency of previously developed blend of natural antioxidants (Dragoev et al., 2004) on the progress of peroxidation processes in "Lukanka" type dry-fermented sausages.

Materials and methods

The model system of "Lukanka" was prepared from: 1) chilled beef type CL 95 stored 72 h *post mortem* at 0 – 4 ^{O}C and frozen to minus 5^{O}C 6 h before grinding and 2) chilled pork sort 50/50, stored 72 h *post mortem* at 0 – 4 ^{O}C and frozen to minus 10^{O}C 6 h before cutting, and 3) frozen bacon to minus 10^{O}C, stored 24 h *post mortem*, before using. The experiments were carried out with "Monastiry's lukanka" which is largely covered representative of dry-fermented sausages in Bulgaria, and is distinguished with comparatively high fat content. The last one is a precondition for the most expressive changes of lipid fraction in comparison with other assortments lukanka. The recipe of the "Monastiry's lukanka" control sample is: beef thigh or shoulder blade - 55 kg, pork sort 50/50 semi fatty meat - 25 kg , bacon from the back - 20 kg, salt – 2.200 kg, potassium nitrate – 0.040 kg, black pepper – 0.300 kg, cumin – 0.200 kg, and red pepper – 0.200 kg.
Some parallel samples were processed: Control sample – without antioxidants; The first experimental sample with addition of 0.124 % or 1.24 g/kg blend of natural antioxidants № 1 (liquid form), which contains 48.39 % LRSE 2 – liquid rosemary extract purchased from "Aromena" Ltd - Sofia (Bulgaria). This preparation represents a 28 – 30 % non distillated alcohol extract with flavonoide concentration 2.12 – 2.64 %. The rosemary extract was combined in the blend with 35.48 % chemical pure routine bought from E. Merck (Darmstadt, Germany) and 16.13 % pure sodium erythorbate under trade mark "Eribate", provided from F.I.A. Food Ingredients Anthes GmbH (Teising, Germany); The second experimental sample with addition of 0.112 % or 1.12 g/kg blend of natural antioxidants № 2 (dry form), which contents 3.58 % DRSK – powder rosemary concentrate, containing approximately 42 % flavonoides, mixed with 78.57 % DKFK - dry powder extract of flower bud of the Japanese acacia (*Sophora japonica*), produced in the Department of

Biotechnology in the University of Food Technology – Plovdiv, Bulgaria. The extract contents 53.33 % quercetine - agucone of the natural glycoside routine and 17.85 % pure sodium erythorbate under trade mark "Eribate", provided from F.I.A. Food Ingredients Anthes GmbH (Teising, Germany).
For analysis were purchased following reagents: 2-thiobarbituric acid, distillated pure chloroform and methanol - from Sigma Chemical Company Ltd. (St. Louis, USA, Deisenhofen, Germany); potassium iodide, sodium thiosulfate and silver iodide were purchased by Fluka Chemie AG (Buchs, Swaziland). All rest chemicals and dissolvents were AR and GPL grade and were supplied by Aldrich Chemical Co (Steinheim, Germany).
The samples of "Monastiry's lukanka" were processed using traditional Bulgarian technology expresses in following. The beef was minced by grinder through bars with diameter of holes 5 mm. The beef, pork and bacon were cut to particle size 3 - 4 mm. The salt and spices were added during cutting. The casings were filled with mass using vacuum filler. The pieces of "Lukanka" were formed by clips automat. The separated pieces were dried 2 days at temperature 8 – 9^{O}C and humidity 95 – 90 %. The drying process continues next 18 days at temperature 9 – 11^{O}C and humidity 85 – 75 %. The dry finish product was packaged and labelled.
The total lipids were extracted from the minced sausages by the Bligh and Dyer method (1959).
The oxidative stability of the extracted lipids was determined by Rancimat method (Ranfft et al., 1988), using apparatus Metrohm 679 Rancimat (Metrohm AG, CH – 9100, Switzerland).
The hydroperoxide concentration was determined when the peroxide value (POV) was established using standard iodometric method, presented as meqvO_2.kg^{-1} lipids (AOAC, 1990).
The content of secondary derivatives express by free malondialdehyde was determined using as indicator TBARS (Thiobarbituric acid reactive substances). The water-acid extraction method was used (Schmedes and Hølmer, 1989). TBARS were presented as mg MDA.kg^{-1} sausage.
Microbiological status. The characterization of the microbiological status of the sausages was made by determination of total number of aerobic micro organisms, and oxidase reducing bacteria (ORB). Those indexes were established by standard cultural methods (Boshkova, 2000).
The results were processed statistically by standard methods using computer program Excel.

Results and discussion

Oxidative stability of lipids. The addition of blend of natural antioxidants (in two examined variants) stabilizes sausage lipids (Fig. 1). On the 30th d the induction period of the lipids of experimental samples is approximately 5 times longer then those of the control sample. Two forms of blend of natural antioxidants identically stabilize the extracted lipids.

Figure 1. Oxidative stability of lipids, extracted from "Monastery's lukanka" processes from 1 d stared at 4^{o}C raw materials when is added the blend of natural antioxidants

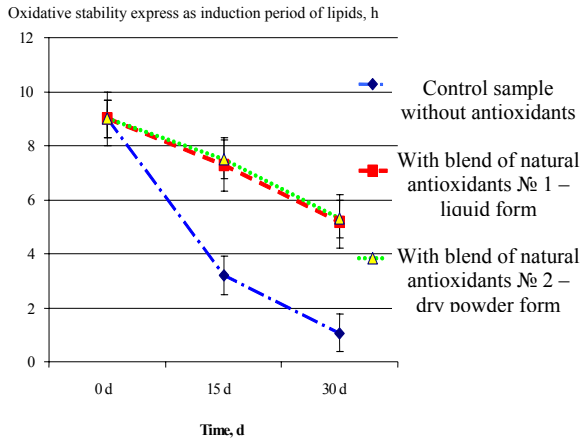
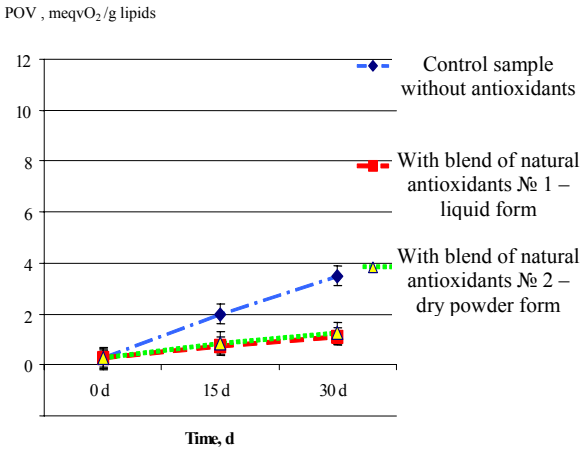


Figure 2. Peroxide value (POV) of lipids, extracted from "Monastery's lukanka" processes from 1 d stared at 4^{o}C raw materials when is added the blend of natural antioxidants

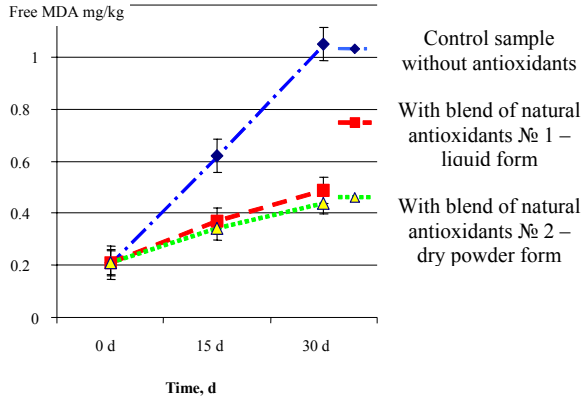


Peroxide value (POV). The addition of blend of natural antioxidants in the mentioned above concentrations in a significant degree suppress accumulation of the primary derivatives of lipid peroxidation (Fig. 2). On the 15th day of drying the hydroperoxide levels in the experimental samples are approximately two times lower than those in control sample, and on the 30th day three times lower. During all examined periods of time two

experimental forms of blend of natural antioxidants do not show statistically different results ($p > 0.05$) regarding hydroperoxide levels.

Thiobarbituric acid reactive substances (TBARS). The addition of blend of natural antioxidants retards dissemination of the chain and transformation of hydroperoxides in secondary derivatives of lipid peroxidation during maturation of the sausages (Fig. 3). The levels of free MDA are almost double time reduced. The accumulation of secondary derivatives of lipid peroxidation delays identically from the two forms of examined blend of antioxidants. The levels of free MDA on 15th d of maturation in the experimental samples are around 40 % lower in comparison with control sample. The level of free MDA in sample № 1 – liquid form of blend on 30th d is lower approximately with 52 % in comparison with the control sample, but in sample № 2 – dry form of blend it is lower around 57 %. Two experimental forms of the blend of natural antioxidants do not show statistically significant differences ($p > 0,05$) regarding TBARS levels, but are statistically significant lower ($p < 0,05$) in comparison with control sample.

Figure 3. TBARS of lipids, extracted from "Monastery's lukanka" processes from 1 d stared at 4°C raw materials when is added the blend of natural antioxidants



The results obtained by us are confirmed by data reported from other investigators (Fernandez and Rodriguez, 1991; Johansson et al., 1994; Chiretti et al., 1997; Chizolini, 1998; Novelli et al., 1998; Zanardi et al., 1998) which are examined typical for Spain, Italy or France dry-fermented sausages and hams. In this case, the indexes of lipid oxidation are within the confines of POV around 2 - 4 meqvO2/кg lipids and TBARS around 0.1 – 0.3 mg MDA/kg.

Microbiological analysis. The tendency of decreasing of the total number of aerobic microorganisms, both in control samples, as well as in two experimental samples were determined (Fig. 4). The addition of blend of natural antioxidants contributes to

Figure 4. Changes of total number of aerobic micro organisms in "Monostery's lukanka" at addition of the blend of natural antioxidants

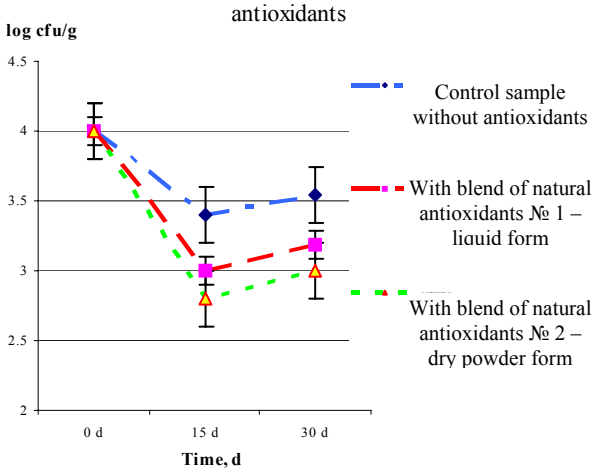
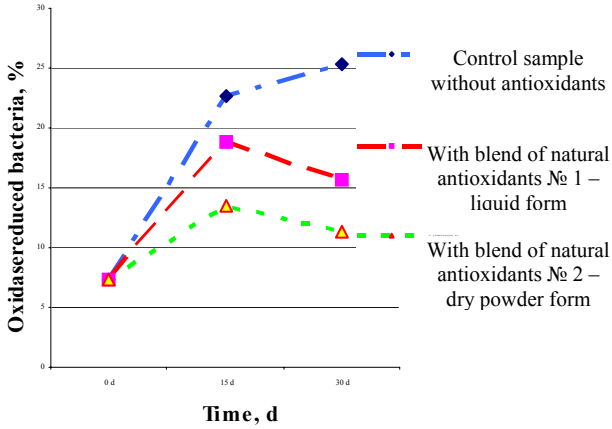


Figure 5. Percentage of ORB in samples "Monastery's lukanka" when is addied the blend of natural antioxidants



statistically significant ($p < 0.05$) decrease of the total number of aerobic microorganisms in "Monastiry's lukanka". The differences of the total number of aerobic micro organisms between two samples with addition of the blend of natural antioxidants are not statistically significant ($p > 0.05$). The percentage of oxidase reducing bacteria increases during maturation of the sausages (Fig.5). In the control sample (30 d) their percentage is statistically significant higher ($p < 0.05$) in comparison with those determined in two experimental samples. The results obtained show, that when the blend of natural antioxidants is added the percentage of oxidase reducing bacteria significantly decreases and the oxidative processes also delay. This increasing is the most significant (around 10 %) on the 15th d. One reason for the considerable decreasing of the hydroperoxides (Fig. 3) in the experimental samples in comparison with the control one is very likely due to decreasing of the percentage of oxidase reducing bacteria.

Conclusions

The two experimental samples of the blend of natural antioxidants stabilized the sausage's lipids and reduced the levels of hydroperoxides and TBARS. The examine blend of natural antioxidants, independently from its forms (liquid or powder), has not clearly bactericide action, because weakly influences on the changes of total number of aerobic micro organisms. The addition of the blend of natural antioxidants mainly affects on the percentage of oxidase reducing bacteria.

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A TECHNOLOGICAL STUDY ON THE LIPID PEROXIDATION IN GROUND MEAT AND PROLONGTION THE SHELF LIVE. CHEMICAL AND MICROBIOLOGICAL PROPERTIES

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Background

In the muscle hydroperoxides accumulate in lipids during refrigeration storage of ground meat at 0 - 4^{O}C (Dragoev and Danchev, 1997). After 5 days at these conditions their levels increase over admissible health recommendations. The secondary products of lipid peroxidation quickly accumulate when the light and oxygen are impact on the ground meat. As a result of this: the lipid oxidative stability considerably decreases, concentration of the haematin iron increases, polyunsaturated fatty acids (PUFA) reduce their levels, and the colour and appearance of the final product changes (Dragoev, 1999).
During the first month of storage of the frozen ground meat at -18ºC, the velocity of formation and development of hydroperoxides are comparatively low, but after that the primary derivatives of lipid peroxidation quickly transform in aldehydes and ketones (Dragoev et al., 1998). The lipid peroxidation processes extends at next boiling (Cheach and Ledward, 1996; Farouck et al., 1991; Raharjo et al., 1993) or cooking (Smith and Alfazar, 1995) of the meat.
In connection with indicated above the investigation of the antioxidative ability of some organic acids as lactic, acetic, and citric and their salts are with an indisputable interest.

Objectives

On the base of bibliography the purpose of this study is to determine the impact of pure sodium lactate parallel with a blend containing sodium acetate, citrate and L-ascorbate on the lipid peroxidation and microbiological status of ground meat.

Materials and methods

Prime and raw materials were purchased from the local market. The liquid sodium lactate type “Purasal S” (produced by “Purac”AG - Germany) was supplied by “Exelpack” Ltd – Sofia. The blend based on sodium acetate “Bombal” (produced by “Van Haas” Ltd - Germany) was furnished by “JVN” Ltd – Sofia.
The meat raw materials were cut on cutter machine type “Müller” (Germany). During the technological process 2 % salt, 3 % soy granulate, previously hydrated with 3 volumes water and the relevant preserving agents were added.
The following samples were prepared: **0** – control sample without preserving agents; **1** – experimental sample with addition of 1,2 % sodium lactate; **2** – experimental sample with addition of 2,0 % sodium lactate, and **3** – experimental sample with addition of 0,5 % blend (composition) containing sodium acetate, sodium citrate and sodium L-ascorbate. The ground meat – mix of beef and bacon = 3:1 w/w were divided in quarters. Each part was stored at two temperature regimes: standard at 0 - 4^{O}C, and irregular at 8 - 10^{O}C. The experiments were made on 0, 4 and 7 d. The samples on the 0 d were examined immediately after preparing the ground meat.
Peroxide value (POV). POV was determined using standard iodometric method, after extraction of total lipids following Bligh and Dyer recommendations (1959).
Thiobarbituric acid reactive substances (TBARS). TBARS were estimated by Schmedes and Hølmer method (1989) and presented as mg free malondialdehyde (MDA)/kg meat.
pH value. The pH was measured by pH-meter MS 2004 (Microsyst, Bulgaria), completed with combined temperature/pH-electrode Sensorex Combination Recorder 450 CD (pH Electrode Station, CA 90680, USA).
Microbiological status. The characterization of the microbiological status of the ground meat was made by determination of:

- *Total number of aerobic microorganisms* was established by cultural method (Boshkova, 2000), and presented as colony forming units per gram ground meat (cfu/g)
- *Coliforms number (CFN).* A definite quantity of ground meat was sowed on the special solid medium. On it coliforms develop typical colonies after cultivation at 37^{O}C. After examinations which conform the presence of coliforms the growing colonies in 1 g ground meat were counted. CFN was calculated by formula: CFN = N. 10 ^{n}, where CFN – the number of cfu coli forms/g; N – number of colonies at the respective dilution; 10^{n} – degree of dilution.
- *Determination of the coagulase positive staphylococcus strains.* Initially an enriched growth on the liquid medium was completed with aim to keep under the concomitant microorganisms. After that the samples for the second time were grown on the solid selective medium (Boshkova, 2000).

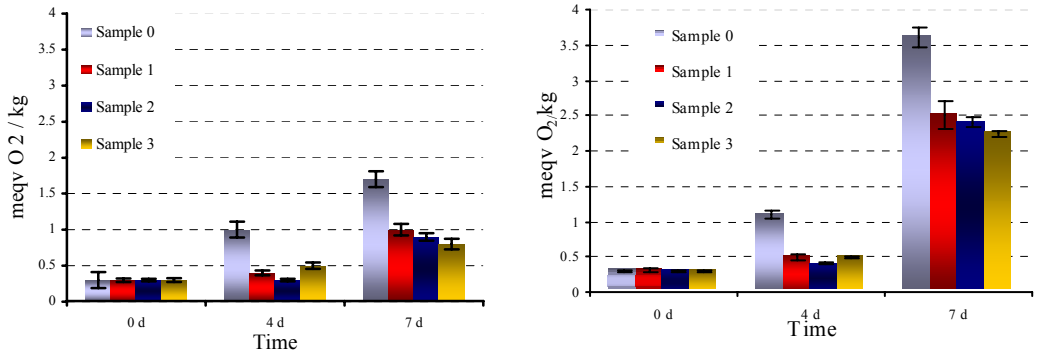
The results were processed statistically by standard methods using computer program Excel.

Results and discussion

Peroxide value (POV). At temperature of storage 0 - 4ºC the accumulation of hydroperoxides in examined samples is restricted in comparison with the control sample 0. In sample 0 (without preserving agents) on the 4th day significant amounts of primary derivatives of lipid peroxidation were found. On the 7th day the POV levels are higher – around 1.5 meqv O_2/kg (Fig.1). The sample 3 – with 0.5 % blend based on the sodium acetate on the 4th day shows POV levels similar of those of samples 2 and 1 – with sodium lactate, but on the 7th day sample 3 contains the least amounts of hydroperoxides.

Figure1. Changes of peroxide value (POV) of ground meat during storage

at 0 - 4ºC at 8 - 10ºC

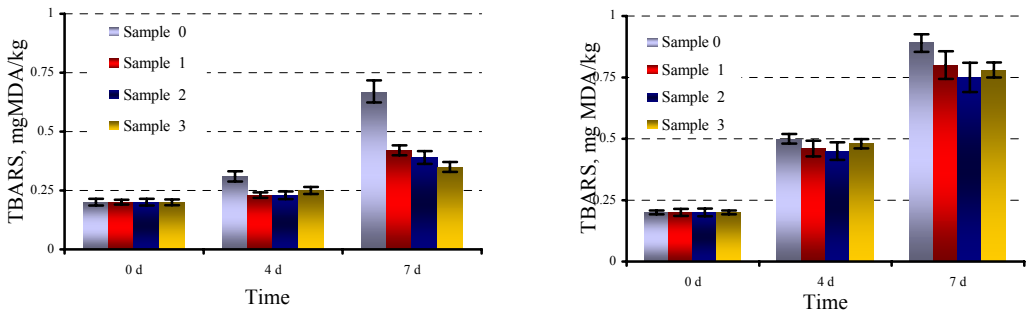


At temperature of storage 8 - 10ºC the situation is different (Fig.1). According POV the experimental samples – 1, 2 and 3 are good to the 4th day. On the 7th day the quality of every sample deteriorate. These results are confirmed by data for sensory analysis (Dragoev et al., 2004). The control sample 0 is the most unstable, while between lipid hydroperoxide levels of the experimental samples 1, 2 and 3 there are not statistically significant differences ($p > 0,05$) (Fig.1).

Thiobarbituric acid reactive substances (TBARS). At temperature of storage 8 - 10ºC the used additives do not prevent the development of deep lipid peroxidation process in ground meat (Fig. 2). The best restriction of TBARS development at 8 - 10ºC shows the addition of 2.0 % sodium lactate (sample 2).

Figure2. Changes of TBARS express as free MDA of ground meat during storage

at 0 - 4ºC at 8 - 10ºC



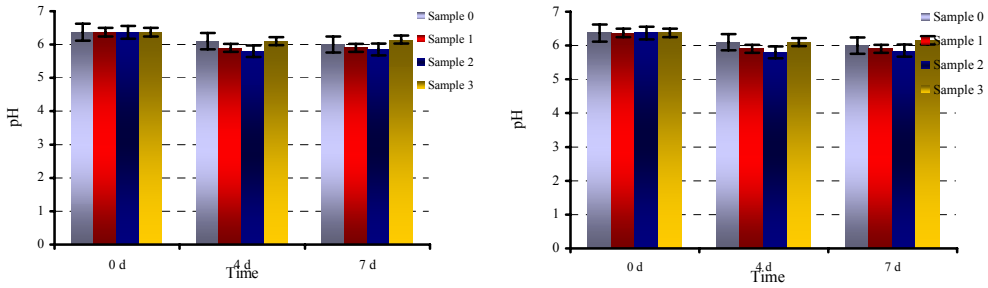
When the ground meat was stored at 0 - 4ºC TBARS slowly increases in comparison with temperatures 8 - 10ºC (Fig. 2). O the 7th day TBARS levels are least in sample 3 – with 0.5 % blend based on the sodium acetate. In the control sample 0 the TBARS is approximately two times higher than in three experimental samples.

The conclusion can be done, that the type and quantity of added preserving agent do not conduct significant differences regarding to oxidative changes of ground meat. Every experimental sample - with 1.2 % sodium lactate (sample 1), with 2.0 % sodium lactate (sample 2) and with 0.5 % blend based on the sodium acetate (sample 3) suppress but can not prevent the development of lipid peroxidation in ground meat. The observed changes are in agreement with results of previous our studies (Dragoev and Danchev, 1997; Dragoev et al. 1998)

pH value. The pH of ground meat decreases in order of 5.8 – 6.1 at 0 – 4^{O}C and 5.1 – 5.4 at 8 – 10^{O}C (Fig. 3). Those results show that the extension of shelf-life of ground meat when the preserving agents are used is a result mainly of the enhanced acidity. It probably oppresses the growth of the putrefactive microorganisms.

Figure3. Changes of pH of ground meat during storage

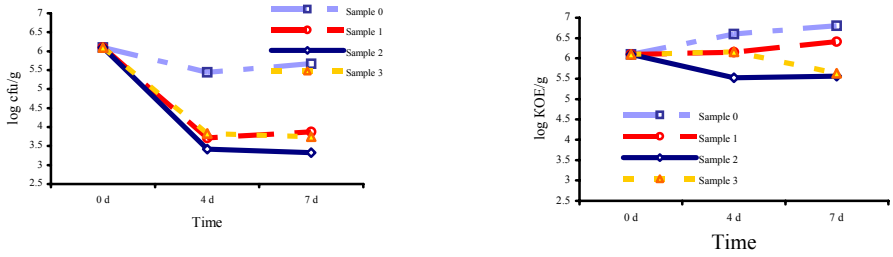
at 0 - 4ºC at 8 - 10ºC



At 8 - 10ºC both the sodium lactate and the blend based on sodium acetate (Fig.3) in practice do not decrease pH of ground meat (fig. 3). The decisive factors of this process are the temperature and initial microbial contamination. The samples with sodium lactate show a little lower pH on the 4th and 7th day of storage, which give them some advantage. Those results do not confirm the sensory properties of ground meat (Dragoev et al., 2004). The samples with sodium lactate - 1, and 2 do not have better flavor and aroma in comparison with control sample 0 and experimental sample 3 – with 0.5 % composition based on sodium acetate.

Figure4. Changes of total number of aerobic microorganisms of ground meat during storage

at 0 - 4ºC at 8 - 10ºC



Microbiological analysis. The results obtained show that the lactate affects significantly quickly on the total number of aerobic microorganisms in comparison with acetate blend, independently from temperature of storage. In spite of higher pH the samples with addition of 2.0 % sodium lactate have a smaller total count of microorganisms (Fig 4).

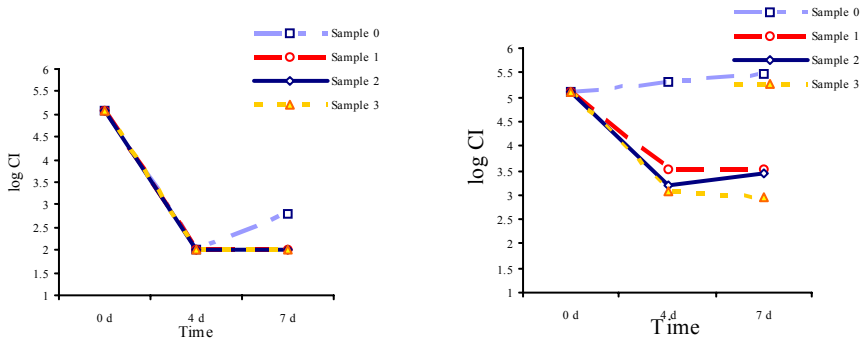
On the 7th day of storage at 0 - 4^{O}C the total number of aerobic micro organisms of ground meat is the best in sample 2 – with 2.0 % sodium lactate, followed from the sample 3 – with 0.5 % blend based on the sodium acetate, and sample 1 - with 1.2 % sodium lactate. On the 7th day of storage at 8 - 10^{O}C control sample 0 don't correspond to the norms for total number of aerobic microorganisms (cfu/g) (Fig. 4).

The tendency determined for the total number of aerobic microorganisms of the samples with preserving agents (Fig. 4) is the same for the CFN at the same conditions (Fig. 5). The control sample doesn't suit requirements for number of coli forms in 1 g meat.

At 0 - 4 ^{O}C the microbiological indices of samples are very good. Every sample, including the control one, correspond to the requirements of the total number of aerobic microorganisms and coliforms. At this temperature the experimental samples are distinguished with good microbiological status. The study implemented for the coagulase positive staphylococcus bacteria demonstrates the supremacy of sodium lactate over acetate composition.

Figure5. Changes of coliform number (CFN) of ground meat during storage

at 0 - 4ºC at 8 - 10ºC



Conclusions

The sodium lactate and the blend containing sodium acetate, sodium citrate, and sodium L-ascorbate can be successfully used as preserving agents with antioxidant action for prolongation of the shelf-life of ground meat at temperature of storage 0 - 4 ^{O}C. In those conditions the shelf-life maximum of ground meat is 7 d.

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METHODS FOR LISTERIA INOCULATION; VALIDATION OF A SURFACE AND A MIXING METHOD

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Background

The development of *Listeria monocytogenes* growth models starts with the initial recovery, proceeds with determining growth and finishes with determining the influence of different factors on growth. If there is no prevalence of Listeria on the product, it can be inoculated with this pathogen. For a comparison of growth between different products it is important to start with consistent low inoculation levels because (i) microbial growth can be dependent on initial numbers and (ii) in practice Listeria contamination will start at low numbers. To study Listeria growth with, for instance, different packaging techniques (Cagri et al. 2002), surface treatments (Naidu et al. 2003), or ingredient compositions (Sabia et al. 2003) different inoculation techniques are used. In the present study, two simple, safe and cost effective methods are compared. The first method consists of inoculating the surface using a horizontal glass plate (HGP). The second method blends the Listeria inoculum into the meat sample (BM) while the meat is chopped.

Objectives

This study was conducted to compare two inoculation methods for *Listeria monocytogenes* on meat; one by surface inoculation of an intact cooked slice of beef and the other by mixing the inoculum while chopping the cooked beef.

Materials and methods

Sample preparation: Beef longissimus steaks (2.5 cm thick) were vacuum packed, heated in a water bath at 70°C for 60 minutes and chilled on ice water. Products were stored for 1 or 2 days at 4°C until used for one of two treatments.
Inoculation: The treatment with surface inoculation (HGP) was carried out using a glass plate (30x30 cm) with a welded metal rim (height = 1 cm) put horizontally using a levelling table. Thirty mL of inoculation suspension was pipetted on the glass plate area, making sure it covered the whole area. The inoculum suspension contained 140.000 CFU/mL *Listeria monocytogenes* ATCC7644 (fresh overnight culture in Brain Heart Infusion broth at 37°C), and a surfactant (Tween 80, 0.01%) was added to facilitate even spreading on the glass plate. The resulting inoculation surface contained 3.67 log CFU/cm^2. One side of the cooked steaks was gently pressed on the glass plate, making sure there was good surface contact. The steaks were again vacuum packed. At the start of the experiment 25 steaks were inoculated, 10 were used at day 0 and after storage at 4°C for 5, 8, 12, 15 and 25 days, 3 steaks were sampled per day.
For the second treatment (BM), 2.0 kg of the cooked meat was chopped until course, using a commercial chopper (RK 30 SL, Kilia, Kiel, Germany). Subsequently, 20 mL of the inoculum containing 10.000 CFU/mL *Listeria monocytogenes* was evenly poured in the chopper. The meat was chopped until very fine. The chopped meat was vacuum packed in portions of 200 g. Ten portions were sampled on the day of inoculation. The others were stored at 4°C for 5, 8, 12, 15 and 25 days. Two portions were sampled per day.
Microbiological analyses: Samples for microbiological analyses were taken with a sterile cork borer, diluted (1:10 w/w) and stomachered in Peptone Physiological Salt solution (2 min). *Listeria monocytogenes* colonies were counted on Rapid L. mono agar (BioRad) after 24 h at 37°C. Total Aerobic Psychrophylic bacteria were counted on Plate Count Agar (Merck) after 3 days at 20°C. BM samples were analysed in duplicate and HGP samples were analysed in triplicate to compensate for possible uneven distribution.
Data was analysed using Microsoft Excel and a two sided T-test was performed.

Results and discussion

The results from the two different inoculation methods (Table 1) were significantly different for recovery percentage ($p<0.05$). For HGP the recovery can be calculated per cm^2, based on the surface of the cork borer (5 cm^2). The HGP method has on average a recovery percentage of 21.8 (SD 4.8) and an inoculation level of 1.67 log CFU listeria/g (SD 0.07) versus a recovery percentage of 15.6 (SD 6.7) and an inoculation level of 1.15 log CFU listeria/g (SD 0.19) for the BM method. Inoculation levels are not statistically compared because the initial loads are not comparable.
The low recovery for BM is possibly explained by a by a damaging effect of the shear forces which occur by chopping at high speed (4000 rpm). The recovery percentage for the HGP method can be explained by the efficiency of transfer of microorganisms from the glass surface to the meat surface. The results of the total aerobic psychrophyles count confirmed the results found with the Rapid L. mono agar, indicating that the selectivity of Rapid L. mono agar had no influence on the results (data not shown).
The growth of Listeria in the vacuum packed beef occurred approximately at the same rate for both inoculation methods (Figure 1). The slope of the growth is 0.02 Log CFU Lm/day for both inoculation methods (0.0219 for HGP and 0.0196 for BM). This rate of growth is low for *Listeria monocytogenes* in cooked beef product compared to the ARS ERRC Pathogen Modelling Program (http://www.arserrc.gov/mfs/PATHOGEN.HTM). The ARS ERRC Pathogen Modelling Program has been developed with broth models. A study form Duffy et al. (1994) has modelled the growth of Listeria in cooked beef. In this study relative long lag phases were determined and growth is modelled over a 35-day period. The products prepared by Duffy et al. contained 2% added water and gelatine for better slicing quality. In this study water loss from the cooking process was not used for the inoculation methods. The slower growth of Listeria monocytogenes in this study can be explained by a lower moisture content in the samples.

Conclusions

The method of surface inoculation using a horizontal glass plate (HGP) resulted in a significantly higher recovery of 21.8% versus 15.6% ($p<0.05$) for the method whereby the meat was chopped (BM) and at the same time the Listeria inoculum was blended in.
The low percentage for the BM method can be explained by a damaging effect of the shear force, which occurs during the chopping process. An inoculation method should not deactivate microorganisms, unless aimed for, because it can have an effect on the growth characteristics. Therefore the BM method is less suitable. An effect of the inoculation method on growth rate was not observed in this study.
For the HGP method there were no indications of a damaging effect on *Listeria monocytogenes*. In addition, the HGP method resulted in the lowest standard deviation in inoculation levels. For easy inoculation with *Listeria monocytogenes* the HGP method is therefore preferred.

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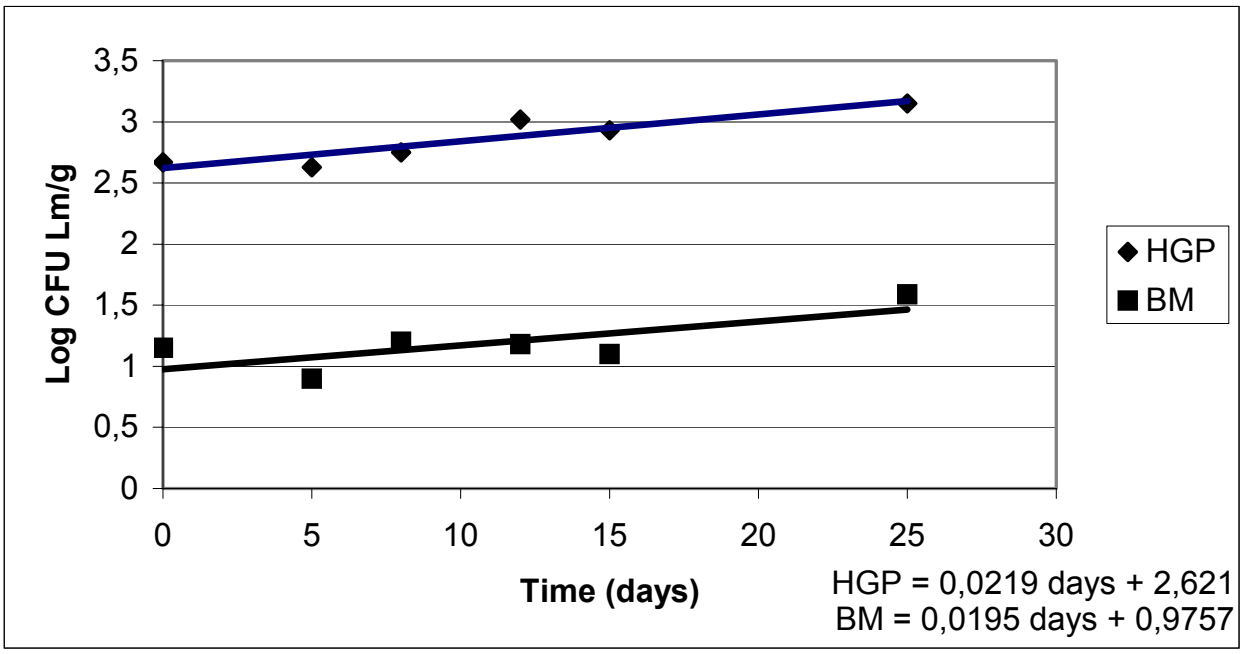
Table 1: *Listeria monocytogenes* counts from two different inoculation methods, HGP and BM

Method	Horizontal Glass Plate			Blending		
	Log CFU/g			Log CFU/g		
	AV	SD	Recovery (%)	AV	SD	Recovery (%)
1	1.68	0.07	25.6	1.40	0.17	25.2
2	1.59	0.18	19.6	1.30	0.00	20.0
3	1.66	0.08	16.7	1.26	0.24	18.2
4	1.70	0.03	26.2	0.80	0.17	6.3
5	1.70	0.13	24.6	0.90	0.17	7.9
6	1.79	0.04	27.1	1.23	0.50	17.1
7	1.58	0.09	15.6	1.36	0.10	22.9
8	1.61	0.09	16.1	1.00	0.30	10.0
9	2.07[1]	0.21[1]	[1]	1.10	0.35	12.6
10	1.74	0.17	24.9	1.10	0.35	12.6
AV	1.67	0.07	21.8	1.15	0.19	15.6

1 outlying observation based on Grubbs equation

Figure 1: Growth of inoculated *Listeria monocytogenes* in vacuum packed cooked beef



EFFECT OF Ar/CO_2 MODIFIED ATMOSTHERE PAKAGING ON TURKEY MEAT CHARACTERISTICS

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Background

Modified atmosphere packaging (MAP) with low storage temperature are important hurdles contributing to microbial and lipid oxidation stability of meat products and increasing their shelf life. Also the use of MAP improves product presentation, facility of storage, distribution, sale and utilisation (Ohlsson, 1994). The success of these technologies depends on the specificity of gas mixtures related to the product, type of meat, the nature and initial quality of meat, the temperature control, the barrier properties of the packaging film and the efficacy of the equipment (Taylor, 1996).
The use of CO_2 enriched atmospheres extends the shelf life of raw poultry by inhibiting the psychotrophic gram-negative bacteria and *Pseudomonas spp.*. Although later meat spoilage and changes on organoleptic characteristics are observed because slower-growing microorganisms (*Carnobacterium spp., Brochotrix thermosphacta, Lactobacillus spp*.) proliferate (Blakistone, 1999).
Apart the CO_2, O_2 and N_2 gas mixtures used on raw meat packaging only carbon monoxide (CO) has been adopted and studied on red meat (Sorheim *et al*., 1997) but its use is interdicted by law. However, Argon (Ar) is one of the gases authorised by the Directive 95/2/CE (1995) to be in contact with food. It's inert, odourless and tasteless, more dense and soluble than N_2. A study of sliced cooked ham packaged with Argon report advantages on oxidation control and microbial proliferation extending the product shelf life (Fachon, 2002).
It is important to clarify the influence of mixtures with Argon on the behaviour of spoilage flora and lipid oxidation of turkey meat.

Objectives

The aim of this work was to study the effect of Ar/CO_2 packaging atmosphere on spoilage flora and lipids oxidation stability of sliced turkey meat stored under refrigeration at 0±1ºC.

Materials and methods

The sampling of breast muscles from turkey carcasses (BIG 6 and BUT 9), selected for deboning according to plant criteria, was performed on different slaughter days under commercial conditions. The breast muscles were sliced with 1.5 cm thickness and a surface area of approximately 90-100cm^2. Scallops were transported in a refrigerated box to the laboratory and in less than one hour were packaged according to different study conditions.
Samples have been individually packaged in an aerobic atmosphere (polystyrene trays wrapped in an oxygen permeable polyvinyl film) and in four different modified atmospheres (MA) containing different gas mixtures as G1=100% N_2, G2=50% Ar/50% N_2, G3=50% Ar/50% CO_2 and G4= 50% N_2/50% CO_2 in "HBX-070" bags (a multilayer film EVOH-based) sealed with a packaging machine (EVT-7-CD, Tecnoprip, Barcelona). The aerobic atmosphere and MA packaged meat have been immediately stored (0±1ºC in the dark) for 12 and 25 days respectively. Each study condition was repeated at least nine times.
On days 0, 5, 12, 19, and 25 the following analyses were carried out:
Microbiological analysis: Total psycrotrophic aerobic counts at 7ºC for 10 days (Plate Count Agar, Merck, Germany), anaerobic count at 7ºC for 10 days (Brewer Anaerobic Agar, Merck, Germany), *Enterobactereaceae* counts in Violet Red Bile agar (VRB agar, Merck, Germany) at 37ºC for 2 days, *Pseudomonas spp*. counts (CFC agar base, Oxoid, UK) after incubation at 30ºC for 2 days, lactic acid bacteria (LAB) counts on Man Rogosa Sharpe Agar (Oxoid, UK) incubated at 30ºC for 3 days and *Brochothrix termosphacta* count in streptomycin, actidione, thallous acetate agar (STAA, Oxoid, UK) incubated for 2 days at 30ºC. Counts were expressed as log cfu/g.

Lipid oxidation evaluation by thiobarbituric acid test (TBA) was performed according to NP-3355 (1990). The total fat content was determined by Soxhlet method according to NP-1224 (1982).

Total fatty acids analysis: Lyophilised muscle samples were weighed into a culture tube and fatty acids were extracted and methylated by the method of Rule (1997). Quantification of fatty acid methyl esters was performed using 2 mg of nonadecanoic acid (C19:0) as internal standard, with analysis conducted by GLC using 30-m fused silica capillary column Omega Wax 250 (Supelco, Bellefonte, PA, USA) with a 0.25-mm internal diameter and a 0.25-mm film thickness. A Varian CP-3800 chromatograph (Varian Analytic Instruments, Walnut Creek, CA, USA), working with helium as gas carrier (1.3ml/min) and a flame ionisation detector, was used. The initial column temperature of 150ºC was held 11 min., increased to 220ºC at 3ºC/min and held 20min.The injector and detector temperature were 250ºC and 220ºC respectively. Peak identification was based on co-chromatography with known standards of fatty acid methyl esters (Sigma, St Louis, MO, USA). All results are presented (weight%) of total fatty acids assuming direct proportionality between peak area and fatty acid methyl ester weight.

Statistical analysis: Data was analysed using SPSS 11.5 for Windows. The comparison between gas mixtures package conditions, for microbial parameters, was performed by model adjustment of a one-way ANOVA for each day. For the parameters total fatty acid and TBA an ANCOVA model was adjusted to each day, considering as covariates the total fatty acid content (mg/g dry mater) and the total fat content (%) respectively. If *F* test from ANOVA or ANCOVA was significant, a LSD test post hoc multiple comparisons for observed Means has been performed. The comparison between days, considering each gas condition, was made by t-test for dependent samples.

Results and discussion

The evolution of microbial flora on sliced turkey meat packaged on different MA study conditions is reported on Figures 1 and 2. The initial contamination of sliced turkey meat (day 0), for total psicrotrophic counts, was approximately 4.6-4.9 log cfu/g. The aerobic packaging of sliced turkey meat allowed psycrotrophic counts to reach 6.9 log cfu/g on 5th day of storage and a higher level on the 12th day, about 9.9 log cfu/g, out of limit microbial criteria acceptability (7 log cfu/g). The facultative anaerobic flora also reached high levels. The dominant flora was *Pseudomonas spp*. followed by *Brochothrix termosphacta* and *Enterobactereaceae*, stated also by Santé *et al.* (1994).

The anaerobic conditions created by packaging with all gas mixtures (G1-4) delayed significantly ($p<0.001$) the development of dominant flora when compared with the aerobic packaging on the 12th days of meat storage. This inhibition of flora development regards particularly the meat packaged on G3 and G4 mixtures with CO_2. In what concerns the lactic acid bacteria (Figure 2, f), a slower growing microorganism group, there was an exception since that all gas mixtures packaging did not have any inibiting effect as it was stated by other authors (Saucier *et al.*, 2000; Blakistone, 1999).

The microbial development on meat MAP conditions G1 and G2 was similar (Figure 1 and 2). Ar, in mixture G2, does not add a microbial inhibitory effect. Despite the delay of flora development, with G1 and G2 gas mixture, had been relevant in particular to *Brochothrix termosphacta* (Figure 2-d), it was the presence of CO_2, on mixtures G3 and G4, which added a significant bacteriostatic effect on *Pseudomonas spp.* (Figure 1-b) and *Enterobactereaceae* (Figure 1-c) development during storage period.

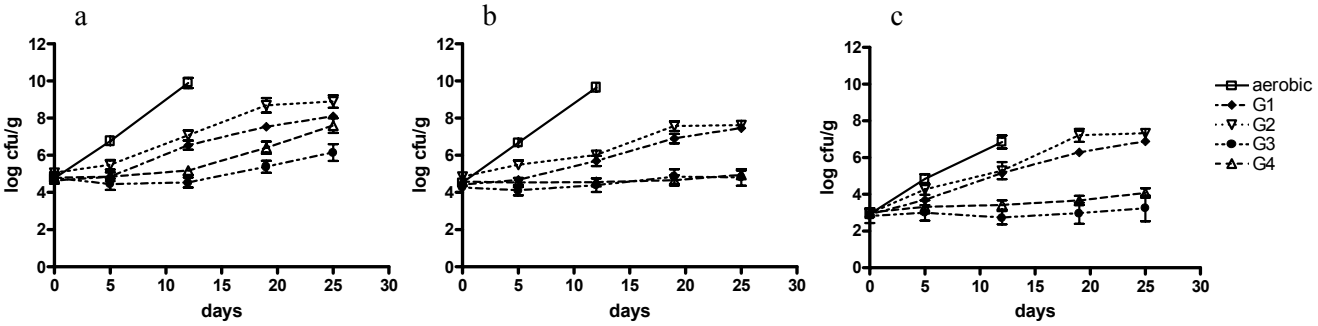

Figure 1: Evolution of total psycrotrophic flora (a), *Pseudomonas spp*. (b) and *Enterobactereaceae* (c) on sliced turkey meat packaged in an aerobic atmosphere and in four different MAP gas mixtures: G1=100% N_2, G2=50% Ar/50% N_2, G3=50% Ar/50% CO_2 and G4= 50% N_2/50% CO_2.

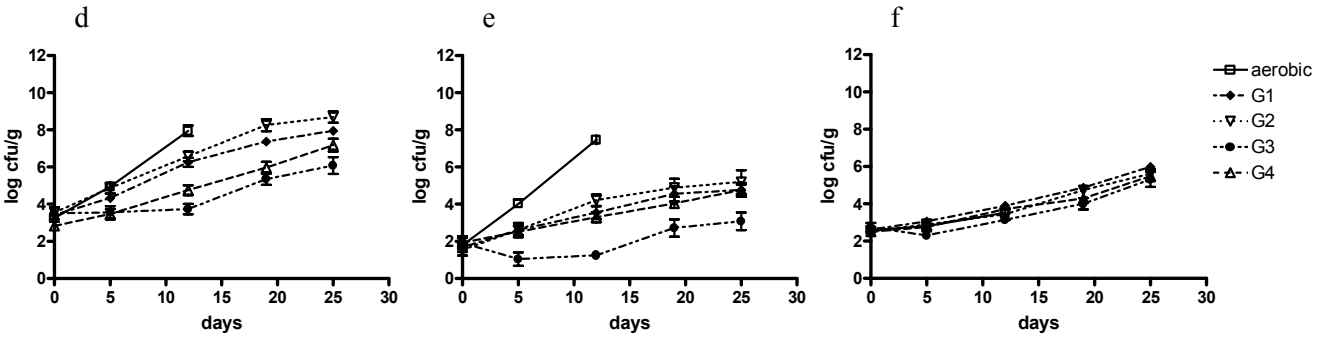

Figure 2: Evolution of total anaerobic flora (d), *Brochothrix termosphacta* (e) and Lactic acid bacteria (f) on sliced turkey meat packaged in an aerobic atmosphere and in four different MAP gas mixtures: G1=100% N_2, G2=50% Ar/50% N_2, G3=50% Ar/50% CO_2 and G4= 50% N_2/50% CO_2.

The mixture G3, with Argon associated to CO_2, was more efficient on delaying flora development than G4, without Ar. In these last study conditions (G3 and G4) there was more than 1 log difference, on the 25th day storage, for total psycrotrophic counts ($p<0.001$), total anaerobic counts ($p<0.05$) and *Brochothrix termosphacta* ($p<0.05$). This synergetic effect of Ar/CO_2 seemed to be related with the development delay of *Brochothrix termosphacta* and consequent relationship on anaerobic and aerobic psicrotrophic flora counts, which was lower than microbial criteria limit.

The microbial shelf life period extension of MA packaging on sliced turkey meat compared with aerobic packaging (5 days shelf life) is one more week for G1 and G2 mixtures, two weeks for G4 and three weeks to G3.

The results of lipid oxidation evaluation by TBA are presented on Figure 3. It was observed for aerobic packaging meat a significant increase ($p<0.05$) of TBA value after 5 days of storage (0.3 to 0.5 mg malonaldehyde /kg). The TBA value of aerobic package meat storage on 12th day has increased (0.7 mg/kg) but there was no significant difference from the 5th day. This last value do not exceed the cut off value of 2 mg malonaldehyde per kg of meat, at which rancidity may be detected by consumers indicated by Wood *et al*. (2003). The TBA results on 5th day of storage from anaerobic meat packaging conditions (G1-4) were slightly lower but not significantly different from those obtained on meat under aerobic package. However, on the 12th day of anaerobic storage conditions (G1, G2 and G3) the TBA values of turkey meat were significantly different ($p<0.05$) from the results obtained on aerobic meat packaging condition with exception to meat packaged with the G4 gas mixture.

The sliced turkey meat, after 19 days of packaging with G1 and G2 gas mixtures, had lower significant TBA values compared with those obtained from meat package with G3 condition. In fact, the meat packaged with G3 mixture had a significant increase of TBA value from the 12th to the 19th day of storage (0.54 to 0.7 mg/kg). The TBA value of meat under G3 package on the 25th of storage was significantly higher ($p<0.05$) than the results obtained from meat on the other anaerobic MAP conditions.

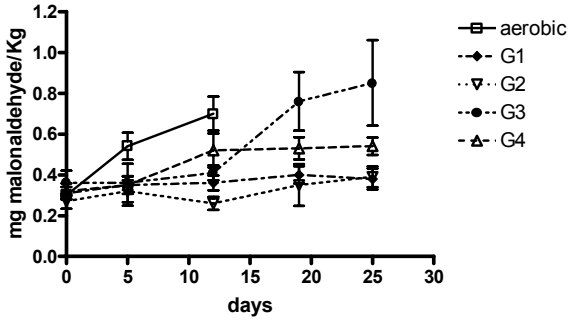

Figure 3 – Development of lipid oxidation measured by TBA content (mg malonaldehyde/kg) on sliced turkey meat packaged in an aerobic atmosphere and in four different MA gas mixtures.

This last observation was not supported by the evolution of polyunsaturated fatty acid content (PUFA) from sliced turkey meat package according to the study conditions (Figure 4).
The quantity of fatty acids on meat packaging under all MA gas mixtures was not affected since there were similar results at the beginning and end of storage. On 12th day of storage was observed a significant difference ($p<0.05$) between the C18:2 n-6 content of the meat packaged with G3 gas mixture (30.2%) and those on meat aerobically packaged (24.8%). In this aerobic condition, the PUFA meat content was significantly lower ($p<0.05$) than the observed on the beginning of storage.
On the 25th day of storage there were no significant differences in the amounts of polyunsaturated fatty acid of meat package with the four different gas mixtures. Comparing the results of total fatty acid from meat under anaerobic package conditions on the 25th day of storage with the control (samples aerobically package on the 12th day of storage), it was observed for the C18:3 n-3 content a significant difference between meat packaged with G1, G2 or G3 gas mixtures and meat aerobically packaged. The loss of polyunsaturated fatty acids was negligible in meat packaged under the anaerobic study conditions without any detectable difference between gas mixtures used on MAP. These accords with TBA values from the different meat anaerobically package, except for G3 condition. From our results, all anaerobic gas mixtures under study were effective on lipid meat oxidative preservation. The presence of Ar on gas mixtures did not seem to have any additional protective effect on lipid turkey meat oxidation.

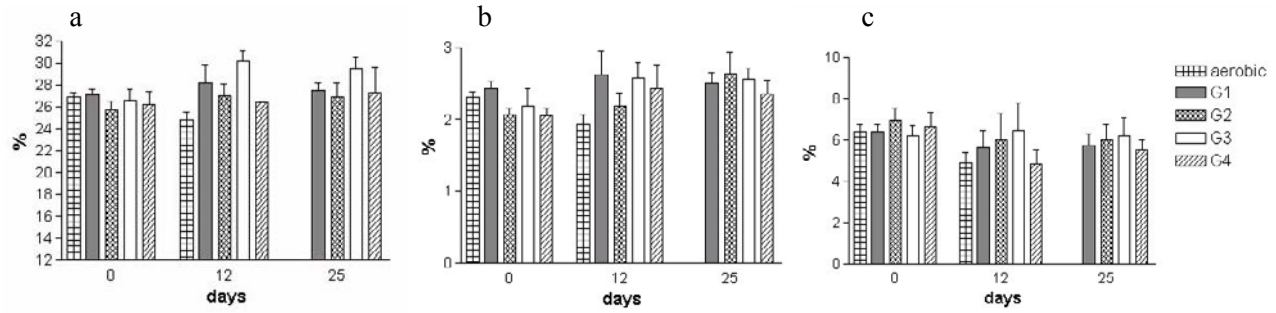

Figure 4: Evolution of fatty acid C18:2 n-6 (a), C18:3 n-3 (b) and C20:4 n-6 (c) on sliced turkey meat packaged in an aerobic atmosphere and in four different MAP gas mixtures.

Conclusions

The mixture with Argon associated to CO_2 was more efficient on delaying flora development than CO_2/N_2 with 1 log difference, on the 25th day storage, for total psycrotrophic counts, total anaerobic counts and *Brochothrix termosphacta*. However, the presence of Ar on gas mixtures did not seem to have any additional protective effect on lipid turkey meat oxidation.

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COMPARISON OF MICROFLORA OF TRADITIONAL FERMENTED SAUSAGES IDENTIFIED WITH TRADITIONAL AND MOLECULAR METHODS

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Background

Fermentation and drying of meat products are probably the most ancient ways of preservation. Fermentation of sausages in a traditional way has got a long history in the European countries. In view of the need to maintain the traditional sensorial quality of dry sausages and assure its safety, it is important to improve the industrial production of fermented sausages on the basis of development of protective cultures with acceptable technological and sensorial characteristics**,** after selection of strains isolated from naturally fermented products.

Key words: fermented sausage, lactic acid bacteria, Micrococci, identification, bacteriocin

Objectives

The objective of the investigation was the isolation and identification of microflora of naturally fermented sausages in different countries, with traditional and molecular methods (Bosnia-Herzegovina, Croatia, Greece, Italy, Hungary and Serbia-Montenegro) and the selection of lactic acid bacteria**,** which will not have negative effect on the sensorial characteristics and can produce bacteriocins which have antimicrobial effect against pathogenic microbes.

Materials and methods

Sausage preparation: Sausages were manufactured according to each country's standard practice without commercial starter cultures. Three batches of sausages were prepared for the experiments. Samples were taken from each batch for chemical and microbiological analysis at 0, 2, 4, 7, 14, 21and 28 days after formulation.

Isolation and characterization of lactic acid bacteria: A total of 150 (50 per batch) isolates were collected from MRS agar plate. Half of the colonies were isolated from ripening days 0 to 7 and the rest from day 14th to the end. The isolates were tested for cell morphology by phase contrast microscopy, Gram reaction, and catalase formation. Gram-positive and catalase-negative strains were subjected into the following physiological and biochemical tests: gas (CO_2) formation from glucose, arginine hydrolysis, growth in 8 and 10 % NaCl, growth at 4, 10, 15, 37 and 45 ^{o}C, slime formation, hydrolysis of arginine, ammonia, gas and slime formation. Sugar fermentation pattern was determined by the API 50 CHL (BioMerieux) and the identification was performed by the computer program APILAB Plus.

Isolation and characterization of catalase-positive cocci: A total of 150 (50 per batch) isolates were collected from MSA agar plate. The isolates were rapidly checked for cell morphology by phase contrast microscopy, Gram reaction and catalase production to ensure their classification to family *Micrococcaceae*. The isolates were subjected to the following tests: sensitivity to novobiocin, production of ammonia from urea, coagulase production, β-galactosidase activity, oxidase reaction, nitrate reduction and acetoin formation. Micrococci were separated from staphylococci on the basis of fermentation of glucose and growth in the presence of erythromycin and lysozyme. The strains were tested with the API Staph (BioMerieux) and the identification was performed by the computer program APILAB Plus.

Molecular identification of the isolated strains using PCR-based methods: DNA extraction*:* Total DNA was extracted using the method of Daud Khaled et al. (1997) with a modification or by the method described by Andrighetto et al. (2001). RAPD-PCR and electrophoresis of RAPD-PCR products were carried out with the oligonucleotide primer M13 (Andrigetto et al., 2001). 16S rDNA amplification and sequencing**:** After grouping of the strains with RAPD-PCR, one representative strain of each group was selected for

identification by 16S rDNA gene sequencing. The primers used for 16S rDNA amplification and sequencing were P1V1 and P4V3 (Klijn et al., 1991).
Antibacterial activity and assay of the isolated lactic acid bacteria: The antibacterial activity of the isolated lactic acid bacteria was assayed by the agar well diffusion assay (AWDA) described by Schillinger and Lücke (1989). The isolated strains were screened against three pathogens, such as *Listeria monocytogenes, Staphylococcus aureus* and *Escherichia coli*, in order to evaluate the potential use of the isolates as protective cultures.

Results and discussion

The results of the microbiological analysis showed that the number of the lactic acid bacteria was generally lower than total viable count in the first days of ripening in all sausages until the 4th day of fermentation. The population of the lactic acid bacteria (LAB) increased rapidly everywhere to about log 8 CFU/g and became the dominant flora of the sausages from this day in all batches in all countries and stayed constant during ripening. The adaptation of lactic acid bacteria to meat environment is well known and this feature made their growth faster. The ripening parameters were different and these are reflected in the composition of LAB. Based on the identification with API 50 CHL and traditional identification keys**,** the dominant microbes were identified as *L. plantarum* in three countries (Bosnia-Herzegovina, Croatia and Greece)*, Lc. lactis* subsp. *lactis* in Italy*, L. sakei* in Hungary and *L. fermentum* in Serbia-Montenegro. Dominant strains were also *L. pentosus* and *L. curvatus* as well as *Leu. mesenteroides* strains. The results of the genetic identification are in a good agreement with the results of other workers. According to the genetic identification**,** the predominant species were *L. sakei, L. plantarum* and *L. curvatus*.
Comparing the results obtained with API 50 CHL and molecular methods**,** it is clear that there were differences between the two identifications (see Table 1 and 3). The database of API 50 CHL identification programme did not contain *L. sakei* strain which has importance in testing of meat industrial strains and was the dominant flora in Italian, Hungarian, Bosnian and Serbian strains by PCR-based methods and second among Greek strains. This strain could be identified only with conventional identification keys and molecular methods. Our results confirm that the API 50 CHL method is not very convenient and may be misleading in the identification of lactic acid bacteria. Molecular genetic techniques showed good reproducibility and gave good results in case of doubtful cases, too**.**
Micrococci population was different among the countries. For example, in the Hungarian sausages the number was very low from the first days (log 2CFU/g) and did not increase further in any of the batches, in fact in batches 1 and 3 it were actually eliminated at the end of ripening process. The average initial micrococci population, was higher in Croatian (log 3 CFU/g), Greek, Bosnian, Italian and Serbian (about log 4 CFU/g) sausages. The tendency was increasing only in Italy, were the final number of population was higher in the end of the ripening process (log 5-6 CFU/g). When comparing staphylococci the dominant strains were *St. xylosus* and *St. saprophyticus* (in 3-3 countries) followed by *St. simulans*, *St. hominis* and *St. capitis*. The API Staph identification gave good results in every country for identification of staphylococci strains; in some cases micrococci needed additional tests.
The bacteriocin producing ability of the isolated strains of all counties were tested against *Listeria monocytogenes, Staphylococcus aureus* and *Escherichia coli* but only 7 strain showed antilisterial activity (Table 4). These strains will be further tested as protective cultures in challenge tests to investigate their inhibition against *Listeria monocytogenes* in sausage models, too.

Conclusions

Based on the API tests, conventional identification keys and molecular methods the dominant *Lactobacillus* and *Staphylococcus* strains isolated from fermented sausages from different countries, the isolated strains were determined and compared. The bacteriocin producing ability of the isolated strains were tested against *Listeria monocytogenes, Staphylococcus aureus* and *Escherichia coli* but only 7 strain showed antilisterial activity. Further investigations are needed to test the inhibition in sausage models.

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Table 1. Comparison of the lactic acid bacteria (%) isolated from naturally fermented sausages of different countries and identified with API 50 CHL kit

Bosnia-Herzegovina	Croatia	Greece	Italy	Hungary	Serbia-Montenegro
L. plantarum 40.7 *L. pentosus* 18 *L. curvatus* 16.7 *L. sakei* 8.7 *L. brevis* 7.3 *P. pentosaceus* 4.7 *Leu. lactis* 3.3. *L. salivarius* 0.6	*L. plantarum* (1*) 34 *L. brevis* 20.7 *L. curvatus* 18 *L. pentosus* 6.7 *L. plantarum* (2*) 5.3 *L. fermentum* 4 *P. pentosaceus* 3.3 *Lc. lactis subsp. lactis* 2 *Leu. mesenteroides* subsp. *mesenteroides* 2	L. *plantarum* 43.3 *L. curvatus* 10.7 *L. pentosus* 10.7 *L. brevis* 8.7 *Lc. lactis subsp. lactis* 6.7 *Leu. mesenteroides* subsp. *mesenteroides* 5.3 *L. rhamnosus* 3.3 *L. sakei* 4 *L. lactis* 4 *L. rhamnosus 3.3* *L. paracasei subsp. paracasei* 1.3 *L. salivarius* 0.7 *E. faecium* 0.7	*Lc. lactis* subsp. *lactis* 26 *L. fermentum 14* *L. plantarum* 11.3 *L. curvatus* 8 *L. brevis* 6 *L. mesenteroides* 5.3 *L. paracasei* 4 *P. pentosaceus* 2.7 *L. mesenteroides/ dextrinicus* 2 *L. lactis* 1.3 *L. acidophilus* 0.7 *P. acidilactici* 0.7 *L. cellobiosus* 0.7 Not identified *18*	*L. sakei* 28.7 *Leu. mesenteroides* ssp. *mesenteroides* 6.7 *Leu. mesenteroides dextranicum* 4.7 *L. sanfrancisco* 4.7 *L. plantarum* 3.4 *L. curvatus* 3.4 *L. delbrueckii* 3.4 *L. alimentarius 3.4* *L. amylophilus* 2.7 *L. bavaricus* 2 *W. viridescens* 2 *L. confosus* 1.4 *L. salivarius* 0.7 *L. acidophilus* 0.7 *L. maltoromicus* 0.7 *L. yamanashiensis* 0.7 *L. halotolerans* 0.7 *L. fructivorans* 0.7 *Leu. citreum* 0.7 *Leu. oenos* 0.7 17 % was unidentified	*L. fermentum* 24 *Leu. mesenteroides* ssp. *mesenteroides* 12.6 *L. brevis* 9.3 *L. delbrueckii* ssp. *delbrueckii* 9.3 *L. curvatus* 7.3 *S. faecalis* 6.6 *Lc. lactis* ssp. *lactis* 6.6 *L. plantarum* 6 *L. cellobiosus* 4.6 *L. collinoides* 4.6 *L. delbrueckii* ssp. *bulgaricus* 2.6 *Leu. mesenteroides* ssp. *mesenteroides* 2.6 *S. faecium* 2 *L. acidophilus* 0.6 *L. paracasei* ssp. *paracasei* 0.6

L. : Lactobacillus, Lc. : Lactococus , Leu. : Leuconostoc, E. : Enterococcus, P. : Pediococcus S. :Streptococcus and in Table 2 St.: Staphylococcus and * means serotype

Table 2. Comparison of the *staphylococci* and *micrococci* (%) isolated from naturally fermented sausages of different countries and identified with API Staph kit

Bosnia-Hercegovina	Croatia	Greece	Italy	Hungary	Serbia-Montenegro
St. saprophyticus 30.7 *St. simulans* 22 *St. xylosus* 16 *St. epidermidis* 10.6 *St. caprae* 10.6 *St. capitis* 6 *St.aureus /intermedius* 2.7 *St. auricularis* 0.7 *St. sciuri* 0.7	*St.xylosus* 29.2 *St. capitis* 25 *St. carnosus* 25 *St. saprophyticus* 20.8	*St. saprophyticus* 34.7 *St. xylosus* 14.7 *St. simulans* 11.3 *St. haemolyticus* 11.3 *St. haemolyticus* 11.3 *St. caprae* 8 *St. capitis* 6 *St. aureus/intermedius* 5.3 *St. sciuri* 3.3 *St. hominis* 2 *St. auricularis* 0.7 *St. warneri* 0.7 *St. cohnii subsp. cohnii* 0.7 *St. cohnii subsp. urealyticum* 0.7 *St. epidermidis* 0.7	*St. xylosus* 74 *St. hominis* 10 *St.warneri* 8 *St. saprophyticus* 3.3 *St. lentus* 2 *St. apidermidis* 1.3 *St. simulans* 0.7 Not identified 0.7	*St. xylosus* 43 *Micrococcus* spp. 16 *St. hominis* 15 *St. lentus* 10 *St. warneri* 6 *St. capitis* 4 *St. epidermidis* 2 *St. haemoliticus* 1 *St. auricularis* 1 *St saprophyticus* 1 *St. cohnii* 1	*S. saprophyticus* 21.1 *St. simulans* 14.4 *St. xylosus* 21 *St. auricularis* 12.2 *St. warneri* 6.7 *St. aureus* 6.7 *St. hominis* 4.4 *St. cohnii* 1.1 *M. varians* 35 *M. nishinomiyaensis* 23.3 *M. lylae* 10 *M. luteus* 6.7 *M. roseus* 10 *Micrococccus* spp. 15

Table 3. Comparison of the lactic acid bacteria (%) isolated from naturally fermented sausages of different countries and identified with PCR method

Bosnia-Herzegovina	Croatia	Greece	Italy	Hungary	Serbia-Montenegro
L. sakei 39.3 *L. curvatus* *L. plantarum* 16.7 *L. brevis* 7.3 *P. acidilactici* 6 *P. pentosaceus* 2.7 *L. alimentarius* 2.7 *Lb. Farciminis* 1.3	*L. plantarum* (1) 51.3 *L. curvatus* 21.3 *L. plantarum* (2) 6.6 *L. brevis* 16 *L. fermentum* 6 *L. pentosus* 4 *Pediococcus pentosaceus* 2 *Lc. lactis* subsp. *lactis* 2	*L. curvatus* 43.3 *L. sakei* 23.3 *L. plantarum* 18 *L. paraplantarum* 4 *E. faecium/durans* 3.3 *L. casei/paracasei* 2.7 *L. farciminis* 2 *Leu. mesenteroides* 2 *L. alimentarius* 1.3	*L. sakei* 42.7 *L. curvatus* 36 *L. plantarum* 6 *L. paraplantarum* 4.7 *L. paraplantarum/ pentosus* 2.7 *Leu. mesenteroides* 2.7 *W. paramesenteroides/ hellenica* 2.7 *Leu. citreum* 0.7 *L. brevis* 0.7 *Lc. lactis subsp. lactis* 0.7 *Enterococcus pseudoavium* 0.7	*L. sakei* 64 *L. curvatus* 7.3 *Leu. paramesenteroides/ W. hellenica* 6.7 *W. viridescens* 6 *Leu. mesenteroides* 4.7 *Ln. kimchii* 2.7 *L. plantarum* 2 *L. plantarum/ paraplantarum* 2 *Leu. citreum* 2 *E. infantar* 0.7 *Enterococcus spp.* 0.7 *St.saprophyticus* 0.7	*L.sakei* 52.7 *L. curvatus ssp. curvatus* 16.7 *L. brevis* 9.3 *L. plantarum/ paraplantarum* 6 *L. parakasei ssp. parakasei* 5.3 *E. fecium/durans* 4.6 *L. johansoni* 2.7 *L. casei MCRF* 2.7

Table 4. Number of isolated LAB which were active against the indicator strains

Indicator microbes	Bosnia-Hercegovina	Croatia	Greece	Italy	Hungary	Serbia-Montenegro
Listeria monocytogenes	0/150 LAB	0/150 LAB	**1/150 LAB**	**6/150 LAB**	0/150 LAB	0/150 LAB
Staphylococcus aureus	0/150 LAB	0/150 LAB	0/150 LAB	0/150 LAB	0/150 LAB	0/150 LAB
Escerichia coli	0/150 LAB	0/150 LAB	0/150 LAB	0/150 LAB	0/150 LAB	0/150 LAB

This research has been carried out under the umbrella of the "SAFETYSAUSAGE" project, which is funded by the E.C. within the framework of the INCO-DEV program (Contract No ICA4-CT-2002-10037). The authors gratefully acknowledge the Institute of Meat Hygiene and Technology (Belgrade), the University of Sarajevo Veterinary Faculty, the Zagreb University Veterinary Faculty for their microbiological results, BEKKA SA, "OVAKO" Meat industry and Hunnia Meat Processing and Service Ltd for the production of sausages and Dr. Rantsios for his overall advice and support**.**

EFFECT OF LACTIC ACID BACTERIA OR GLUCONODELTALACTONE ON TEXTURAL AND PHYSICOCHEMICAL PROPERTIES OF CALCIUM CHLORIDE MARINATED BOVINE BRACHIOCEPHALICUS MUSCLE

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Background

Natural meat proteolysis seems to be one of the main factors that contribute to tenderisation process during meat aging. Two enzymatic systems are involved in meat tenderness: calpains and cathepsins. However, there are some discrepancies regarding to the cooperative action of both enzymatic systems. It is accepted that these proteolytic systems are similar in all muscles, independently of animal species. Calpain activation could be done by injecting or dipping the meat with/in $CaCl_2$ solutions. Many meat cuts considered as tough due to their high collagen content have a relatively low price during commercialisation. An alternative to obtain more tender meat is the calcium chloride marination, by means of the activation of the calcium dependent or calpain proteolytic system. Acidulating agents produce a high pH reduction. In this way, glucono-δ-lactone in water is hydrolyzed to gluconic acid, and is employed to produce gels in many conditions. In other hand, lactic acid bacteria has been suggested to become protective cultures in meat products due their ability to inhibit the growth of other microorganisms by many ways, like lowering pH by lactic and acetic acid production, competition for the nutrients, or producing hydrogen peroxide, bacteriocins, or antibiotics.

Objectives

We are proposing the calcium chloride marination beside to the application of acidulating agents in order to, first, activate the calpain system, and secondly, decrease pH enough, affecting on a remaining cathepsin activity or a protein degradation by the denaturing effect of low pH, and thus improving meat tenderness.

Materials and methods

A complete factorial design was employed to determine the effect of the acidulating agent, lactic acid bacteria (LAB) or glucono-δ-lactone (GDL), and storage time on texture, water holding capacity, pH and myofibrillar fragmentation index of meat samples. The PROC GLM procedure of the SAS Statistical System v. 8 (SAS Institute, Cary, NC) was employed in order to determine significant differences. Results are the means of three replications. Bovine *brachiocephalicus* muscle was obtained within 48 h *postmortem* in a local slaughterhouse. Muscles were cut in 150 g samples and were marinated in a 150 mM CaCl2 solution at 4°C in a vacuum tumbling machine. After marination, one lot of samples were immersed in a LAB suspension with an optical density of 1.0 (*Pedioccocus pentosaceus* PC-111016, Christian Hansen A/S, Hoersholm, Denmark) at 4°C during 15 minutes. Other lot of marinated samples was immersed in a GDL solution (1% w/v, Lot: 070199-22, Glocona America Inc., Janesville, WI) at 4°C during 30 minutes. Finally, a third lot of samples were keep as control under same storage conditions. All samples where then vacuum packed and stored at 4°C during 1, 5, 10 and 15 days. Warner-Bratzler shear force (WBSF) was determined in a texture analyser model TA-HDi (Texture Technologies, Scarsdale, NY/ Stable Micro Systems, Surrey, UK) equipped with a 50 kg load cell. Samples of approx. 6.0x1.2x1.2 cm were cooked in hot water until an internal temperature of 70°C, as described by Wheeler et al. (1979). Cooked samples were compressed at a constant speed rate of 120 mm/min with the Warner-Bratzler razor, reporting maximum force detected during the test. The pH value was determined using 10 g of sample homogenized with 100 ml of distilled water in a Warning blender during one minute with an Oakton potentiometer EP 2500 (Oakton Corp., Singapore). Water holding capacity (WHC) was determined in agree to the methodology reported by Hamm (1975), with some modifications. Meat samples (300 mg) were placed between two Whatman No. 1 filter papers and compressed during 10 minutes with 1 kg weight between two Plexiglas plates. Water holding

capacity was reported by weigh difference as the percentage of humidity retained in the paper. Sodium dudecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), in denaturing conditions, was performanced in a Mini Protean II Slab cell (Bio Rad, Richmon, CA). Acrylamide concentrations were 4% for stacking and 12% for resolving gels (Bollag & Edelstein, 1991). Myofibrillar fragmentation index was determined using the technique reported by Olson et al. (1976). In order to determine the effect of inoculated LAB on microbial populations, a microbiological analysis were made during storage time. Total coliforms were determined in violet red bile (VRB) agar plates incubated at 35°C during 24 h. LAB were determined in Mann-Rugose-Sharp (MRS) agar plates incubated at 35°C during 24 h. After pertinent dilutions, the colony forming units (CFU) per g of sample were reported in control and LAB treated samples.

Results and discussion

Control pH remained constant during all the storage period since after 48 h *postmortem* and vacuum packed little biochemical changes are expected at 4°C. As expected, LAB and GDL had a significantly effect ($P<0.01$) on pH. WHC was significantly affected ($P<0.01$) by the LAB or GDL treatments during the storage (Figure 1). Control samples (only marinated with $CaCl_2$ solution) presented a little increase in WHC during storage time. LAB treated samples presented higher WHC values than the GDL treatments. The results indicate that the samples treated with acidulating agents had lower WHC values than control sample, since the acidity reduced the pH close to the myofibrillar proteins isoelectric point. Furthermore, low pH values could result in a myofibrillar protein denaturation altering their ability to retain water (Aktas & Kaya, 2001). The texture of the samples was affected by the treatment ($P<0.01$), but not by the storage time ($P>0.05$). GDL treated samples were tougher (high shear force values) than LAB or control (Figure 1). Meat control tenderness increased with storage time, mainly by the $CaCl_2$ effect (Pérez-Chabela et al., 1998). LAB samples force presented a drastic reduction after the 5-day of storage, reaching the same WBSF values than control samples at the 15 day. GDL samples were stronger and more difficult to shear, almost twofold than control samples. In our experiment, GDL samples were tougher than LAB ones. It could be explained from the view that the GDL reduced drastically and quickly the pH, denaturing proteins instead to promote some kind of myofibrillar weakening. When meat pH is below 5.5, meat could be softer due to protein degradation or loss of the structure. MFI increases with storage time and was higher for control samples than for LAB or GDL samples ($P<0.01$)(Figure 2). Control samples presented higher values on the first storage day, with an important increase on day 10. LAB and GDL samples were similar during the first 10 days but LAB had high values at the end of the sampling period. Lin et al. (2000) reported that a high MFI in mule duck marinated in red wine correspond to the acidic environment provided by the red wine, where the cathepsins or lysosomal enzymes could be responsible for protein degradation of myofibrils in acid environment (Saunders, 1994), and MFI is directly related to tenderness (Culler et al., 1978). Microbial population evolution was depicted in Figure 3, where the LAB population increased in LAB treated samples and control, whereas the coliforms population was decreasing in LAB samples. The reduction in coliforms population was important due to inoculated LAB after $CaCl_2$ marination, whereas in control samples in spite of the detection of an relatively high naturally occurred LAB population, coliforms grew with storage time with no apparent relationship with the native LAB. An important natural occurring LAB population was found in control samples. LAB inoculation could contribute in extending shelf life of meat products due to their ability to inhibit pathogens and deteriorative microorganisms by the nutrient competition, displacing them form their ecologic niche and/or the production of antimicrobial substance as lactic or acetic acids, diacetyl, hydrogen peroxide and bacteriocines (Requena & Peláez, 1995; Helander et al. 1997).

Conclusions

The GDL concentrations not affected meat tenderness, probably because of high acidic conditions generated. LAB incorporation had a minor effect on meat tenderness, but reduced the coliforms population. In the present experimental conditions employed, a maximum degradation, and hence tenderness, were reached at the 5th day of storage with LAB as acidulating agent.

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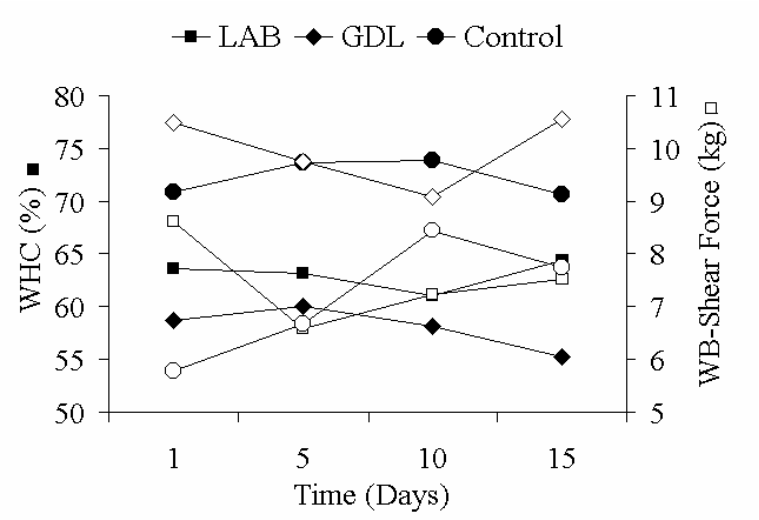

Figure 1. Water holding capacity (WHC) and Warner-Bratzler shear force in marinated meat samples during storage.

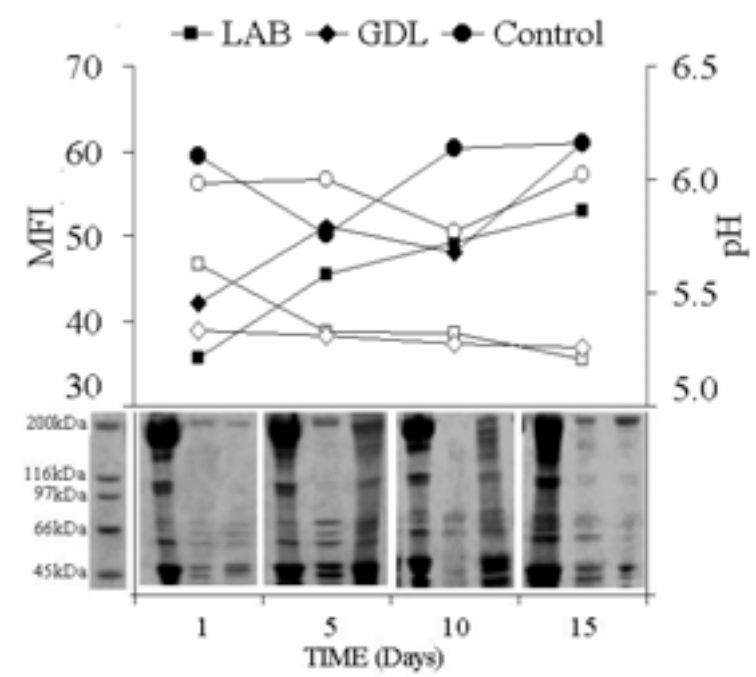

Figure 2. Myofibrillar fragmentation index (MFI), pH and SDS-PAGE for the marinated meat samples during storage (Left: MWM. For each time: Line 1: Control, Line 2: LAB, Line 3: GDL).

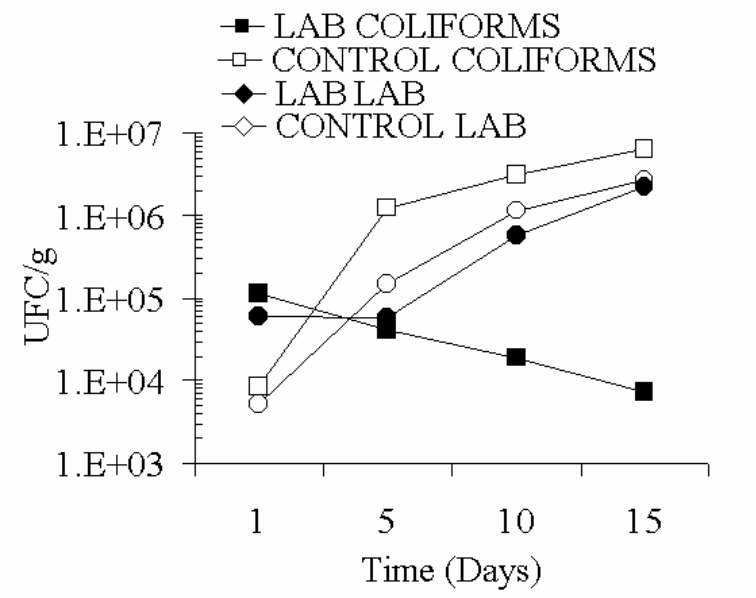

Figure 3. Microbial population in Control and LAB treated samples during storage.

COMPETITIVE INHIBITION OF MEAT SPOILAGE BACTERIA AND PATHOGENS

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Background

Brochothrix campestris was isolated from soil and grass and first described in detail by Talon and co-workers (1988). Subsequently, it was found to produce a broad spectrum bacteriocin (brochocin-C) inhibitory to a range of gram-positive bacteria including *Brochothrix thermosphacta*, *Listeria* spp. and lactic acid bacteria (Siragusa and Cutter, 1993). Purified brochocin-C was also active against vegetative cells and spores of *Bacillus* and *Clostridium* spp., including *C. botulinum* (McCormick *et al*., 1998).

The cost associated with the production of bacteriocins and inactivation in food environments often precludes their practical application as food preservatives. Thus, the use of viable, bacteriocin-producing cells as biopreservatives in meats is a reasonable alternative (Lücke, 2000). Unfortunately, it has been assumed that *B. campestris* would not be an acceptable strain for use in meats and research has been undertaken to heterologously express brochocin-C in "food grade" lactic acid bacteria such as *Carnobacterium piscicola* (McCormick *et al*., 1998; Garneau *et al*., 2003). This technique is tedious and it is difficult to develop strains in which the heterologous expression of brochocin-C is stable.

Preliminary, inoculation studies had shown that *B. campestris* could grow on pork adipose tissue at 4°C without producing undesirable odours or changes in the appearance of the fat. Also, there were no known published reports of the competitive inhibition of bacteria by *B. campestris* in broths. Thus, the current study was designed to provide needed data on the competitive effects of *B. campestris* co-inoculated with gram-positive spoilage bacteria and pathogens.

Objectives

This study was undertaken to determine the ability of *B. campestris* ATCC 43754 to control the growth of *B. thermosphacta* B2, *Lactobacillus sake* 1218 and *Listeria monocytogenes* when co-cultured in APT broth at 4°C.

Materials and methods

B. campestris ATCC 43754 was purchased from the American Type Culture Collection (Manassas, VA, USA). *L. sake* 1218 (Leisner *et al*., 1996) and *B. thermosphacta* B2 (Greer and Dilts, 2002) were meat spoilage strains. Two of the *L. monocytogenes* strains were isolated from a pork processing facility, one was a poultry isolate (ATCC 19111) and the fourth was a clinical isolate of *L. monocytogenes* Scott A. (Health Canada, Ottawa, ON). Inocula were prepared by growing the individual strains in APT broth (Difco, Becton Dickinson Co., Sparks, MD, USA) at 25°C for 18 h.

Growth studies were conducted in APT broth incubated at 4°C for 7 to 13 d. All experiments were conducted under aerobic conditions with the exception of mixed cultures with *L. sake* where growth was also determined under anaerobic conditions in a BBL anaerobic jar with 5-10% CO_2 (BBL Anaerobic System, Becton Dickinson). *B. campestris* ATCC 43754 was diluted to give an initial population of about 6 to 7 log CFU/ml and the target species were at initial numbers of 3 to 4 log CFU/ml. Prior to dilution, the 4 *L. monocytogenes* strains were mixed by combining equal volumes of the individual 18 h cultures.

The growth of the 3 target species (*B. thermosphacta* B2, *L. sake* 1218 or the 4 strain mixture of *L. monocytogenes*) was determined alone and in mixed culture with *B. campestris* ATCC 43754. To recover and enumerate *B. thermosphacta* during co-culture with *B. campestris* a modified MRS medium (Wilkinson and Jones, 1977) was utilized in the presence or absence of 0.1% potassium tellurite. *B. thermosphacta* B2 grows to produce black colonies on this medium while the growth of *B. campestris* ATCC 43754 is inhibited. Tryptone-phytone-yeast extract agar containing 0.02% erioglaucine (TPYE, Greer and Dilts,

1997) was used to enumerate *L. sake* 1218 during co-culture with *B. campestris* ATCC 43754. This medium would inhibit the growth of *B. campestris* ATCC 43754 while *L. sake* 1218 would produce pale green colonies. PALCAM-agar (Difco) was used to enumerate *L. monocytogenes* during co-culture with *B. campestris* ATCC 43754. *L. monocytogenes* produced black colonies on this medium (Corry *et al*., 1995) and *B. campestris* ATCC 43754 was inhibited. Streptomycin-thallous acetate-actidione agar (STAA, Corry *et al*., 1995) enabled the recovery of *B. campestris* ATCC 43754 while not permitting the growth of *L. sake* 1218 or *L. monocytogenes*. All media were inoculated using the spread plate technique with the exception of TPYE where Hydrophobic Grid Membrane Filtration was used.

Bacterial numbers were converted to common logarithms and reported as log CFU/ml. The significance of treatment differences was determined by analysis of variance using the general linear model procedures of the SAS Institute.

Results and discussion

Biopreservation of meats with bacteriocinogenic lactic acid bacteria (LAB) and their bacteriocins has been the subject of authoritative reviews (Stiles, 1996; Lücke, 2000). Although *B. campestris* ATCC 43754 was known to produce a broad spectrum bacteriocin (Siragusa and Cutter, 1993) the current study was the first to show that this organism could competitively restrict the growth of meat spoilage bacteria and pathogens in mixed cultures at 4°C (Tables 1 to 4) resulting in a significant reduction in bacterial numbers ($P < 0.05$).

The competitive strain, *B. campestris* ATCC 43754, increased by 2 log CFU/ml by about 5 d of incubation to reach a maximum population approximating 9 log CFU/ml. During co-culture with *B. thermosphacta* (Table 1) or *L. monocytogenes* (Table 2), *B. campestris* was bacteriostatic. This was evident in a reduction in the rate of growth of the target strains producing a 3-4 log CFU/ml reduction ($P < 0.05$) in bacterial numbers by the end of the incubation period. Lactobacilli have also been shown to inhibit the growth of *B. thermosphacta* (Shay *et al*., 1984) and *L. monocytogenes* (Schillinger *et al*., 1991) in broths and meat products.

In mixed culture with *L. sake* 1218 under aerobic conditions, *B. campestris* ATCC 43754 was initially bactericidal and *L. sake* could not be recovered from the culture media after 1 and 4 d of incubation (Table 3). Thereafter, *L. sake* 1218 began to grow but the numbers remained at least 4 log CFU/ml lower by the end of the incubation period when compared to those determined during the growth of *L. sake* 1218, alone (Table 3). Under anaerobic conditions, *B. campestris* ATCC 43754 demonstrated a prolonged bactericidal effect and no *L. sake* 1218 could be recovered from mixed cultures with *B. campestris* ATCC 43754 following 2 to 12 d of incubation at 4°C (Table 4), while *L. sake* 1218 alone grew to 9 log CFU/ml. Leisner *et al*. (1996) reported that *L. sake* 1218 could be inhibited in APT broth and in vacuum packaged beef by *Leuconostoc gelidum* UAL 187 and the inhibition of bacterial growth was of a sufficient magnitude to delay the sulphide spoilage by *L. sake* 1218.

Conclusions

The growth of *B. thermosphacta* B2, *L. sake* 1218 or a 4 strain mixture of *L. monocytogenes* was significantly inhibited in mixed culture with *B. campestris* ATCC 43754 during the incubation of APT broth at 4°C. Depending upon the target strain in the mixture and the gaseous atmosphere, the inhibition was either bacteriostatic or bactericidal. Additional research is necessary to transfer the system to meats and to insure that the competitive organism, *B. campestris* ATCC 43754, does not produce spoilage.

Acknowledgements

The authors gratefully acknowledge the valuable support of Loree Verquin.

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Table 1. Aerobic growth of *B. thermosphacta* B2 in mixed culture with *B. campestris* ATCC 43754 in APT at 4°C

Time (d)	Log CFU/ml[1]		
	B. campestris	*B. thermosphacta*	*B. thermosphacta* with *B. campestris*
0	7.04 ± 0.14	3.44 ± 0.24	2.25 ± 0.12
1	7.52 ± 0.33	3.14 ± 0.26	2.48 ± 0.40
2	8.16 ± 0.25	4.92 ± 0.07	3.01 ± 0.24
3	8.51 ± 0.28	5.68 ± 0.28	3.91 ± 0.13
4	8.69 ± 0.09	6.60 ± 0.04	4.01 ± 0.34
5	8.73 ± 0. 07	6.97 ± 0.12	4.32 ± 0.30
6	8.97 ± 0.08	6.97 ± 0.44	3.80 ± 0.40
7	8.90 ± 0.09	7.82 ± 0.15	4.28 ± 0.26

[1]Data are means and standard errors for 2 trials and 5 replications.

Table 2. Aerobic growth of a *L. monocytogenes* pool in mixed culture with *B. campestris* ATCC 43754 in APT at 4°C

Time (d)	Log CFU/ml[1]		
	B. campestris	*L.* monocytogenes[2]	*L. monocytogenes*[2] with *B. campestris*
0	6.90 ± 0.08	4.42 ± 0.05	4.45 ± 0.04
1	7.06 ± 0.09	4.64 ± 0.04	4.65 ± 0.04
3	8.44 ± 0.05	5.28 ± 0.07	5.34 ± 0.07
6	9.21 ± 0.09	6.36 ± 0.08	5.88 ± 0.14
9	9.10 ± 0.11	7.62 ± 0.09	5.92 ± 0.15
11	8.75 ± 0.18	8.68 ± 0.09	6.01 ± 0.08
13	8.45 ± 0.17	9.09 ± 0.04	5.80 ± 0.13

[1]Data are means and standard errors for 2 trials and 6 replications.
[2]Pool of 4 strains.

Table 3. Aerobic growth of *L. sake* 1218 in mixed culture with *B. campestris* ATCC 43754 in APT at 4°C

Time (d)	Log CFU/ml[1]		
	B. campestris	*L. sake*	*L. sake* with *B. campestris*
0	6.42 ± 0.05	3.22 ± 0.05	2.81 ± 0.33
1	6.49 ± 0.05	3.58 ± 0.11	0[2]
4	8.46 ± 0.14	6.18 ± 0.11	0
6	8.69 ± 0.02	5.91 ± 0.05	4.51 ± 0.18
8	8.81 ± 0.08	8.43 ± 0.11	3.91 ± 0.03

[1]Data are means and standard errors for 2 trials and 5 replications.
[2]<1 bacteria/ml.

Table 4. Anaerobic growth of *L. sake* 1218 in mixed culture with *B. campetris* ATCC 43754 in APT at 4°C

Time (d)	Log CFU/ml[1]		
	B. campestris	*L. sake*	*L. sake* with *B. campestris*
0	6.92 ± 0.03	2.91 ± 0.05	2.74 ± 0.06
2	7.68 ± 0.03	3.36 ± 0.01	0[2]
5	9.00 ± 0.04	5.87 ± 0.01	0
7	8.80 ± 0.03	6.29 ± 0.04	0
9	8.92 ± 0.03	8.04 ± 0.03	0
12	8.93 ± 0.07	9.21 ± 0.05	0

[1]Data are means and standard errors for 2 trials and 5 replications.
[2]<1 bacteria/ml.
Incubation was in a BBL anaerobic jar (Becton Dickinson) in an atmosphere containing 5-10% CO_2.

THE EFFECT OF DISINFECTANTS ON *LISTERIA MONOCYTOGENES* ADHERENT TO MEAT BIOFILM

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Background

Listeria monocytogenes is the major problem concerning the safety of ready-to-eat (RTE) cooked meat products. When L. monocytogenes occurs in RTE cooked meat products, it is due to after-contamination when the products are handled and packed, because L.monocytogenes occurs in the environment. The occurrence in the environment can be persistent, because L. monocytogenes has the ability to form biofilms on food-processing surfaces. Biofilm is composed of meat residues and microbial polymers formed during bacterial growth. Bacteria adherent to a biofilm is highly protected against chemical compounds such as disinfectants.
It is very important to be aware of the ability of the disinfectants to penetrate a biofilm, so that the right disinfectant can be chosen when problems with biofilm arise in the production plant. It is important that the disinfectants are evaluated in an experimental set-up closely related to the environment in the production plant.
Fatemi et al.(1999) and Krysinski et al. (1992) measured the effect of disinfectants and sanitizers on L. monocytogenes cells adherent to a biofilm attached to stainless steel. Both methods included a biofilm produced in tryptone soya broth (TSB). When using the laboratory broth, the nutrients are different from the nutrients available for the microorganisms in the production plant. Krysinski et al. (1992) enumerated surviving L. monocytogenes by the plate count method after dislodging the cells from the stainless steel. Fatemi et al. (1999) enumerated viable L. monocytogenes in the biofilm by the direct agar overlay method.
The present study introduces a method to create a L. monocytogenes biofilm composed of meat residues normally present on the surfaces of equipment in meat production plants and describes a method to measure the bacterial counts on stainless steel using a Malthus apparatus.

Objectives

The objective of this study is to test the ability of five different disinfectants to inactivate *L. monocytogenes* cells adherent to a biofilm under simulated production site conditions.

Materials and methods

L. monocytogenes DMRICC 3001, originally isolated from the meat environment, was grown in BHI broth at 30°C.
Biofilm was formed on stainless steel coupons (1cm x 2.5cm) by immersing the coupons in a meat sausage/water emulsion (1:4) inoculated with 1% *L. monocytogenes* and incubated at 30°C for 6 days. The coupons were then rinsed with sterile water to remove unattached cells and meat.
Detergents: 4% Deptal FM2, alkaline detergent (NaOH, KOH), Hypred and 5%, P3-topax, 66, sanitizer (NaOH: 5-15%, sodium hypochlorite: <5% active chlorite, alkylaminoxide: <5%), Ecolab.
Cleaning methods: Before disinfection, coupons with biofilm were cleaned in the alkaline detergent or the sanitizer by scrubbing for one minute with a cloth soaked in one of the detergents or by soaking the coupons in the detergent solution for 15 minutes. After cleaning the coupons were rinsed in 10 ml sterile water.
Disinfectants: Deptal Mycoside S (QAC) (Didecyldemethylammoniumchloride) 0.3% for 20 minutes (Hypred), Betane Plus (QAC) (QAC compounds: 5-15%, poly(hexamethylen)biganid, HCl:<5%) 1% for 10 minutes (Ecolab), Desinfect Maxi (QAC) (N,N-didecyl-N,N-dimethylammoniumchloride:5-15%, propan-1-nol:<5%) 1% for 5 minutes (Novodan), sodium hypochlorite (12.4% active chlorite), 0.16% for 5 minutes (Novadan), P3 oxysan (Acetic acid: 15-30%, H_2O_2:5-15%, alkylsulfonate: 5-15%, peraceticacid: <5%, phosphonacid: <1%) 0.1% for 5 minutes (Ecolab).
Disinfection: The coupons were disinfected by soaking in the disinfectants. The concentration and time treatment were chosen from the respective data sheets from the chemical manufacturer. After disinfection, the coupons were rinsed with 10 ml sterile water and air-dried for 5 minutes. Untreated coupons were soaked

in sterile phosphate buffer (pH 7.2) for 5 minutes. All coupons were soaked in a neutralizing liquid for 5 minutes before they were analyzed in the Malthus apparatus
Growth of *L. monocytogenes* was determined by measuring the time until *Listeria monocytogenes* started producing CO_2 in the Malthus apparatus. The correlation between time and cfu/g biofilm was calculated to be: X (log cfu/g)=÷0.29 xY(time until growth) + 9.32 (R=0.99).

Results and discussion

Cleaning with the sanitizer followed by disinfection with pH neutrale quaternary ammonium chloride compounds (QACs) resulted in a complete inactivation of L. monocytogenes adherent to the meat biofilm (see figure 1). There was no difference between the two cleaning methods. QAC is a large four-chain molecule with a central N-atom. The chemcial composition of the three investigated QACs varied in two of the sidechains, but this did not affect the ability of the QACs to inactivate L. monocytogenes. The most important properties for the QAC's ability to penetrate biofilms are resistance to organic matter and low surface tension. The cleaning method could not be evaluated because of the complete inactivation of L. monocytogenes.
The same results were seen when cleaning with an alkaline detergent before disinfection with QACs (figure 2).
Cleaning with the sanitizer by scrubbing followed by disinfection with sodium hypochlorite reduced the count of *L. monocytogenes* by 3.5 log cfu/g compared to untreated coupons (figure 1). Cleaning by soaking the coupons in the sanitizer resulted in a 1 log reduction compared to untreated coupons (figure 1). This indicates that a mechanical treatment is an important factor in reducing bacterial counts.
Cleaning with the alkaline detergent by scrubbing, followed by disinfection resulted in a 1.7 log reduction compared to untreated coupons (figure 2). This is a lower reduction compared to cleaning with the sanitizer, the reason being that the sanitizer contained disinfectant (hypochlorite), which meant that the coupons were disinfected twice.
The lower inactivation of *L. monocytogenes* resulting from disinfection with hypochlorite compared to QACs can be explained by the fact that sodium hypochlorite is very sensitive to the presence of organic matter, especially protein.
Disinfection with peroxide after cleaning with the sanitizer resulted in a 1.5 log reduction of *L. monocytogenes* compared to untreated coupons (figure 1). The cleaning method (soaking or scrubbing) had no influence on the inactivation of *L. monocytogenes*.
When cleaning with the alkaline detergent by scrubbing followed by disinfection with peroxide, the reduction of *L.monocytogenes* was 1.5 log cfu/g, which is similar to cleaning with the sanitizer followed by disinfection.
Soaking in an alkaline detergent, followed by disinfection with peroxide only reduced the count of *L. monocytogenes* by log 0.5 cfu/g compared to the untreated coupons. When scrubbing before disinfection with peroxide, a minor increase in log reduction was seen. This could be explained by the fact that some of the organic matter was removed and the scrubbing opened the biofilm, which meant that the peroxide could penetrate the biofilm more easily. The main explanation for peroxides low capability to inactivate *L. monocytogenes* is that peroxide is very sensitive to the presence of organic matter.
In the present study in a strong biofilm the effect of sodium hypochlorite and peroxide is almost the same, and the effect of the QACs is much higher than that of sodium hypochlorite and peroxide. The result for peroxide differed from the results of Fatemi et al. (1992), who reported a better effect of peroxide compared to hypochlorite in TSB biofilm covered with a layer of milk. Kysinski et al. (1999) reported a high effect of peroxide and one neutral QAC and a low effect of hypochlorite on *L. monocytogenes* culture attached to stainless steel in a TSB biofilm. Hypochlorite generally has a low ability to inactivate bacteria in biofilm. The ability of peroxide to penetrate and inactivate bacteria biofilms seems to vary according to the chemical matrix of the biofilm.
The results of these experiments demonstrate how important it is to be aware of the disinfectant's ability to inactivate bacteria adherent to biofilm. The disinfectants used in this study are normally capable of inactivating planktonic cells completely (LeChevallier et al.,1988). The knowledge from this study is useful when high bacterial counts are detected after cleaning and disinfection, since could indicate problems with biofilm in the production site. In such a situation, it is important to choose the right disinfectant to solve the problem.

Conclusions

From the experiments, the following conclusions can be made:
QACs can completely inactivate L. monocytogenes adherent to a strong meat biofilm with approximately log 7 cfu/g.
Sodium hypochlorite and peroxide have a low ability to inactivate L. monocytogenes in a strong meat biofilm.

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Figure 1. Log *Listeria monocytogenes*/g after cleaning with sanitizer followed by disinfection with 3 different QACs, sodium hypochlorite and peroxide (n=4).

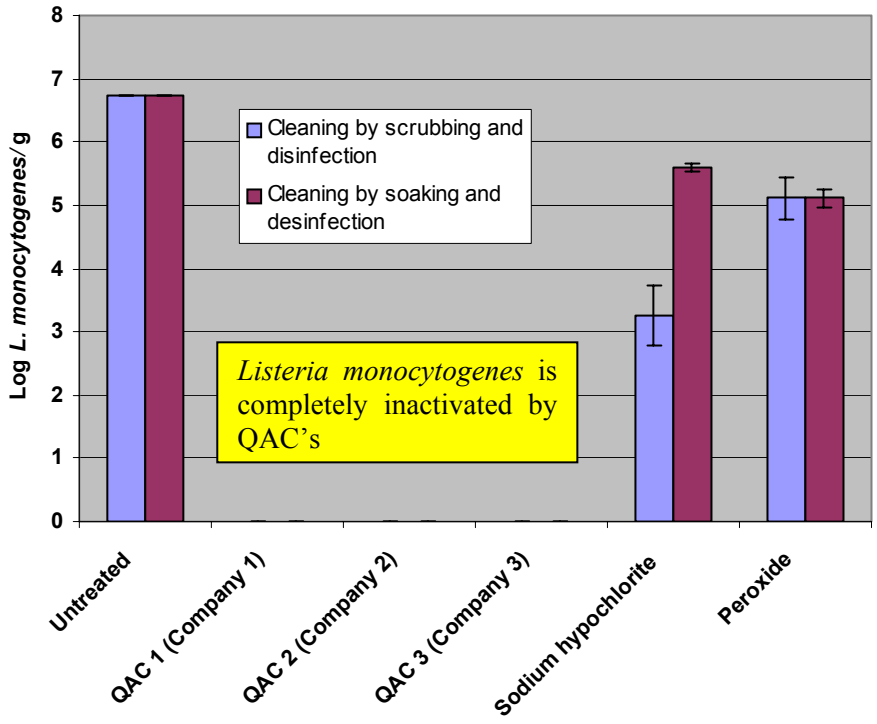
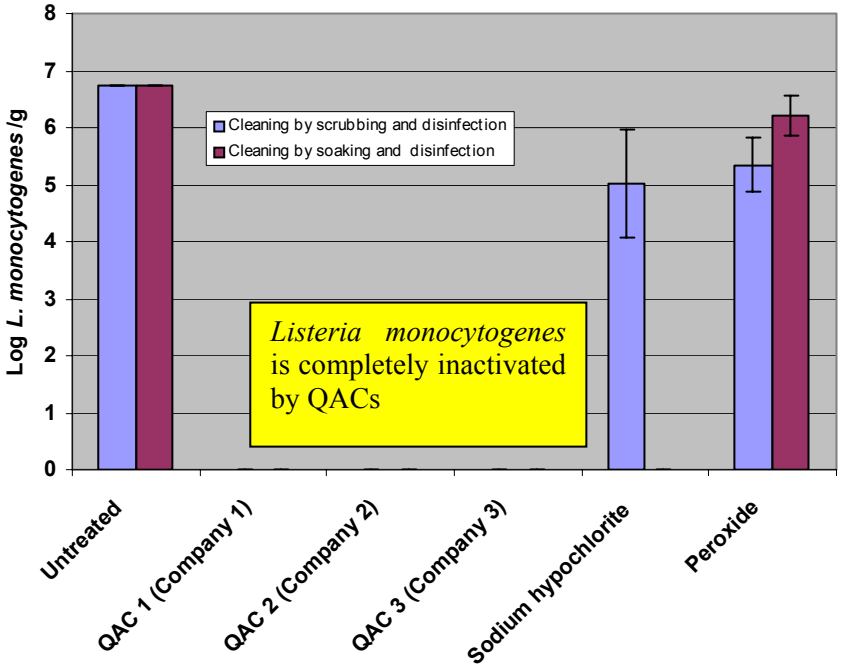


Figure 2: Log *L. monocytogenes/g* after cleaning with the alkaline detergent followed by disinfection with 3 different QACs, sodium hypochlorite and peroxide (n=4).



MOLECULAR EPIDEMIOLOGY AND DISINFECTANT SUSCEPTIBILITY OF *LISTERIA MONOCYTOGENES* FROM MEAT PROCESSING PLANTS AND HUMAN INFECTIONS

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Background

Previous studies have shown that many *Listeria monocytogenes* isolates from heat-treated meat products seem to have other origins than fresh meat (Boerlin and Piffaretti., 1991; Nesbakken et al., 1996).

Objectives

The purpose of this investigation was to obtain knowledge of sources, routes of contamination and genetic types of *L. monocytogenes* present along the production line in the meat processing industry, and to compare meat industry isolates and human isolates.

Materials and methods

We have investigated the molecular epidemiology of *L. monocytogenes* from the meat processing industry producing cold cuts and from cases of human listeriosis by discriminative pulsed-field gel electrophoresis (PFGE). A subset of the isolates was also investigated for susceptibility to a disinfectant based on quaternary ammonium compounds (QAC) frequently used in the meat processing industry. Of the 218 isolates from four meat-processing plants, 197 were from two plants responsible for nearly 50% of the production of cold cuts in the Norwegian market. The strain collection included historical routinely sampled isolates (1989-2002) and isolates systematically sampled through a one year period (November 2001-November 2002) from fresh meat and production environments in three plants in Norway. Human strains included all available reported isolates from Norwegian patients in selected time periods.

Results and discussion

No isolates were obtained in samples from employees (throat (n=70), faeces (n=45)). The *L. monocytogenes* PFGE data showed a large genetic heterogeneity, with isolates separated into two genetic lineages and further subdivided into 56 different PFGE profiles. Certain profiles were observed on both sides of production (before and after heat treatment) indicating contamination of end products by fresh meat or fresh meat environments. While fresh meat isolates almost exclusively grouped within lineage I, isolates from end products showed a more balanced distribution between lineage I and II. Ten profiles were common among isolates from human and meat industry. Typing of human isolates identified a previously unrecognised outbreak. Generally, a higher QAC resistance incidence was observed among isolates from the meat processing industry than among human isolates although large plant to plant differences were indicated. No correlation between resistance and PFGE profile or resistance and persistence was observed.

Conclusions

Additional factors than fresh meat seem to be responsible for contamination at the end product side. No positive samples detected from throat and faeces of employees indicated employee carriage as a minor factor in colonisation and spread of *L. monocytogenes* in the plants. The results indicate an overall higher prevalence of resistant clones in the food industry compared to isolates from human cases.

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HYGIENIC CONDITIONS OF A MEXICAN LOCAL SLAUGHTERHOUSE

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Background

Healthy cattle are a reservoir for the major foodborne pathogens like *Escherichia coli* O157, *Salmonella spp*., *Listeria spp*. and *Campylobacter spp*. and these microorganisms may be transferred onto the meat during slaughter and dressing of the carcasses (Vanderlinde and Shay, 1998). Apart from pathogenic bacteria, isolated spoilage microorganisms include lactic acid bacteria, *Pseudomonas spp.*, *Acinetobacter spp.* and *Moraxella spp.* (Kraft, 1992). Microorganisms are in the intestinal tract of the animals, on its hide, hair and hooves (Gill and Newton, 1978). Generally, the internal surfaces of the carcasses are sterile, but during the slaughtering process, defects on dressing and skinning led to the contact of carcasses with dust, dirt and feces, and facilitate the contamination of the meat with the microorganisms deposited on the surface of the animals.

Equipment and utensils used in the slaughtering plants play an important role in the cross-contamination of carcasses. For example, in the case of swine cattle although bacteria on the skin are largely destroyed by scalding, the skin is recontaminated with spoilage and pathogenic bacteria during passage of the carcass through the dehairing equipment (Gill and Bryant, 1993). Workers could be also another microbial contamination source.

In this way, safety programs have been adopted in the slaughtering plants in order to improve meat safety. Good manufacturing practices (GMP) emphasize sanitary effectiveness and hygienic practices during the processing of foods. Hazard analysis critical control point (HACCP) is mainly directed to identify and control foodborne pathogens. In USA every slaughter plant that operates under federal inspections is committed to establish and carry out a HACCP program as well as to apply sanitation standard operating procedures (SSOP) (USDA, 1996). In the European Union the UE Commission Decision (2001/471/EC) requires validated HACCP systems in the slaughter plants and conduct regular checks on general hygiene. However none of these safety programs are compulsory in slaughterhouses in Mexico, especially in non TIF (Federal Inspection Type) slaughterhouses. Therefore, microbial counts in these establishments are expected to be quite high but no studies are related to the subject.

Objectives

The aim of this work was to conduct a microbiological evaluation of the slaughtering process at a small slaughterhouse located in Hidalgo State, Mexico.

Materials and methods

The slaughter house involved in the study is located in Pachuca, Hidalgo State, Mexico, and it has one line for pigs and one line for cattle. Over a nine-month period the local slaughtering establishment was sampled eight times, four times in each line. At each sampling time, nine carcasses were randomly selected immediately after slaughter and dressing and sampled. Four zones of 100 cm^2 were strongly swabbed with cheese cloth previously moistened with buffered peptone and placed in a same sterile Stomacher bag constituting a single composite sample. In case of swine, zones swabbed were ham, back, belly and jowl and in beef carcasses the zones swabbed were leg, belly, breast and jowl. Also knives used for the bleeding, scrapping, skinning and evisceration as well as saws used to split the sternum and carcasses of beef and hands of different personnel working were sampled by swabbing technique. The water from the scalding process and used to wash the carcasses was also analysed.

The swab samples were cultured and enumerated for the presence of total viable count (TVC), coliforms, *E. coli*, *Salmonella* and *Staphylococcus aureus*. Plate count agar (PCA) was used to enumerate total viable count, Petrifilm Coliforms/*E. coli* (3M) was used to enumerate coliforms and *E. coli* and Baird Parker agar to enumerate *Staphylococcus aureus.*

For detection of *Salmonella spp.*, 100 ml of buffered peptone water was used for preenrichment at 37ºC for 18 h. Selective enrichment was done in Rappaport-Vassiliadis (RV) broth and tetrathionate broth. Isolation and identification were performed by plating on xylose lysine desoxycholate (XLD) agar and modified brilliant green agar (BGAM). One suspect colony on each plate was inoculated on triple sugar iron agar (TSI) and lysine iron agar (LIA) for identification. Also multivalent serum agglutination was performed to confirm the presence of *Salmonella*.

Results and discussion

A total of 158 samples were analysed (74 samples in pork line and 84 samples in beef line). Results of TVC (total viable count), coliforms and *E. coli* are shown in tables 1 and 2. Total viable counts were recovered from all samples and coliforms and *E. coli* from most samples. Generally speaking, carcasses, utensils and workers were more contaminated in pig line than in beef line. However, counts in carcasses were higher than those found by Gill et al. (2000) in beef and pig carcasses. Anyway TVC values found in pig carcasses were all higher than 4 Log cfu/cm^2 which for example according to Commission Decision 2001/471/EC would be considered unacceptable. In beef carcasses TVC counts ranged from 3.26 to 7.00 Log cfu/cm^2 and but coliforms were lower than 1.5 Log cfu/cm^2 or were not present except in five samples and *E. coli* counts were not detected in half samples. High counts in utensils and worker hands indicated absence of good manufacturing practices which facilitate the contamination of carcasses. Respecting the water used to clean the carcasses the presence of coliforms and *E. coli* could be explained due to the fact that the water prior to be used is placed in an open container and could be contaminated during the slaughtering process.

Respecting *Salmonella*, 31% of the samples were positive for *Salmonella spp* in the porcine line and 11% were positive in the bovine line. It is well documented that *Salmonella* enters into the slaughterhouse through the animals, especially pigs and although scalding step could reduce the presence of this pathogen, subsequent operations as dehairing, manual scraping and polishing and evisceration contribute significantly to cross-contamination and could explain the prevalence of *Salmonella* in the carcasses, knives and hands of the workers. The percentage value found in pork line is slightly higher than the value found by Korsak et al. (1998) in four pork slaughterhouses; however, these authors did not found *Salmonella spp* in the beef slaughterhouses sampled. *Staphylococcus aureus* was not detected in any sample. This microorganism in foods usually indicates contamination from skin, mouth or nose of food handlers.

Conclusions

High microbial counts present in carcasses, utensils and personnel working indicated poor hygienic conditions in the slaughtering establishment and implementation and maintenance of Good Manufacturing Practices (GMP) and Hazard Analysis Critical Control Points (HACCP) should be the strategy in order to assure the microbial safety of meat.

Acknowledgments

This work has been supported by a project from PROMEP (Programa para el mejoramiento del perfil del profesorado) of the Government of Mexico.

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Table 1. Microbial results of the samples obtained from carcasses, utensils, workers and water at pork slaughtering process.

Microbial count	Sampling site	Number of samples	Mean	Standard deviation	No
Total viable count	Carcasses (Log cfu/cm$^{2)}$	32	5.01	0.84	-
	Utensils (Log cfu/utensil)	15	6.77	0.84	-
	Workers (Log cfu/hand)	19	7.13	0.98	-
	Scalding water (Log cfu/ml)	4	3.70	1.01	-
	Cleaning water (Log cfu/ml)	4	2.89	1.18	-
Coliforms	Carcasses (Log cfu/cm$^{2)}$	32	2.54	1.47	-
	Utensils (Log cfu/utensil)	15	3.72	2.42	2
	Workers (Log cfu/hand)	19	4.88	1.53	-
	Scalding water (Log cfu/ml)	4	0.70	0.58	3
	Cleaning water (Log cfu/ml)	4	0.38	1.29	2
E. coli	Carcasses (Log cfu/cm$^{2)}$	32	1.26	0.74	-
	Utensils (Log cfu/utensil)	15	2.19	1.52	3
	Workers (Log cfu/hand)	19	3.54	1.09	-
	Scalding water (Log cfu/ml)	4	0.24	0.46	3
	Cleaning water (Log cfu/ml)	4	0.15	0.52	3

No: number of samples from which bacteria were not recovered

Table 2. Microbial results of the samples obtained from carcasses, utensils, saws, workers and water at beef slaughtering process.

Microbial count	Sampling site	Number of samples	Mean	Standard deviation	No
Total viable count	Carcasses (Log cfu/cm$^{2)}$	36	4.39	1.04	-
	Utensils (Log cfu/utensil)	16	6.09	1.38	-
	Saws (Log cfu/saw)	8	6.67	1.55	-
	Workers (Log cfu/hand)	20	6.74	1.05	-
	Cleaning water (Log cfu/ml)	4	4.61	0.32	-
Coliforms	Carcasses (Log cfu/cm$^{2)}$	36	1.11	1.81	7
	Utensils (Log cfu/utensil)	16	2.15	2.94	7
	Saws (Log cfu/saw)	8	4.38	2.64	-
	Workers (Log cfu/hand)	20	3.95	2.22	2
	Cleaning water (Log cfu/ml)	4	3.23	1.28	-
E. coli	Carcasses (Log cfu/cm$^{2)}$	36	0.44	0.95	18
	Utensils (Log cfu/utensil)	16	0.67	1.85	11
	Saws (Log cfu/saw)	8	0.58	1.53	5
	Workers (Log cfu/hand)	20	2.47	1.93	5
	Cleaning water (Log cfu/ml)	4	2.84	1.25	-

No: number of samples from which bacteria were not recovered

STUDY OF CONTAMINATION VARIATION LEVEL OF STAPHYLOCOCCUS AUREUS IN POULTRY SLAUGHTER PREMISES WITH HACCP SYSTEM

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Background

Staphylococcus aureus is part of the microflora of chickens, commonly associated with bruised or infected tissue, nasal passages, skin surfaces, and arthritic joints (Mead and Dodd, 1990; NACMCF, 1997). Low levels of *S. aureus* are commonly found on the surface of poultry and throughout poultry processing premises (Thompson *et al*., 1980; Noterman *et al*., 1982; Mead and Dodd, 1990). Typically, these are poultry-associated strains which can be differentiated from human isolates (Gibbs *et al*., 1978a,b). Poultry strains do not seem to be important in the aetiology of poultry product-associated staphylococcal intoxications and may be considered as unimportant in terms of food safety (Isigidi *et al*., 1992). Instead, most staphylococcal outbreaks appear to be related to contamination of cooked products by infected food handlers followed by improper holding temperatures (Bryan, 1980). Prevalence of *S. aureus* in broiler carcasses in the United States was found to be 64% based on a nationwide baseline survey (USDA FSIS, 1996a). The survey did not identify whether the isolates were poultry-associated strains or human strains. A New Zealand survey found *S. aureus* to be present in 3.6% of 48 ready-to-eat poultry product samples tested with 7 samples (0.5%) found to contain excessive numbers (Campbell and Gilbert).

Objectives

The purpose of this study was to detect of prevalence rate, variation of contamination level contamination frequency of *Staphylococcus aureus* and critical points on slaughtering line.

Materials and methods

30 samples is taken in each of slaughtering stages of poultry slaughter house in the city of Tabriz. Samples were :

A. 50ml water of scalder.
B. 25cm^2 skin swab
C. meat sample after defethering
D. meat sample after eviscerating
E. meat sample after cold water washing.
F. water of chiller.
G. meat sample after chilling.
H. meat sample from markets.

For sample preparations and culture of *Staphylococcos aureus* with standard plate count is used standard methods of Institue of standards and Industrial Research of Iran, no: 356,1194. T-test for quantitative data and Mc nemar test for qualitative data are used.

Results and discussion

Staphylococcus aureus count (spc) in each of premises sampling indicated in table 1

Table 1: A) water of scalder, B) skin swab, C) meat sample after defethering , D) meat sample after eviscerating E) meat sample after cold water washing, F) water of chiller, G) meat sample after chilling, H) meat sample from market

Sampling stage	sample	mean	***Std. Deviation***	***Variation***
A	30	10^3×1	10^3× 2.61	10^6×1.6
B	30	10^3× 2.88	10^3×2.6	10^6×6.8
C	30	10^4 × 1.23	10^4×1.5	2.24E+8
D	30	10^4× 3.42	10^4×3.9	1.54E+9
E	30	10^4 × 1.97	10^4×2.19	4.82E+8
F	30	10^3 × 8.5	10^4×2.19	4.82E+8
G	30	10^4 × 1.06	10^4×1.02	1.06E+8
H	30	10^4× 1.55	10^4×1.79	3.21E+8

Increasing the mean of *staphylococcus aureus* contamination level after each of premises such as defethering, eviscerating and immersion chilling is significant ($p<0.05$). But, decrease of staphylococal contamination level in cold water spray washing is not significant ($p>0.05$)(chart1)

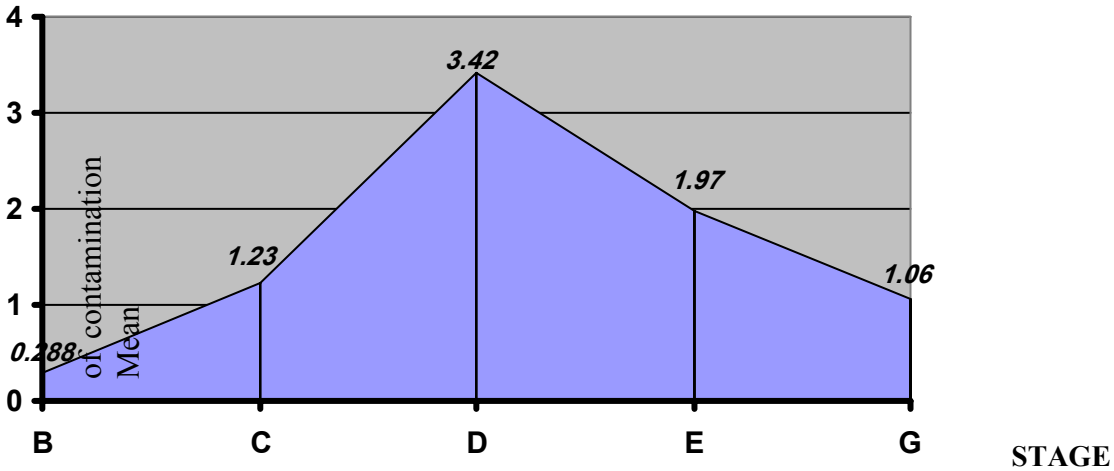

CHART 1: Variation of *staphylococcus aureus* contamination level in B) skin swab, C) meat sample after defethering , D) meat sample after eviscerating E) meat sample after cold water washing, G) meat sample after chilling,

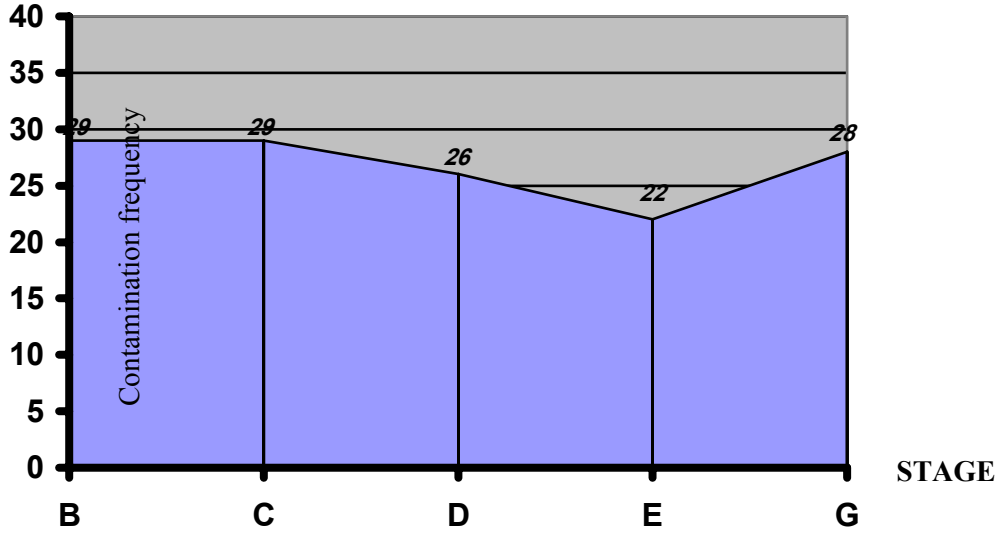

CHART 2: Variation of *staphylococcus aureus* contamination frequency in B) skin swab, C) meat sample after defethering, D) meat sample after eviscerating E) meat sample after cold water washing, G) meat sample after chilling,

Staphylococal contamination frequency after immersion chilling compared with cold water spray washing was showed significant increase ($p<0.05$)(chart 2). Finally *Staphylococcus aureus* contamination prevalence rate in each of poultry slaghter premises is showed in chart 3.

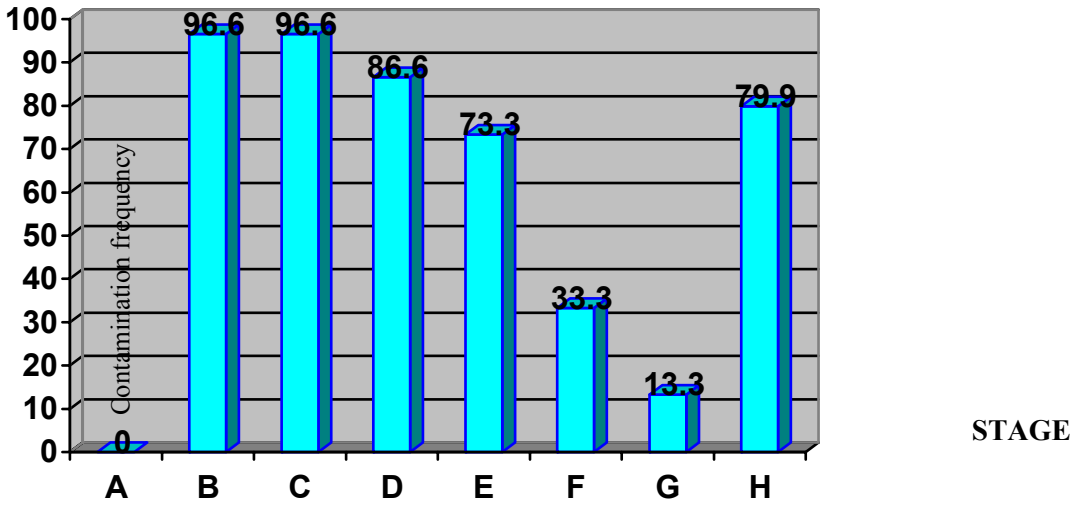

Chart 3: A) water of scalder, B) skin swab, C) meat sample after defethering , D) meat sample after eviscerating E) meat sample after cold water washing, F) water of chiller, G) meat saple after chilling, H) meat sample from market

Conclusions

Finally results show that, stages of defethering, eviscerating cold spray washing and immersion chilling are seriously critical points in slaughter houses. Any control processes were not applied on them. Thus, they result in increasing contamination in poultry meat.

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KINETICS OF COLOUR LOSS FOR SLICES OF FERMENTED SAUSAGE DISPLAYED IN LIGHT OF VARYING INTENSITY

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Background

Surface colour is an important attribute that consumers use to judge acceptability of meat and meat products. For cured meats, the pink, pink-red, to red-maroon colour is viewed as an indicator of product freshness (Issanchou, 1996). Nitrosylhemochrome, the pigment of cured meats, is sensitive to light in the presence of oxygen. Protecting sliced cured meats, such as fermented sausages, from oxygen is normally done by proper packaging. Colour stability of cured meats is retained for 5-8 weeks when low oxygen transmission rate (OTR) films, generally <17 cc/m^2/24 hr, are utilized with vacuum packaging (Lin et al., 1980; Yen *et al*., 1988; Kartika *et al*., 1998). Light-induced fading occurs more rapidly if residual oxygen remains in packaged cured meats (Andersen *et al*., 1988) as can occur with inadequate vacuum-packaging and if the packaging film has too high an oxygen transmission rate (Lin *et al*., 1980, Yen *et al*., 1988, Kartika *et al*., 1998).

Since colour fading of cured meats is basically a chemical reaction in which nitrosylhemochrome is gradually destroyed by oxidation, the rate of colour change due to destruction could be evaluated as a function of storage temperature of the product. The Arrhenius relationship describes rate constant dependency on temperature and is very useful in determining quality losses in foods (Labuza, 1984). Since a time-temperature relationship reflects an energy input, a light intensity-time relationship would also reflect an energy input at the product surface and potentially resemble an Arrhenius type of relationship for reactions associated with colour loss.

Objectives

The objective of this study was to determine if an Arrhenius-type relationship exists for surface colour fading or colour loss for packaged slices of product placed in light display with lighting of different intensities. Rate constants for CIE L*a*b* as well as CIE C*and h* were obtained for product packaged in a non-oxygen barrier film so that O_2 was not restricted as a reactant in degradation of nitrosylhemochrome.

Materials and methods

Fresh commercial fermented sausage was obtained that analyzed 47.7% moisture, 17.1% protein, 29.7% fat, 3.36% ash, 4.06% NaCl (AOAC, 1995) and had a pH of 4.6. The sausage was classed as “summer sausage” and had been stuffed in chub form weighing approximately 1.36 kg each. The sausage was approximately 7 cm in diameter and was sliced to provide samples 3 mm in thickness. Two slices, each an experimental sample, were vacuum-packaged (72.4 cm Hg) side-by-side in a flexible film pouch using a Turbovac packaging machine (Turbovac B.V., Netherlands). The packaging film had an oxygen transmission rate (OTR) of 12,000 cc/m^2/24hr at 23^{o}C, 0%RH and 1 atm (Cryovac Division of Sealed Air Corporation, Duncan, SC). A high OTR film was used so that oxygen was not a limiting factor for colour fading. This made it possible to examine colour fading or loss of colour stability due to light intensity effects.

The packaged samples were immediately placed in the dark at 2±1^{o}C for 25 min and then initial colour values (0 hr) were measured. The packages were then placed on display under four light intensities: 660, 927, 1822 and 3172 lux at 2±1^{o}C. Continuous lighting was provided by Cool White fluorescent lights. Two packages (4 slices) were placed under each light intensity. Within each of three study replications, a new set of four slices in two packages as described above were prepared and used for colour evaluation.

CIE L* (lightness), +a* (redness), and +b* (yellowness) were measured using a Minolta Chroma Meter CR-300 (Minolta Corporation, Ramsey, New Jersey) at 0.5 hr intervals during display for 2.5 hr using 5 randomly chosen surface points on each slice. CIE C* (chroma), related to colour intensity, and CIE h* (hue

angle), the measure of colour in the CIE L*C*h* space were calculated based on +a* and +b*. The Chroma Meter was calibrated with a white calibration plate and CIE Illuminant C was the illuminant source.

Kinetic analysis was conducted using the general linear model regression analysis procedure of SAS (1996). First order reaction kinetics were applied and a linear fit of colour characteristic data at each light intensity was tested using the following first order relationship: $\log y = b_o + b_1 x$, where y is the specific colour coordinate value, x is the time of light display (hr), b_1 is the slope and b_o is the intercept. First order rate constants were calculated from the slopes and then used in a second regression analysis to test for an Arrhenius-type relationship due to light intensity given as: $\log k = b_o + b_1(1/\text{lux})$, where k is the rate constant (hr^{-1}), b_1 is the slope, and b_o is the intercept. The coefficient of regression (R^2) and significance of b_1 in the Arrhenius type relationship were used in determining acceptance or rejection of the fit of the data to first order kinetics. In addition to regression analysis, Arrhenius-type plots were prepared from the data.

Results and discussion

The main effects of light intensity, time of display, and the light intensity*time of display interaction were significant factors ($P<0.001$) for decreases in summer sausage surface lightness (CIE L*), redness (CIE +a*) and chroma (CIE C*) and an increase in hue (CIE h*) as determined by general linear models analysis of variance (SAS, 1996). For surface yellowness (CIE +b*), only the interaction was significant. Northcutt et al. (1990) reported that time of light exposure was significant for L*, a* and b* when vacuum-packaged turkey bologna was similarly displayed in a high OTR film. Generally, as light intensity for display increased in the present study, there was a decrease in colour intensity (CIE C*) as the visual colour (CIE h*) shifted from its initial "redness" toward "brown" discoloured characteristics. The major visual hue change shown to occur was associated principally with the redness (CIE +a*) component, typical for highly pigmented cured products, and not the yellowness (CIE +b*) aspect of the hue.

The regressions for rate of change in surface redness, chroma, and hue (TABLE 1), fit a first order reaction mechanism rather than zero or other order and this is in agreement with previous studies of Northcutt et al. (1990) and Mattos et al. (2003). Summer sausage surface lightness (CIE L*) had non-significant ($P>0.05$) slope changes and there was no overall consistent slope pattern for yellowness (CIE +b*) as light intensity increased. All slopes for the response variable redness (CIE +a*), hue (CIE h*), and chroma (CIE C*) were significant ($P<0.0001$). The major observation of the first order rate constants for the significant colour components is that ***the rate constants increase as light intensity increases***. Therefore, light intensity is similar to the temperature effect usually studied for effects on traditional chemical reactions.

In the Arrhenius relationship, a linear relationship exists between the log of the rate constants and the reciprocal of the absolute temperature. When the rate constants of each colour characteristic were used in linear regression analysis for relationship to the reciprocal of light intensity, the coefficients of determination (R^2) showed an acceptable relationship for CIE +a*, h* and C* but not for CIE L* and b* (TABLE 2). Data from TABLE 1 were used to generate Arrhenius-type plots for redness (CIE a*) and hue (CIE h*) (FIGURE 1) to demonstrate that the Arrhenius concept for reaction dependency on temperature is also valid for dependency on light intensity, another form of energy affecting surface colour stability of summer sausage.

Conclusions

Fermented summer sausage slices vacuum-packaged in a high oxygen transmission film and placed in display at 2^oC under fluorescent light intensities in the range of 660 to 3173 lux produced decreases in product surface redness and chroma and increases in hue. Plotting first order rate constants of the colour attributes against the reciprocal of light intensity indicated an Arrhenius-type reaction rate fit. This finding confirmed that the Arrhenius concept for reaction dependency on temperature is also valid for dependency on light intensity, another form of energy that can negatively affect the colour stability of cured meats.

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TABLE 1. Linear regression and correlation coefficients for CIE colour characteristics of vacuum-packaged summer sausage displayed for up to 2.5 hr under different light intensities.

Colour Coordinate	Light Intensity (lux)	Coefficient			Standard Error	Rate Constant
		b_o	b_1*10^2	r	b_1*10^2	$k*10^2$ hr^{-1}
Lightness (CIE L*)	660	1.72	0.0091ns	0.07	0.067	0.2096
	927	1.72	0.0072ns	0.06	0.063	0.1258
	1822	1.73	-0.0002ns	0.00	0.065	0.0046
	3173	1.72	0.0130ns	0.10	0.068	0.2994
Redness (CIE a*)	660	1.23	-2.348***	0.68	0.133	5.408
	927	1.26	-3.021***	0.77	0.131	6.958
	1822	1.25	-4.403***	0.84	0.149	10.141
	3173	1.25	-5.428***	0.85	0.179	12.500
Yellowness (CIE b*)	660	1.05	-0.299*	0.14	0.116	0.689
	927	1.04	0.060ns	0.03	0.114	0.138
	1822	1.05	-0.034ns	0.02	0.109	0.079
	3173	1.04	0.248*	0.13	0.098	0.570
Chroma (CIE C*)	660	1.33	-1.751***	0.67	0.103	4.032
	927	1.33	-2.086***	0.72	0.161	4.804
	1822	1.32	-2.968***	0.81	0.113	6.835
	3173	1.32	-3.451***	0.81	0.134	7.971
Hue (CIE h*)	660	1.49	1.632***	0.56	0.132	3.757
	927	1.50	2.428***	0.74	0.116	5.592
	1822	1.50	3.360***	0.81	0.127	7.739
	3173	1.51	4.287***	0.87	0.128	9.874

Linear regression model: $\log y = b_o + b_1 x$ where y = colour attribute, x = display time (0 to 2.5 hr), b_o = intercept, and b_1 = slope. Correlation coefficient (r) of the association between x and y are included. Rate constant $k = | b_1 | * 2.303$. *** $P<0.0001$, * $P<0.05$, ns $P>0.05$.

TABLE 2. Regression coefficients of determination (R2) for colour characteristics of vacuum-packaged summer sausage using the Arrhenius-type relationship of rate constants to light intensity of display.

Colour Attribute	Colour Coordinate	Regression Coefficient		
		b_o	b_1	R^2
Lightness	CIE L*	-3.50	444.5	0.11
Redness	CIE a*	-0.82	-299.4	0.99
Yellowness	CIE b*	-2.78	221.7	0.42
Chroma	CIE C*	-1.03	-249.2	0.99
Hue	CIE h*	-0.91	-333.7	0.99

Linear regression model: $\log y = b_o + b_1 x$ where y = k (first order rate constant), x = 1/light intensity (lux), b_o = intercept and b_1 = slope.

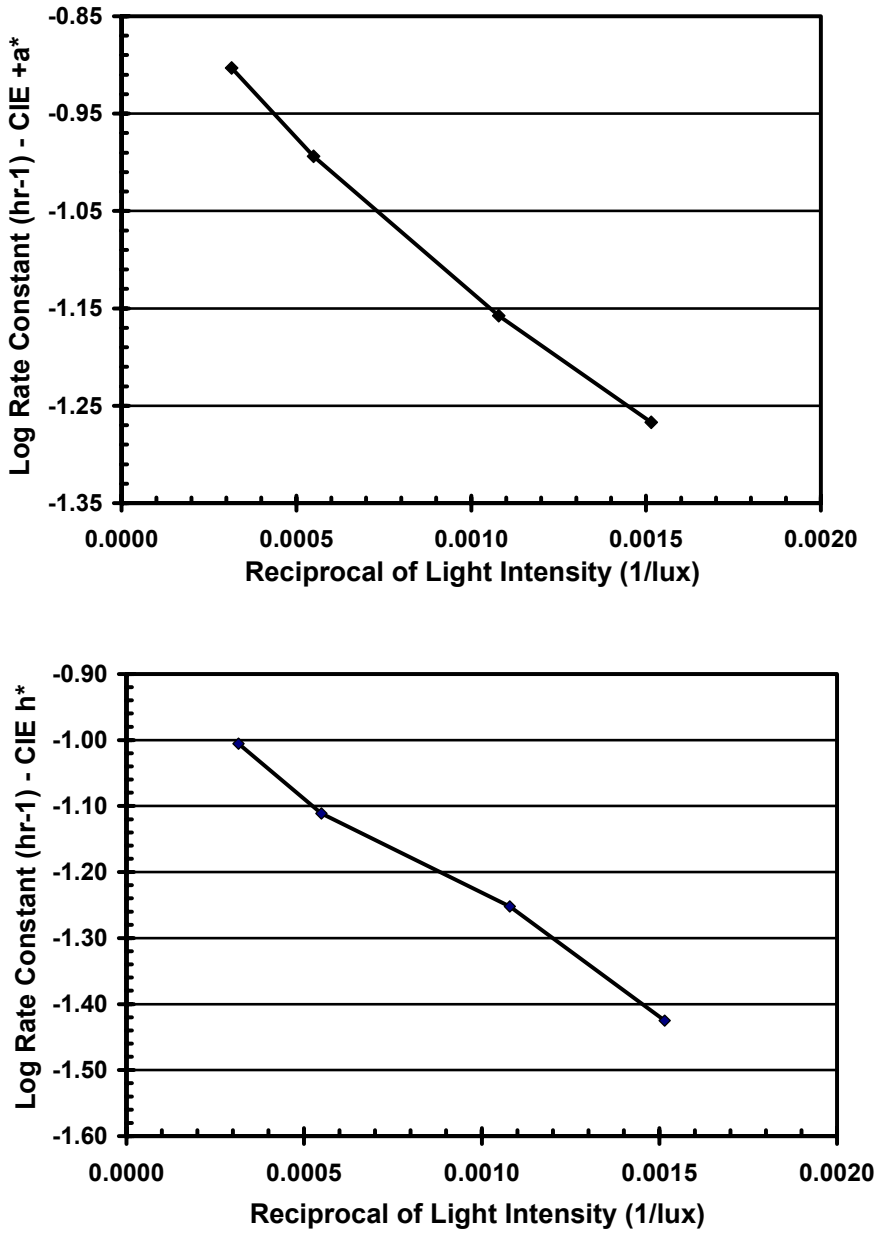

FIGURE 1. Arrhenius-type plots showing the dependency of the first order rate constants (taken from absolute values of slopes) for redness (CIE +a*) decrease and hue (CIE h*) increase as a function of light intensity.

QUANTITATIVE ASSESMENT OF ANTIBIOTIC AND ANABOLIC AGENTS IN BROILER CHICKEN MEAT

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Background

Antibiotics and anabolic agents are widely used for treatment of bacterial diseases or as growth promoters in intensive poultry production. Although most of the countries around the world banned a wide range of antibiotics and growth promoters, many of those are still being indiscriminately used (Weirup, 2001). Administration of antibiotics or anabolic agents to broiler may lead to deposition of residual material in meat and consequently presents a potential human health hazard. Over-use of antibiotics in poultry feeding and /or treatments and its contribution to the generation of antibiotic-resistant strains of bacteria has become a worldwide problem in the treatment of human infectious diseases (Millar et al., 1998; Neely and Holder, 1999).

Objectives

The objectives of this study were to detect and quantify residue levels of some widely used antibiotics (streptomycin and tetracycline) and anabolic agents (oestradiol and testosterone) in broiler chicken meat.

Materials and methods

Thirty-four fresh and frozen broiler chicken carcasses representing 16 local and imported products collected between April and May 2003 were used. Four samples (10 to 20 grams) from the breast muscles including meat and skin were homogenized then stored in sealed plastic bags at -80^0C. The samples were randomly allocated to one of four residual assays; streptomycin, tetracycline, oestradiol and testosterone. The Enzyme Linked Immunosorbent Assay (ELISA) technique was used to detect residues in the samples. For each assay, a specific kit was used to provide quantitative negative or positive detecting results after extracting and preparing samples according to the type of the residue. The sample was mixed thoroughly and then kept in a sealed plastic bag at -80^oC. Frozen samples were left at room temperature (20^oC) for two hours before starting extraction. Five to ten grams from each sample were mixed with 10-25 ml of special buffer (organic solvent), which has been prepared specifically for each analysis, for 30 minutes using a shaker, and then it was homogenized. The homogenized sample was transferred to screw cap glass test tube and centrifuged at 3000 to 4000 rpm for 10 minutes at 15^oC. The supernatant was decanted into another flask, then the buffer was dried using rotary evaporator. The residue was redissolved in the solvent. Finally, 5 ml of the filtrate were purified with RIDA C18 column and stored at -20^oC. The concentration of the hormone or antibiotic in ppb/ppt corresponding to the absorbance of each sample was read from the calibration curve.

Results and discussion

The results of oestradiol-17-ß, testosterone, streptomycin, and tetracycline concentration levels in broiler chicken meat samples are presented in Table 1. Fifteen out of 16 samples (94%) contained oestradiol-17ß but of these only four samples (24%) contained residues that exceeded tolerance levels of 100 nanogram/kg specified by the National Residue Control Plan. Salem, et al. (2003) found that feeding 100 µg/bird/day of ethynyl oestradiol to chickens resulted in the highest residue levels of 81.9 µg/kg in the skin rather than in the muscle (22.9 µg/kg). They concluded that the use of oestradiol resulted in its accumulation in different tissues and organs, even if the treatment was stoped for 4 weeks and even when the chicks meat was boiled for one and a half hours. In the present study, seven samples contained 52.4 micrograms/kg of oestradiol-17-ß, which were above the average level in muscle and skin chicken sample reported by Salem (2003). Although, most of oestrogenic compounds have been now banned in most countries because of their apparent carcinogenic (cancer-causing) activity (McCutcheon, 1989), this study revealed that residues of oestradiol-17-ß were present in broiler meat. The naturally occurring androgen testosterones, and its synthetic analogues such as trenbolon acetates (Finaplix®), are effective growth-promoting agents in

domestic animals. Fifteen (94%) out of 16 samples contained testosterone residues, but all were less than the tolerance level of 100 nanogram/kg. These values were less than the limit specified for meat samples by the German Residue Control Plan (100 nanogram/kg).

All meat samples (100%) tested in the present study showed residues of streptomycin (75-155 ppb), but these were below tolerance levels of 500 microgram/kg as specified by the maximum residue limit-European Union regulation. Tetracyclines are also extensively used throughout the world for treatment of bacterial infections in poultry. They act by modifying metabolism of bacteria in the intestine and consequently suppress sub-clinical disease and improving digestive efficiency. All the samples tested in the present study contained showed various levels of tetracycline concentrations. All samples contained tetracycline that ranged between 46.2- 47.2 micrograms/kg, which was lower than the maximum residue limit specified for meat samples by European union (100 micrograms/kg). This indicates that tetracycline was used at least once during chicken lifetime as treatment against bacterial infections or as growth promoters.

Conclusions

This study indicated that broiler chicken meat products contained various amounts of residues of antibiotic and anabolic agents. Although, levels of some of these did not exceed the maximum residue limit in most cases, they still poise human health hazard. Residues of antibiotics may cause development of resistant pathogenic strains.

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Table 1. Levels of accumulated testosterone (ppt)[1], oestradiol (ppt), streptomycin (ppb)[2] and tetracycline (ppb) residues in broiler chicken meat.

Sample #	Testosterone	Oestradiol-17ß	Streptomycin	Tetracycline
1	29.5	5	125	46.8
2	30	28	135	47.2
3	24	45	135	46.8
4	5	95	115	47.2
5	70	95	105	46.4
6	40	90	105	46.4
7	0	6	125	46.8
8	27	100*	150	47.2
9	5	45	135	46.8
1	25	28	75	47.2
11	30	200*	145	46.4
12	38	44	125	46.4
13	15	100*	105	46.4
14	12	0	85	46.4
15	24	200*	155	47.2
16	40	28	155	47.2

* Above the detection limit
[1] ppt: nonaogram/kg
[2] ppb:microgram/kg.

DNA-FINGERPRINTING OF *CAMPYLOBACTER* SPP. FROM POULTRY AND GENETIC RELATIONSHIP OF SELECTED STRAINS TO HUMAN ISOLATES

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Background

An important source of infection with *Campylobacter* is considered to be raw poultry meat. A lot of broiler flocks are infected with *Campylobacter* spp. (Newell & Wagenaar, 2000), in Germany about 41% (Atanassova & Ring, 1999). Chicken colonized with *Campylobacter* spp. excrete large amounts of bacteria (Stern et al., 1995). After colonized birds have entered the slaughterhouse processing lines *Campylobacter* can be found on the chicken carcasses throughout the slaughtering process and result in contamination of equipment, working surfaces, process water and air (Jacobs-Reitsma, 2000). On the other hand case-control studies have identified the handling and consumption of poultry as a major risk factor for *Campylobacter* infections. To evaluate the spread of *Campylobacter* through the slaughterline and possible genetic relationship between isolates from poultry products and human isolates DNA fingerprint techniques are needed. Macrorestriction analysis by pulsed-field gel electrophoresis (PFGE) has been used successfully for inter- and intraspecies differentiation of *Campylobacter* (Ribot et al., 2001) and PFGE is one of the most discriminatory genotypic typing methods currently available for subtyping *Campylobacter* species.

Objectives

The aim of this study was to differentiate isolates of *Campylobacter* spp. at various stages throughout processing with DNA fingerprinting techniques. Genotyping of the selected strains by PFGE should determine subtypes which were common for carcasses and offal and prove if cross-contamination within and between flocks occured. To assess the impact of poultry products as vectors for sporadic *Campylobacter* infections in Germany, in this study the relatedness of *Campylobacter* spp. strains occurring in a 3-month period within the city of Berlin on retail chicken products and in cases of human campylobacteriosis by pulsed-field gel electrophoresis (PFGE) were analysed.

Materials and methods

Eight poultry flocks from different farms were sampled at different dates: flock „a“ and „b“ in June, flock „c“ and „d“ in July,; flock „e“ in August, flock „f“,„g“ and „h“ in September, and flock „g“ in October. Cloacal swabs were taken at the beginning of the slaughter, and neck skin samples before evisceration were taken from each flock. Crates for living birds were sampled before and after washing, carcasses after scalding and neck skin after air spray chilling in some flocks, additionally.
Detection of thermophilic *Campylobacter* spp. was performed by selective enrichment in Preston-broth (Bolton, 1983) following isolation on Preston-agar (Oxoid, CM 67 plus selective supplements SR 117 + SR 48. Oxoid GmbH, Wesel, Germany). One presumptive *C.* isolate from each selective agar plate was identified to species level on the basis of phase-contrast microscopy (characteristic morphology and motility), Gram stain, catalase and oxidase production, growth at 25°C and 43°C, indoxyl acetate hydrolysis, hippurate hydrolysis, and susceptibility to nalidixic acid and cephalotin. All isolates were stored at -80°C in a freezer using the Microbank™ system (PRO-LAB Diagnostics, Cheshire, UK). *C. jejuni* type-strain ATCC 33560^T and *C coli* type-strain ATCC 33559^T were used as controls.
A subset of 154 *Campylobacter* spp. strains were choosen from each flock at each processing station for ribotyping in a previous study (Vollmer, 1996) and the same strains were collected for subtyping with pulsed-field gel electrophoresis.
For the evaluation of the genetic relationship between poultry product isolates and human isolates *Campylobacter* spp. strains were isolated in Berlin, Germany, from January to March 2002. 31 *C. jejuni* and 6 *C. coli* from chicken product samples and 40 *C. jejuni* and 7 *C. coli* from cases of human campylobacteriosis were subtyped.

Poultry food samples (chicken) were taken at retail level. Besides different meat samples (breasts, drumsticks, and wings), sampling included liver, gizzard and heart, presenting the broad range of chicken products consumed regularly by german consumers. Isolation of *C.* spp. from foods was performed in general accordance with the guideline ISO 10272 (International Organization for Standardisation, 1995). Human isolates originated from ambulant cases of diarrhea (stool samples) occurring in Berlin and have been isolated following standard laboratory methods.
Isolates were subtyped by PFGE using the restriction enzyme *Sma*I and *Kpn*I. Strains from poultry products and human isolates were only additionally analysed with with *Kpn*I when identical *Sma*I profiles were found. Preparation of DNA-containing agarose blocks for PFGE was adapted to a "CAMPYNET" prototype standard protocol (http.//campynet.vetinst.dk/PFGE.html) with following exceptions: 500mµl of cell suspension were mixed with 500µl warm 2% inCert-agarose (FMC BioProducts). DNA was cut for 18h with *Sma*I and *Kpn*I at appropriate temperature according to manufacturers instructions (New England Biolabs). Digested DNA plugs were loaded on a 1% SeaKem GTG agarose gel (FMC BioProducts). DNA fragments were separated on a CHEF DR-III apparatus (BioRad) in 0.5xTBE-buffer at 10°C for 22.5h (*Sma*I) or 23h (*Kpn*I). Ribotyping was performed and described in a previous study (Vollmer, 1996).
Computer analysis was carried out with GelCompar 4.1 (Applied Maths). Macrorestriction profiles were evaluated and assigned to arbitrarily-defined "profile groups", using a cut off of 90%.

Results and discussion

A number of genetically distinct *Campylobacter* strains were isolated at different sampling stations per flock. Twenty-one different ribotypes (RT), 24 *Sma*I macrorestriction profiles (MRP)-types and 25 different *Kpn*I MRP-types or profile groups (PGs) were identified. Regarding the distribution of types within the two *Campylobacter* spp. the 13 *C. coli* isolates yielded two different PFGE patterns (PFGE (*Sma*I / *Kpn*I), types X / X and Y / Y), and two different ribotypes (RT 14 and RT 16, respectively). 141 *C jejuni* isolates were divided into 22 distinct PFGE *Sma*I, 23 different PFGE *Kpn*I, and 19 distinct RT profiles, respectively. During the processing line at least one *Campylobacter* subtype appeared only in one flock, and was mainly isolated from cloacal swabs, e.g. PG P/P from flock „f", (Tab. 1). In nearly all flocks sampled, at least one additional *Campylobacter* subtype was recovered from the carcasses or offal at different processing steps compared to the subtypes isolated from cloacal swabs (Tab. 1). Some *Campylobacter* strains with identical profile groups were isolated from different flocks at different times. Strains with PG U / U (*Sma*I / *Kpn*I) were recovered in flock „b" and „h", and strains with PG C / C in flock „b" and „e"in June and September.
It is assumed that *Campylobacter* isolates on carcasses and offal with identical macrorestriction profiles (MRPs) like those detected in cloacal swabs were flock-specific and derived from cross-contamination within flocks during processing, e.g. defeathering or evisceration. The detection of farm-specific *Campylobacter* clonal isolates that occurred in successive rotations strongly indicates that those clones persist on the individual farms, either inside the broiler house or in the near environment (Petersen & Wedderkopp, 2001). Analysis of *Campylobacter* colonization of one flock during the rearing period and contamination of the carcasses at the slaughterhouse by *flaA* typing showed that for example in studies of Rivoal et al. (1999) seven of eight strains isolated from the chicken feces at the farm were also present at the slaughterhouse at neck skins taken directly after evisceration at the processing plant. In addition four other *fla*A-types were collected from these batch only at the slaughterhouse. These results confirm that contamination of broilers during processing was mainly due to the rupture of the gastrointestinal tract during evisceration (Rivoal et al., 1999) and to a lesser extent from other sources. In our study for example, in flock "d" we found only the MRP F and the RT 07 from cloacal swabs on carcasses and offal.
Genotyping by PFGE of poultry product isolates and human isolates identified 15 *Sma*I patterns that occurred in more than one isolate (n=50). As shown in table 2, the same *Sma*I pattern was found in up to 7 isolates. After the second analysis using the restriction enzyme *Kpn*I, 56 of the 84 isolates in total had a unique *Sma*I/*Kpn*I PFGE-profile, whilst 28 had a profile they shared with 1 to 5 other strains. The *Sma*I/ *Kpn*I -profiles III-6, V-12, and XI-24 encompassed human and poultry isolates. Two subtype groups with identical profiles were formed by strains of human origin only (VIII-19 and XIV-31) and 4 groups encompassed only strains of poultry origin (I-1, II-4, VI-15, XII-25). Genotyping by PFGE revealed a high degree of diversity, which is a well-known phenomenon for bacteria of the genus *Campylobacter*. But despite the weak clonality of *C.* spp., 10.6% of human isolates were genetically identical with isolates found in the same geographical region and time frame on retail chicken products.

Conclusions

The populations of *C. jejuni* and *C. coli* are extremely diverse and this diversity is refelcted in a broad spectrum of subtypes. Distinct flocks could be colonized with different types of *Campylobacter*. One flock could be colonized by multiple genotypes whereas strains within individual flocks coexisted rather than excluding each other. In addition more than one strain in each flock may have potential epidemiological importance. Concerning the possible genetic relationship of poultry product isolates and human isolates it can be concluded that retail chicken products are an important source for sporadic human infections with *C.* spp. in Germany.

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Tab. 1: PFGE profile groups (PG) and ribotypes (Vollmer, 1996) of isolates from selected flocks of poultry processing

	Flocks					
	F			G		
Point of sampling	Isolates (n)	PFGE-Grp. *Sma*I/*Kpn*I	Ribotype	Isolates (n)	PFGE-Grp. *Sma*I/*Kpn*I	Ribotype
transport crate / flock	2	I/I	6	nd		
transport crate before washing	not done (nd)			3	V/V	11
				1	G/G	12
transport crate after wash step	nd			5	V/V	11
				4	G/V	12
				1	S/S	13
cloacal swabs	1	P/P	19	1	V/V	11
carcass after scalding	nd			2	L/L	22
neck skin before evisceration	3	P/P	19	nd		
neck skin after chilling	5	P/P	19	2	V/V	11
	1	**Q/Q**	**19**			
offal swab	3	P/P	19	4	V/V	11
				1	G/G	12

Table 2: *Kpn*I-subtypes of 43 *C. jejuni* and 7 *C. coli* strains from poultry products and human origin which clustered in *Sma*I-profiles present in more than one isolate

Species	***Sma*I profile**	***Kpn*I profile**	**Origin of isolates**	**N isolates**
C. jejuni	I	1	chicken	2
C. jejuni	II	2	human	1
C. jejuni	II	3	chicken	1
C. jejuni	II	4	chicken	3
C. jejuni	III	5	human	1
C. jejuni	III	6	1 human 1 chicken	2
C. jejuni	III	7	human	1
C. jejuni	III	8	chicken	1
C. jejuni	IV	9	human	1
C. jejuni	IV	10	human	1
C. jejuni	IV	11	human	1
C. jejuni	V	12	2 human, 4 chicken	6
C. jejuni	V	13	human	1
C. jejuni	VI	14	human	1
C. jejuni	VI	15	chicken	2
C. jejuni	VII	16	human	1
C. jejuni	VII	17	chicken	1
C. jejuni	VIII	18	human	1
C. jejuni	VIII	19	human	2
C. jejuni	IX	20	human	1
C. jejuni	IX	21	human	1
C. jejuni	X	22	human	1
C. jejuni	X	23	human	1
C. jejuni	XI	24	2 human, 1 chicken	3
C. jejuni	XII	25	chicken	6
C. coli	XIII	26	chicken	1
C. coli	XIII	27	human	1
C. coli	XIV	28	chicken	1
C. coli	XIV	29	chicken	1
C. coli	XV	30	human	1
C. coli	XIV	31	human	2

INFLUENCE OF FERMENTATION ON CREATINE AND LACTATE CONCENTRATIONS IN WHITE EFFLORESCENCES ON DRY FERMENTED SAUSAGE

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Background

Undesired white efflorescences or surface blooms on packaged dry fermented sausages have been repeatedly observed in recent years. Since around the mid-1980ies this phenomenon was noticed with products packed in polyethylene (PE) bags under protective atmospheres such as nitrogen. The problem is observed worldwide wherever dry fermented sausages are produced (Kroeckel, unpublished). The efflorescences are known to be due to racemic magnesium-di-lactate derived from microbially produced D,L-lactate and meat-borne L-lactate (Kuehne *et al.*, 1986) and/or meat-borne creatine (Kroeckel *et al.*, 2003). Dew point changes during storage of the packaged sausages apparently favour the formation of these blooms.

Objectives

There is no published work so far on the nature of efflorescences on different types of dry fermented sausages. Also, it is not clear how the formation of the coatings can be prevented. To get more insight into the conditions necessary for the generation of the efflorescences their appearance and composition have to be related to sausage parameters such as type of sausage, physical characteristics as well as chemical and microbiological composition. The present study aimed at collecting this kind of informations and detecting links between these parameters and bloom formation.

Materials and methods

Different types of sausages with characteristic efflorescences were obtained from various national and international manufacturers. From all sausages the composition of the blooms were analysed. On a limited number of samples further analyses were performed to varying extent. Creatine and lactate were determined as described earlier (Kroeckel *et al.*, 2003). Physical (pH, a_w, water content) and microbiological parameters were determined using standard methods. Mg^{++} in the efflorescences was determined complexometrically.

Results and discussion

Rapidly fermented smoked salamis produced in Germany

Paprika salamis of manufacturer H. All salamis (samples no. 1 – 6 in Tab. 1) were vacuum packed upon arrival. The white efflorescences became visible within minutes after removal of the packaging foil and reached maximum intensity within 3 – 4 hours. According to the manufacturer, the sausage recipe contained pork, nitrite curing salt, spices (including paprika extract), sugars (lactose, maltodextrine, dextrose). The white blooms were found to contain 60 – 64 % of D,L-lactate and only 0.3 % of creatine (Tab. 1). The magnesium content in the blooms of sausage no. 2 was 8.7 %. The sausage with the strongest efflorescence (80-90 % of the casing surface affected) had the lowest water activity (sample no. 2 in Tab. 1). The sausage with the highest water activity and the highest water content (sample no. 1 in Tab. 1) showed almost no efflorescence, although the lactate content inside the sausage was even higher than in sample no. 2. Bloom formation was stronger in older sausages, i.e. those with lower residual shelf life (RSL). Therefore, bloom formation does not only depend on water activity but also on storage time after packaging. The extent of blooming was higher in sausages without dry fringe which indicates that the formation of a dry fringe possibly reduces blooming. Microbiological analysis showed remainder of starter culture lactic acid bacteria (*Pediococcus pentosaceus* and *Lactobacillus plantarum*) and other LAB. D(-) and L(+) lactate were found in similar ratios in the blooms and inside the sausages. However, the concentrations outside were 13 – 20 times higher than inside. D(-) lactate is derived exclusively from microbial activities, in contrast to L(+) lactate which is present already to some extend in *post rigor* muscles, usually at concentrations below 1%. Taking into account the participation of magnesium and considering the hydrated form of Mg-D,L-lactate, the tri-

hydrate, the value of 63.9 % of lactic acid for sample no. 6 would correspond to about 91 % of [Mg-D,L-lactate] x 3 H_2O in the white blooms.

Sample no.	1	2	3	4	5	6
Type	spicy	spicy	spicy	sweet	sweet	sweet
Lot no.	62	54	54	72	72	72
RSL[a] , days	50[b]	41	41	62	62	62
Dry fringe [c]	+	-	-	++	+	+
Surface blooms % [c]	5 - 10	80 - 90	60 - 70	40 - 50	40 - 50	40 - 50
Water content %	36.9	28.7	nd	nd	nd	35.1
Water activity	0.8824	0.8572	0.8634	0.8730	0.8662	0.8636
pH	4.32	4.52	4.62	4.47	4.55	4.55
Creatine %						
in sausage	0.64	0.32	nd	nd	nd	0.45
in surface bloom	nd	0.30	nd	nd	nd	0.30
Lactic acid %						
in sausage						
L(+)	2.27	1.48	nd	nd	nd	2.71
D(-)	2.45	1.49	nd	nd	nd	2.36
L(+) + D(-)	4.72	2.97	nd	nd	nd	5.07
in surface bloom						
L(+)	nd	30.1	nd	nd	nd	31.8
D(-)	nd	29.4	nd	nd	nd	32.1
L(+) + D(-)	nd	59.5	nd	nd	nd	63.9
Bacterial counts [d]						
Pc. pentosaceus	5.00	7.54	7.67	7.7	7.60	8.00
Lb. plantarum	6.53	6.00	6.48	6.70	5.70	6.95
other LAB	5.70	7.65	7.68	7.90	8.04	7.85

[a] residual shelf life; [b] estimatet from Lot no.; [c] estimatet by eye; [d] $\log_{10}$ CFU/g sausage;

Tab. 1: Chemical and microbiological characteristics of paprika salamis from manufacturer H showing white surface blooms after removal from the vacuum packs.

Paprika salami of manufacturer R. One sample (calibre: 45 mm, pH 5.0) was obtained in modified atmosphere package. In the bloom a lactic acid concentration of 57.9 % (D : L = 1.0) was found. Magnesium amounted to 7.0 % and creatine was below the detection limit (< 30 µg/g). Inside the sausage D,L-lactic acid amounted to 2.0 % (D : L = 0.95). Thus, the scraped off crystalls corresponded to 58 - 69 % Mg-D,L-lactate or 74 - 87 % of its tri-hydrate. Microbial analysis revealed 2 x 10^7 CFU/g LAB and 10^4 CFU/g *Micrococcaceae*.
Smoked salami of manufacturer GH. Blooms from three sausages (calibre: 50 mm) were found to contain 50.5 % D,L-lactic acid (D : L = 1.0), 7.8 % magnesium and 23.4 % creatine. Assuming that racemic Mg-di-lactate is present as its tri-hydrate, the analytical results explain 100 % of the composition of the efflorescences.

Slowly fermented salamis and thin calibre dry fermented sausages

French saucisse of manufacturer B. All salamis arrived packed in PE foils under modified atmosphere. Recommended storage was below 18 °C. White efflorescences were visible already before removal of the sausages from the packages. According to the manufacturer, the sausage recipe contained pork, common salt plus nitrate, spices, sugars (lactose, dextrose, sucrose), lactic acid bacteria (LAB) and ascorbate. Microbiological analysis showed reminder of starter culture bacteria (*Lactobacillus plantarum, Micrococcaceae*) and other LAB (Tab. 2). Both, D(-) and L(+) lactic acid were present in the sausages in similar ratios but in comparatively small concentrations indicating that only a mild fermentation had taken place before further ripening (drying). The relatively high pH of 5.7 is a typical result of this kind of salami

technology. The residual shelf life was comparable to the paprika salami sample no. 2 in Tab. 1 while the water activity was even lower.

Sample no.	1	2	3
Type	collagen casing	natural casing	natural casing
RSL[a] , days	52	52	52
Surface blooms % [b]	50	50	50
Water content %	32.4	nd	nd
Water activity	0.8425	nd	nd
pH	5.68	nd	nd
Creatine %			
in sausage	0.67 - 1.15	nd	nd
in surface bloom	95	90	93
Lactic acid %			
in sausage			
L(+)	0.79	nd	nd
D(-)	0.88	nd	nd
in bloom	nd	nd	nd
Bacterial counts [c]			
Lb. plantarum	6.83	nd	nd
Lb. sakei	4.84	nd	nd
other LAB	5.14	nd	nd
Micrococcaceae	5.67	nd	nd
Yeasts	4.74	nd	nd

[a] residual shelf life; [b] estimatet by eye; [c] $\log_{10}$ CFU/g sausage;

Tab. 2: Slow fermented salamis (saucisses) in modified atmosphere packages showing white surface blooms already before removal from the packages.

The creatine concentration inside the sausage was at the upper limit of what should be expected and, the efflorescence was composed of creatine only. Magnesium was not detected in the blooms. These results suggest that at pH values around 5.7 only minor amounts of Mg-D,L-lactate is formed. Also, creatine is more stabile at pH 5.7 than at pH 4.5 (see paprika salamis in Tab. 1).

German thin calibre dry fermented sausages. Salami-style sausages in sheep gut casings (10 mm calibre) were obtained in a PE package under protective atmosphere with a residual shelf life of 24 days. According to the product label the sausages were smoked and were composed of pork, nitrite curing salt, spices, glucose, taste enhancer and wort. The surface blooms were not visible upon arrival but were inducible by subjecting the packages to repeated temperature changes (8 °C / 20 °C). The efflorescences formed consisted almost entirely of creatine (83 %) and did not contain any lactate. The pH value of the sausages was around 5.8 – 5.9, i.e. even higher than the pH of the French saucisse in Tab. 2. The concentration of D,L-lactate in the sausage was 10.9 mg/g (D : L = 0.36). The D:L ratio indicated that hardly any microbial lactic acid formation had taken place, i.e. the sausages were more dried than fermented. The LAB count was less than 10^4 CFU/g, probably because of the low water activity of 0.812. The creatine and creatinine contents of the sausage were 2.4 mg/g and 0.4 mg/g, respectively.

Other thin calibre sausages. Creatine was present in the surface crusts of vacuum packaged ‘beersticks’ from New Zealand and, in the efflorescences of four loose sausages that apparently had been subjected to storage conditions favouring bloom formation. The pH values of the sausages ranged from 5.4 to 6.1. The white coating obtained from sample no. 1 in Tab. 3 also contained some D,L-lactate which, however, was about 10 times lower in concentration than creatine. Interestingly, there was hardly any D(-) lactate inside this sausage. This finding is in agreement with the low LAB count which suggests a drying-type process rather than a fermentation-type process. However, the LAB count also may have been reduced by the low water activity in this sample. In contrast, samples no. 3 – 5 showed a typical bacterial count of a fermentation microflora.

Sample	Hungarian 'Kolbasz'-style sausage	'Beersticks' from New Zealand	Thin calibre dry fermented sausage from organic production (Germany)		
Sample no.	1	2	3	4	5
Calibre, mm	20	10	10	10	10
Smoked	+	+	+	+	+
Packaged	-	vacuum	-	-	-
Meat	porc, beef	porc, beef	porc, beef	lamb, porc	porc, beef
Surface blooms % [c]	50	10-20	50	50	50
Water content %	29.9	nd	nd	nd	nd
Water activity	0,8386	nd	nd	nd	nd
pH	6.15	nd	6.01	5.71	5.37
Creatine %					
in surface bloom	13	8.5	2.9	2.6	2.1
Lactic acid %					
in sausage					
L(+)	0.9	nd	nd	nd	nd
D(-)	0.04	nd	nd	nd	nd
L(+) + D(-)	0.94	nd	nd	nd	nd
in surface bloom					
L(+)	0.7	< 1.6	< 1.6	< 1.6	< 1.6
D(-)	0.8	< 1.6	< 1.6	< 1.6	< 1.6
L(+) + D(-)	1.5	< 1.6	< 1.6	< 1.6	< 1.6
Bacterial counts					
Lactic acid bacteria	< 4	nd	8.2 [c]	7.9 [c]	8.5 [c]
Micrococcaceae	< 2	nd	6.0	5.2	6.0

[a] estimatet by eye; [b] $\log_{10}$ CFU/g sausage; [c] *Lactobacillus sakei*;

Tab. 3: Characteristics of some thin calibre dry fermented sausages from different origin.

Conclusions

The fermentation process determines the nature of the surface blooms on dry fermented sausages while bloom formation itself is determined by physical factors such as water activity of the sausages and storage conditions. Racemic lactic acid is the major component of efflorescences on dry sausages which were subjected to a rapid fermentation in the presence of added starter cultures and fermentable carbohydrates. In contrast, slowly fermented dry sausages and thin calibre dry sausages which are more dried than fermented may contain creatine as the sole component. High pH values in the sausage favour creatine blooms while low pH values favour Mg-di-lactate blooms. Appearance of the efflorescences is correlated with water activities of the sausages in the range of 0.81 – 0.88. Some efflorescences may contain both, creatine and Mg-D,L-lactate. The results of this study may help to develop strategies for preventing undesired efflorescences on dry fermented sausages.

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PHYSIOCHEMICAL PROPERTIES OF DRY-CURED HAMS FROM TAIWAN BLACK PIGS AND LYD HYBRID PIGS

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Background

Taiwan black pigs is a local two-way crossed pig breed in Taiwan area, which is crossbred by Taoyuan pigs and Duroc pigs (75%)(Tai *et al.*, 1992). Taiwan black pigs need be fed for 10-12 months and the average marketing live weight is 140-150kg. Meanwhile, LYD hybrid pigs are generally raised for meat pigs in there and need be fed for 5.5-6.0 months for marketing (average live weight: 110-115 kg). According to Tai *et al.*(1997) and Liu and Hsu (2000) the fat content and marbling degree of Taiwan black pigs were higher than that of LYD pigs but the moisture content was lower than LYD pigs.
Dry-curd ham constitutes one of the most traditional meat products, which after a long processing time become a tasty and flavorful products as a consequence of a great number of both physico-chemical and biochemical transformation such as an increase of small peptides, free amino acids and free fatty acids. A clear relationship has been established between the quality of the raw ham from different pig breeds and the sensory quality of the dry cured products (Antequer *et al.*, 1994; Aristory and Toldr'a, 1995; Berdague' *et al*., 1993 ; Gou et al., 1995; Parolari et al., 1994).

Objectives

The purposes of this study was to manufacture dry-cured ham from ham part of Taiwan black pigs (Taoyuan pigs x Duroc pigs(75%), feeding period: 10months) and LYD hybrid pigs (Landrace x Yorkshire x Duroc(50%), feeding period: 5.5 months) and investigate the change of the physiochemical properties of dry-cured hams during processing and ripening (300days).

Materials and methods

Ham : twenty Taiwan black pigs hams weighing 12-14kg and twenty LYD crossbreed pigs hams weighing 10-11kg were selected to manufacture dry –cured ham in this study. Processing: The hams were refrigerated for one day at 2-4℃ and then nitrified with a dry salt mixture of 10g of NaCl, 0.1g of $NaNO_2$ and 0.15g $NaNO_3$, per kg of ham. After 24hr they were covered with salt for a period. Afterwards the hams were washed in cold water and hung at 3-5 ℃in an atmosphere with a relative humidity between 80-85% for 60days, increasing the temperature to 13-15℃ and decreasing relative humidity to 65-70% then they remained at these conditions until the end of ripening period (300days). Sampling: three Taiwan black pigs and three LYD crossbreed pigs hams were sampled at each of the following periods: 24hr postmortem, resting (60days=aging 0 day) and aging (60, 120, 180, 300). When fat and skin had been removed the lean tissue (including *M. rectus femoris, M. vastus medialis, M. vastus lateralis, M. semitendinosus, M. semimembranosus, M. biceps femoris*) was taken out by a stainless sampling tube (cone dimeter:2.5cm) from meat side of hams, the lean tissues were divided into two parts , one centimeter distance from meat side was external part sample, overpassed one centimeter was internal part sample. Physicochemical assay: yield and weight losses of hams were evaluated relative to their initial fresh weight. The proximate contents of raw hams were determined by AOAC (1990). The moisture, NaCl concentration, water activity (A_w), protein hydrolysis index of the dry hams were tested in this experiment. Finally, the sensory panel test of dry-cured hams was evaluated after the ripening of 300 days. A scale from seven to zero was employed for all sensory attributes in this study.

Results and discussion

The results showed that the crude fat of raw and the end dry-cured hams from Taiwan black pigs were higher than that of LYD hybrid pigs, but moisture protein and ash content were not significantly different between the two breeds ($p > 0.05$). The yield of dry-cured ham was 62.35% for Taiwan black pigs and 63.43% for

LYD hybrid pigs, respectively. The pH value of dry cured ham from the two breeds significantly increased with ripening time ($p<0.05$) but no difference was found between the breeds. In the meantime, the moisture, internal and external water activity of dry-cured ham from the two breeds had no significant difference but significantly decreased ($p < 0.05$) during ripening period. The color of raw hams from the two breeds showed light red color, and then became dark red in the end products. The salt content of dry cured ham from the two breeds significantly increased with ripening time ($p < 0.05$) and was 6.97and 6.90% respectively in the end products. The nitrite residue of dry cured ham from the two breeds significantly decreased with time ($p < 0.05$). The proteolysis index was 25.97% for the end products of Taiwan back pigs, and 24.82% for the end products of LYD hybrid pigs at the ripening 300 day. However, the sensory panel scores of dry cured ham from Taiwan black pigs were better than those of LYD hybrid pigs.

Conclusions

Hams from Taiwan black pigs have some advantages over those from LYD hybrid pigs in dry cured ham production: a higher fat content and marbling and a higher proteolysis index. The only sensory difference between hams was found in flavor trial. The Taiwan black pig is possibly better for dry-cured ham production under the processing condition used in this study.

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Table 1. The chemical compositions of raw meat and the end dry-cured ham from Taiwan black pigs and LYD hybrid pigs

	Moisture (%)		C protein (%)		C fat (%)		Ash (%)	
	Raw	The end	Raw	The end	Raw	The end	Raw	The end
Black	75.86^{ax}	48.35^{bx}	18.54^{bx}	35.85^{ax}	3.63^{ax}	5.59^{ax}	1.14^{bx}	7.19^{ax}
LYD	75.77^{ax}	47.66^{bx}	19.59^{bx}	36.35^{ax}	3.00^{bx}	4.42^{ax}	1.14^{bx}	7.52^{ax}

Black：Taiwan Commercial black pig. LYD：Hybrid pig. Mean ± SD., n = 3.
The end：Ripening for 300 days.
$^{a-b}$：Means within the same row without the same superscript are significantly different（$p < 0.05$）.
$^{x-y}$：Means within the same column without the same superscript are significantly different（$p < 0.05$）.

Table 2. The change of moisture and water activity in external and internal part of dry-cured ham from Taiwan black pigs and LYD hybrid pigs during ripening period

	Raw ham	Ripening time （days）				
		0	60	120	180	300
Moisture			%			
Black	75.86 ± 1.33^{ax}	65.42 ± 4.28^{bx}	58.70 ± 3.61^{cx}	55.17 ± 3.36^{dx}	51.17 ± 4.44^{ex}	45.32 ± 1.12^{fx}
LYD	75.77 ± 1.82^{ax}	63.34 ± 5.34^{bx}	56.00 ± 2.87^{cx}	52.63 ± 1.99^{dx}	48.12 ± 3.38^{ex}	44.22 ± 2.40^{fx}
external part						
Aw						
Black	0.93 ± 0.01^{ax}	0.89 ± 0.01^{bx}	0.88 ± 0.01^{bx}	0.81 ± 0.03^{cx}	0.77 ± 0.03^{cy}	0.79 ± 0.01^{dx}
LYD	0.94 ± 0.01^{ax}	0.88 ± 0.00^{bx}	0.87 ± 0.01^{cx}	0.80 ± 0.01^{dx}	0.79 ± 0.03^{dx}	0.80 ± 0.01^{dx}
internal part						
Aw						
Black	0.97 ± 0.01^{ax}	0.92 ± 0.01^{bx}	0.91 ± 0.01^{bcx}	0.91 ± 0.01^{cx}	0.89 ± 0.01^{dx}	0.87 ± 0.00^{ex}
LYD	0.96 ± 0.01^{ax}	0.92 ± 0.01^{bx}	0.91 ± 0.01^{bx}	0.91 ± 0.01^{bx}	0.90 ± 0.01^{cx}	0.87 ± 0.01^{cx}

The foot note was the same as Table 1

Table 3. The change of color (L, a and b value) of dry-cured ham from Taiwan black pigs and LYD hybrid pigs during ripening period

	Raw ham	Ripening time （days）				
		0	60	120	180	300
Black						
L	36.26 ± 2.90^{a}	34.66 ± 5.39^{ab}	31.70 ± 6.28^{c}	34.36 ± 2.43^{abc}	32.36 ± 5.46^{bc}	21.15 ± 2.94^{d}
a	12.71 ± 4.43^{cd}	11.83 ± 4.52^{d}	12.14 ± 3.97^{d}	15.97 ± 4.45^{b}	19.61 ± 4.69^{a}	15.00 ± 3.17^{bc}
b	7.59 ± 1.81^{a}	6.92 ± 1.95^{ab}	5.81 ± 2.05^{b}	6.91 ± 2.07^{ab}	5.96 ± 2.38^{b}	3.42 ± 1.24^{c}
LYD						
L	35.31 ± 2.93^{a}	33.13 ± 4.49^{a}	34.25 ± 5.00^{a}	29.35 ± 2.93^{b}	35.86 ± 7.30^{a}	24.49 ± 3.59^{c}
a	8.81 ± 3.45^{d}	13.09 ± 5.42^{c}	13.22 ± 4.39^{c}	16.03 ± 3.07^{b}	19.91 ± 7.69^{a}	16.12 ± 3.17^{b}
b	6.90 ± 1.73^{ab}	7.71 ± 2.00^{a}	5.68 ± 2.49^{b}	6.22 ± 1.61^{b}	6.69 ± 3.29^{ab}	4.42 ± 1.38^{c}

Black：Commercial black pig. LYD：Hybrid pig. Mean ± SD., n = 3.
$^{a-c}$：Means within the same row without the same superscript are significantly different（$p < 0.05$）.

Table 4. The change of salt content, pH and nitrite residue of dry-cured ham from Taiwan commercial black pigs and LYD hybrid pigs during ripening period

	Raw ham	Ripening time （days）				
		0	60	120	180	300
Salt		---------- % ----------				
Black	0.76±0.10 ^{dx}	3.97±0.59 ^{cx}	4.92±0.34 ^{bx}	5.13±0.60 ^{bx}	5.42±0.60 ^{bx}	6.97±0.69 ^{ax}
LYD	0.66±0.08 ^{dx}	3.51±0.23 ^{cx}	4.88±0.78 ^{bx}	5.06±0.88 ^{bx}	5.20±0.79 ^{bx}	6.90±0.61 ^{ax}
pH						
Black		6.30±0.19 ^{cx}	6.63±0.10 ^{abx}	6.50±0.22 ^{abcx}	6.43±0.05 ^{bcx}	6.70±0.37 ^{ax}
LYD		6.36±0.15 ^{bx}	6.71±0.23 ^{ax}	6.47±0.06 ^{bx}	6.44±0.08 ^{bx}	6.67±0.13 ^{ax}
Nitrite		----------ppm----------				
Black		10.08±4.44 ^{ax}	4.17±2.04 ^{bx}	2.39±1.06 ^{bcx}	3.05±1.60 ^{bcx}	1.94±1.06 ^{cx}
LYD		10.79±1.59 ^{ax}	4.38±2.02 ^{bx}	1.74±0.60 ^{cx}	1.51±0.57 ^{cy}	0.25±0.41 ^{cx}

The foot note was the same as Table 1

Table 5. The score of sensory panel test of dry-cured ham from Taiwan black pigs and LYD hybrid pigs

	Color	Flavor	Mouth feeling	Salty	Hardness	Pastiness	Crumbliness	Overall acceptability
Black	4.50±0.85 ^{x}	4.71±0.17 ^{x}	4.43±0.19 ^{x}	4.14±0.46 ^{x}	4.00±0.14 ^{x}	4.07±0.14 ^{x}	4.21±0.12 ^{x}	4.71±0.32 ^{x}
LYD	4.86±0.86 ^{x}	4.14±0.23 ^{y}	4.21±0.42 ^{x}	4.07±0.20 ^{x}	4.29±0.38 ^{x}	4.21±0.58 ^{x}	3.57±0.50 ^{x}	4.29±0.49 ^{x}

Black：Commercial black pig. LYD：Hybrid pig. Mean ± SD., n＝14.
$^{x-y}$：Means within the same column without the same superscript are significantly different（$p < 0.05$）.

CHARACTERIZATION OF *STAPHYLOCOCCUS AUREUS* ISOLATED FROM A SLAUGHTERHOUSE FOR PIGEONS BY RAPD-PCR AND REA-PFGE

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Background

S. aureus is a common etiologic factor of foodborne infections and intoxication and is a significant marker of food quality and surface cleanliness (Losito *et al*., 2004). Recently, a variety of molecular methods have been used to clarify staphylococcal identification, including RAPD-PCR and REA-PFGE (Restriction Endonucleases Analysis-Pulsed Field Gel Electrophoresis) (Matthews *et al*., 1997). However, no information is available concerning the intraspecies differentiation of *S. aureus* isolated from a slaughterhouse for pigeons by these genetic procedures.

Objectives

In the present study, RAPD-PCR and REA-PFGE were used to characterize *S. aureus* strains originating from a slaughterhouse for pigeons and to assess the practical value of these typing methods for the discrimination among strains.

Materials and methods

Bacterial strains. The source of isolation of the 38 wild strains used in this study is shown in Table 1. Strains were grown in Brain Heart Infusion broth (BHI) (Oxoid Ltd., Basingstoke, Hampshire, UK) at 37°C. Before DNA extraction, cultures were streaked on BHI agar plates and grown overnight at 37°C.

DNA isolation for PCR assays. DNA extraction was carried out from a single colony by using an InstaGene Matrix (Bio-Rad Laboratories, Hercules, CA) following the conditions described by the supplier. About 25 ng of DNA were used for PCR amplification.

Identification of *S. aureus* strains by PCR. The thermostable nuclease (*nuc*) was amplified for the identification of *S. aureus* strains. Sequences of the primer set for the *nuc* gene were first described by Brakstad *et al*. (1992) (Table 2).
PCR mixtures were prepared as described in Blaiotta *et al*. (2004). After the denaturing step for 3 min at 95°C, 35 amplification cycles (5 s at 95 °C for the denaturing step, 30 s at 58 °C for the annealing-extension step) were performed for detection of *nuc* gene. The PCR products were resolved by agarose (2 % w/v) gel electrophoresis at 7 V cm^{-1} for 2 h.

RAPD-PCR amplifications. RAPD-PCR analysis was performed in a total volume of 25 µl using primers PRIM 239 (CTGAAGCGGA) and XD9 (GAAGTCGTCC) in the same reaction. RAPD-PCR mixtures were prepared as reported by Moschetti *et al*. (2000). Amplification was carried out in a programmable heating incubator for 40 cycles (1 min at 94 °C, 1 min at 31 °C, 2 min at 72 °C per cycle). Finally, a 7 min extension period at 72 °C was performed. Amplified products (25 µl) were resolved by electrophoresis on a 2 % (wt/vol) agarose gel at 7 V cm^{-1} for 2 h.

REA-PFGE (Restriction Endonucleases Analysis-Pulsed Field Gel Electrophoresis). Genomic DNA of high molecular weight was prepared as previously described Blaiotta et al. (2001). Inserts of intact DNA were digested in 200 µl of appropriate buffer supplemented with 40 U of *Sma* I (Promega). Electrophoresis of the restriction digests was performed by using the CHEF system (Bio-Rad Laboratories, Hercules, CA, USA) with 1% (wt/vol) agarose gels and 0.5 x TBE as running buffer, at 10 °C. Restriction fragments < 560 kb were resolved in a single run, at constant voltage of 6 V cm^2 and an orientation angle of 120° between electric fields, by a single phase procedure for 24 h with a pulse ramping between 1 and 50s.

Results and discussion

Results obtained by RAPD-PCR and REA-PFGE are depicted in Table 3. Examples of RAPD PCR and REA-PFGE patterns are showed in Fig.1 and Fig.2 respectively. RAPD PCR technique produced 9 different pattern-types among the 38 strains of *S. aureus* analysed. The most common RAPD PCR profile was the A one. The strains mostly isolated from the defeathering machine and worker's overall during all samplings produced this pattern. The number of the patterns went up to 25 using REA-PFGE. The strains isolated from the defeathering machine during the three samplings showed six different REA-PFGE patterns; one of them was isolated before slaughtering. The four strains from worker's hand produced three REA-PFGE patterns. The profile G was the only one common to different samplings. It was found on worker's overall during the first and the third sampling.

Conclusions

Results obtained during this study confirm that REA-PFGE technique is more discriminatory than RAPD (Raimundo *et al*., 2002; Al-Thawadi *et al*., 2003). A good discrimination among the strains is a very important step for the contamination monitoring above all in the slaughterhouse environment where the microbial contamination levels are always high. Our results underline the elevated differentiation percentage of the strains of *S. aureus* present in a slaughterhouse for pigeons. The findings also point out that the REA-PFGE technique could be a useful tool to reveal the contamination sources.

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Tab.1. tested surfaces contaminated by *St. aureus*

Tested surfaces	I sampling		II sampling		III sampling	
	B	**A**	**B**	**A**	**B**	**A**
Crop rinsing tube	+	+				
Defeathering machine		+	+	+		+
Leg defeathering machine (I finger)		+			+	+
Leg defeathering machine (V finger)	+	+				
Gutting machine		+				+
Tunnel entrance		+				+
Tunnel exit		+				+
Worker's hand	+	+				
Worker's overall		+		+		+

B: before slaughtering; **A**: after slaughtering

Table 2. PCR primers for the *nuc* gene used in this study

Primer	Sequence (5'-3')	References	Target gene	Product (bp)
nucF	GCGATTGATGGTGATACGGTT	Bakstad et al. 1992	*nuc*	279
nucR	AGCCAAGCCTTGACGAACTAAAGC			

Table 3. RAPD PCR and REA-PFGE types of *S. aureus* strains used in the present study.

Strains	**Source**	**D/P**	**Sampling**	**PCR *nuc***	**RAPD PCR**	***Sma*I PFGE**
2D	Defeathering machine	D	III	+	A	?
3D1	Leg defeathering machine (I finger)	D	III	+	A	A
5D1	Gutting machine	D	III	+	B	O
6D1	Tunnel entrance	D	III	+	A	B
7D1	Tunnel exit	D	III	+	A	C
9D3	Worker's overall	D	III	+	A	G
10D2	Defeathering machine	D	III	+	A	B
8	Tunnel entrance	D	I	+	B	J1
9	Leg defeathering machine (I finger)	D	I	+	B1	J3
10	Gutting machine	D	I	+	A2	T
11	Leg defeathering machine (IV finger)	P	I	+	A	G1
12	Defeathering machine	D	I	+	A	D
13	Tunnel exit	D	I	+	A	D
14	Leg defeathering machine (I finger)	D	I	+	B2	N
15	Leg defeathering machine (IV finger)	D	I	+	B4	S
16	Tunnel entrance	D	I	+	A	D
17	Defeathering machine	D	I	+	B2	P
18	Crop rinsing tube	D	I	+	B3	R
19	Leg defeathering machine (IV finger)	D	I	+	B1	J3
20	Leg defeathering machine (IV finger)	P	I	+	A	E
21	Crop rinsing tube	D	I	+	B	M
22	Tunnel exit	D	I	+	B1	J3
23	Crop rinsing tube	P	I	+	A	F
24	Worker's hand	P	I	+	B	J2
25	Worker's hand	P	I	+	B	L
26	Worker's hand	D	I	+	A	E1
27	Worker's overall	D	I	+	B2	Q
28	Worker's hand	D	I	+	A	E1
29	Worker's overall	D	I	+	A	G
101	Worker's overall	D	II	+	A	H
102	Worker's overall	D	II	+	A	H
103	Worker's overall	D	II	+	A	H
104	Defeathering machine	D	II	+	A	H
105	Defeathering machine	D	II	+	A1	I
106	Defeathering machine	D	II	+	A1	I
107	Defeathering machine	D	II	+	A	?
108	Defeathering machine	D	II	+	A	H
109	Defeathering machine	P	II	+	A3	K

Fig.1. RAPD-PCR patterns

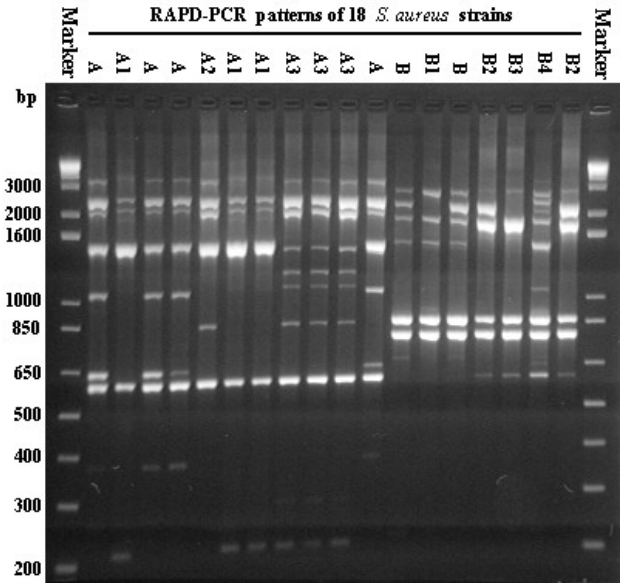
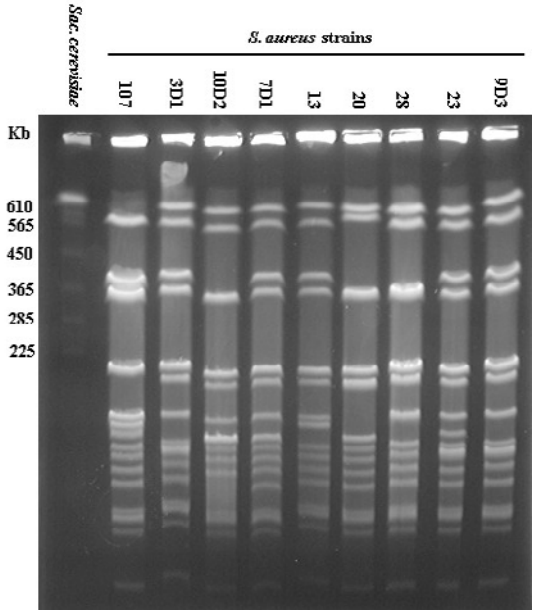


Fig. 2. REA-PFGE patterns



COLONISATION OF PIGLETS BY GENOTYPES OF *CAMPYLOBACTER COLI*

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Background

In Northern Ireland (NI) *Campylobacter* spp. are the principal cause of gastro-enteritis (Anon. 1995) with *Campylobacter jejuni* responsible for 90-95% of cases and *Campylobacter coli* causing the majority of the remainder, as is the case in most developed countries. A local survey of campylobacters in deep tissues of freshly eviscerated porcine liver (Moore and Madden, 1998) showed that 50% of the *Campylobacter* spp. isolated were *C. coli* however only 6% of livers were contaminated. In contrast 71.7% of samples of retail pig liver in England and Wales carried *Campylobacter* spp. with *C. coli* dominant, being found in 42.4% of samples. Subsequent investigations of pig faeces showed high *C. coli* carriage rates, as reported elsewhere (Blaser *et al.* 1983). Pigs are a natural reservoir of *C. coli* (Weijtens *et al*. 1993) and hence pork products may represent a risk to consumers. Skirrow (1991) concluded that *C. coli* from pigs are probably an important source of clinical infection in countries where pork is eaten salted or lightly cooked.

Objectives

Pigs are known to carry a wide range of *C. coli* genotypes and one pig may carry several (Weijtens *et al*. 1997). A detailed sampling of a single litter, and their mother, over the first 10 weeks of life was undertaken to allow a better understanding of the colonization of pigs by distinct *C. coli* genotypes.

Materials and methods

All media used were obtained from Oxoid, Basingstoke, UK, and all chemicals from Sigma-Aldrich, Fancy Road, Poole, UK.

Sampling of pigs. Pigs were sampled in the farrowing unit of an experimental farm. Litters were not allowed to associate but no exceptional measure were made to avoid cross-infection of the litters. Anal sampling of pigs was based on the method previously described (Madden *et al*. 2000). Swabs were locally made and consisted of cotton gauze fixed to a flat wooden support and had a surface area of approximately 6cm^2. Swabs were moistened in sterile peptone saline diluent prior to use and each pig was swabbed once. The swab was then broken into a sterile disposable universal. All samples were stored at 4^{0}c for transport to the laboratory and analysis commenced within two hours of sampling.

Six specific piglets in a single litter were selected using the unique identifier tattooed on its ear and sampled 6 times, using anal swabs, over the first 10 weeks of life. The sow was also sampled on each visit.

Isolation of *Campylobacter* spp. Swabs were streaked onto modified charcoal cefoperazone desoxycholate agar (mCCDA) (Bolton and Robertson, 1984) and incubated (42^{0}C) in a Don Whitley Mk III anaerobic cabinet (Don Whitley Scientific Ltd, Shipley, Yorks., UK) using a gas mix of 5% O_2, 10% CO_2, 85% N_2. Plates were examined daily for up to 5d and colonies characteristic of *Campylobacter* spp. selected and purified. Five isolates per plate were picked from each of the six piglet samples and twenty from those of the sow. All colonies morphologically characteristic of campylobacters were subjected to initial phenotypic characterisation and all *Campylobacter* spp. identified to species level using standard biochemical tests (Bolton and Robertson, 1982). They were then stored using 'Cryovials' (Technical Service Consultants, Heywood, Lancs. GB) at -80^{0}C.

Genotyping. Polymerase chain reaction/restriction fragment length polymorphism (PCR/RFLP) typing of the *flaA* gene was conducted using the method of Nachamkin *et a.l* (1993) whilst random amplification of polymorphic DNA (RAPD) was performed as previously described (Madden *et al*. 1996). Pulsed field gel electrophoresis (PFGE) was by the method of Thomson-Carter *et al*. (1993) using *Sma* 1. All profiles were analysed using Gelcompar (V 4.1) software.

Results and discussion

Sampling was initiated 3d after the piglets were born and at that time the sow and 3 piglets carried *Campylobacter coli*, the remaining 2 piglets yielded no campylobacters. Subsequently *Campylobacter* spp. were isolated from all samples. All isolates from the piglets (n=165) were identified as *C. coli*. The carriage rate of 100% from 17d of age onwards is higher than that reported by Weijtens *et al.* (1997), who found 85% positive after 4 weeks and Steinhauserova *et al.* (2001) who reported 41% of healthy piglets up to 8 weeks old. However Weijtens *et al.* (1997) noted that significant differences in carriage rates were found between farms, hence this could account for the differences reported. The sow died when the piglets were 14d old and was replaced by a foster sow. The latter was sampled when the piglets were 17d and 31d old and carried *Campylobacter lanienae*, with 20 isolates and 19 isolates being found out of 20 samples taken on the respective days. One *C. coli* isolates was found on the second sampling and subsequently all isolates were of this species. Overall 120 isolates from the sows were studied.
When the piglets were 27d old some piglets from a neighbouring litter briefly gained access to the pen, and on day 31 four of the neighbouring piglets were sampled to obtain isolates for comparative purposes.
Genotyping was conducted by RAPD, PCR-RFLP and PFGE and *C. coli* isolates were characterized according to their initial source of isolation i.e. mother, neighbour or other. Based on this characterization the genotypes isolates obtained from the piglets and the sow showed the same trend hence the mean is shown below, Figures 1 and 2.

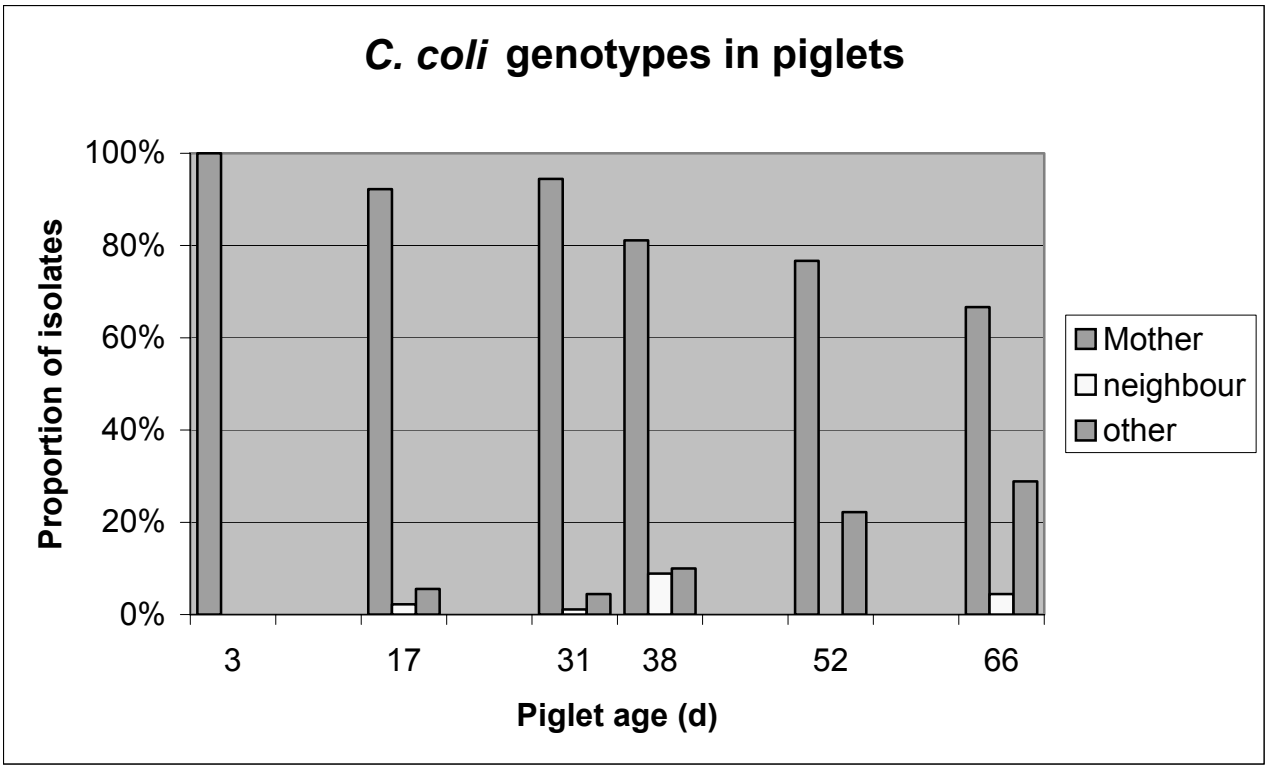

Figure 1. Distribution of *C. coli* isolates from piglets time (piglet age), based on the mean number of genotypes from three methods applied (PCR-RFLP of *flaA*, PFGE and RAPD).

The piglets show a clear trend of initially being colonized solely by *C. coli* strains from the mother but then, over the study period of 66 days, acquiring some from other sources causing replacement of the maternal genotypes. These results contrast with those of Hume et al. (2002) who reported that, in a study of 99 C. coli isolates from 3 sows and 17 piglets, there was no pattern of shared PFGE genotypes between sows and their respective piglets, nor between littermates. However in that study broth enrichment was used and enrichment conditions can affect the range of *Campylobacter* species (Madden *et al.* 2000) and genotypes (Madden et al. 2003) found. Weijtens *et al.* (1997) reported that a large diversity of campylobacter genotypes (determined by ERIC-PCR and RFLP) was found in piglets and their sows, as was the case in this study. In addition they noted that identical genotypes were found in piglets and their mothers and hence suggested that piglets became infected via their mothers, which is in agreement with the findings of this study (Fig. 1). However ,

the study reported here also shows that colonization is highly dynamic and point sampling will only record what is happening at one instant, in a very dynamic situation.

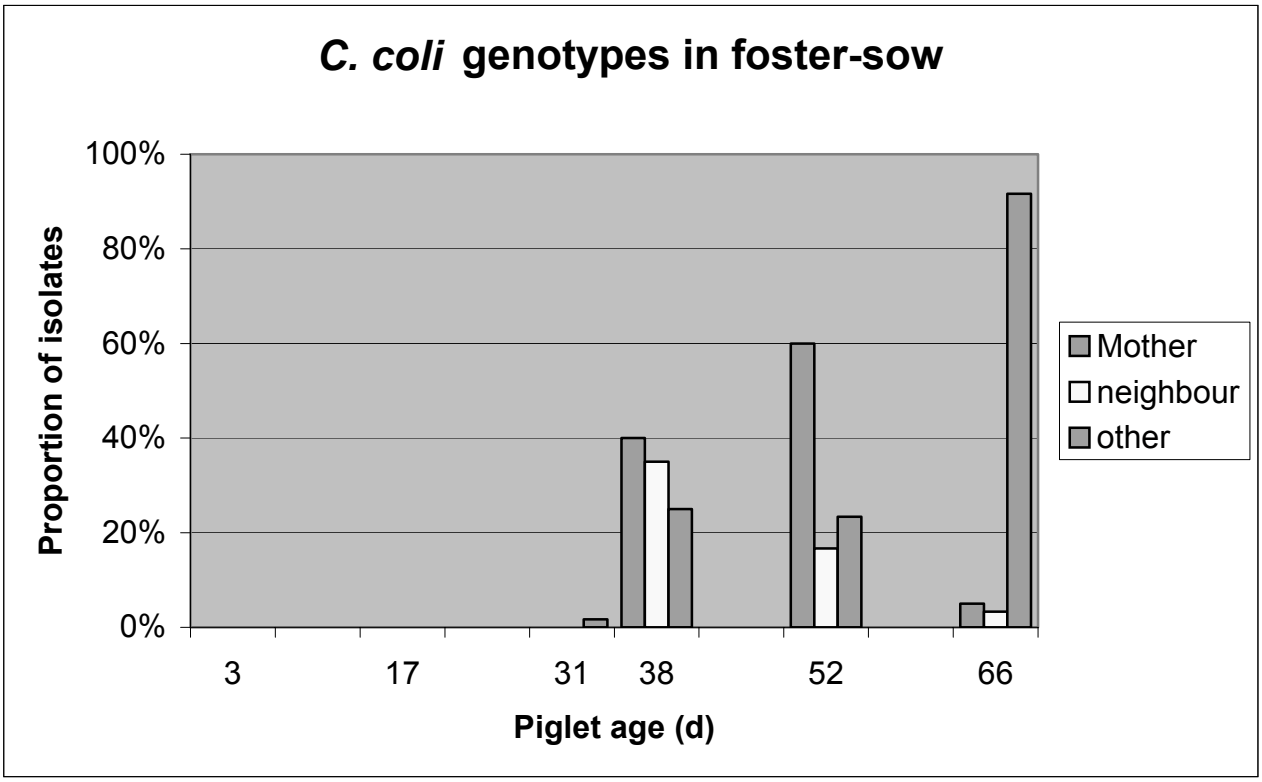

Figure 2. Distribution of *C. coli* isolates from foster-sow (introduced at age 15d of piglets) with piglet age, based on the mean number of genotypes from three methods applied (PCR-RFLP of *flaA*, PFGE and RAPD). Sampling on 17d and 31 d of age yielded 39 campylobacters, but only 1 *C. coli*.

The dynamic nature of colonization is illustrated by the results seen with the foster mother, (Fig 2). On arrival this sow was dominated by a *Campylobacter* spp. which was completely replaced (within 24d) by *C. coli* genotypes, the majority of which had been found in the original mother or the neighbouring litter. Thus whilst transmission from the sow to the litter appears to occur the process can also occur in reverse with the sow being infected from the litter, or nearby litters. Colonization by *C. coli* is highly dynamic in nature and displacement of dominant genotypes appears to occur regularly, even in adults.

Conclusions

Pigs and piglets have been reported as carrying several genotypes of *Campylobacter coli.* This study confirms that finding and shows that piglets are infected by their mother but can subsequently acquire other genotypes in a matter of days. The dominant genotypes in the piglets studied changed constantly over the 10 week sampling period, but the trend was for other genotypes to replace the maternal ones. In addition the sow can acquire new genotypes from her piglets and surroundings, and also displays a constant change in the dominant genotypes detected. Such a dynamic system means that attempts to use genotyping of *C. coli* in epidemiological studies involving pork products are unlikely to succeed. However piglets may prove a useful system in which to study genetic changes of *C. coli.*

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OPTIMISATION OF THE ISOLATION OF *CAMPYLOBACTER SPP.* FROM RETAIL PACKS OF RAW POULTRY

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Background

In Northern Ireland (NI) *Campylobacter* spp. are the principal cause of gastro-enteritis (Anon. 1995) with *Campylobacter jejuni* responsible for 90-95% of cases and *Campylobacter coli* causing the majority of the remainder, as is the case in most developed countries. Local surveys of campylobacters in retail packs of poultry have shown levels of 65% (Flynn *et al.* 1994) and 57% (Wilson, 2002). In order to conduct a survey to determine the current level the most effective current isolation procedures were required. Accordingly one of the most commonly used campylobacter enrichment broths, Preston broth, was selected for comparison in a trial with a much newer medium, Bolton broth. Locally Preston broth had shown up to 100% efficiency in recovering campylobacters from pigs (Madden *et al.* 2000).

Objectives

This investigation sought to compare Bolton (Oxoid, Basingstoke, UK) and Preston (Bolton and Robertson, 1982) enrichment broths for their ability to recover campylobacters from retail packs of raw poultry using the manufacturers protocols. Both overall recovery rates and species diversity were to be evaluated. With the more efficient medium defined a study could then be undertaken to determine the effect of enrichment broth volume (90ml or 225ml) on recoveries of campylobacters. The standard 1:9 ratio of samples to broth (w/v) was to be used in both cases. Use of the lower volume would significantly reduce costs where large numbers of samples were to be analysed. Finally the consequences of the choice of incubation temperature, 37^{O}C or 42^{O}C, and duration, 24h or 48h, on the subsequent isolation rates of campylobacter species were to be investigated.

Materials and methods

All media used were obtained from Oxoid, Basingstoke, UK, and all chemicals from Sigma-Aldrich, Fancy Road, Poole, UK.

Sampling of retail poultry packs.

Retail packs of chilled poultry were purchased from local supermarkets and butchers. In supermarkets the EC processor codes and pack 'sell by ' dates were used to ensure a diverse range of samples. Analyses commenced within 2h of purchase.

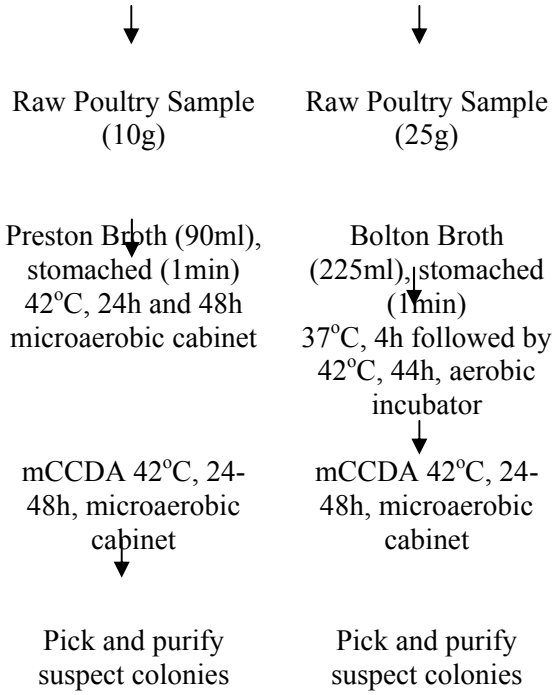
Comparison of methods for the isolation of *Campylobacter* spp.

Modified charcoal cefoperazone deoxycholate agar (mCCDA) was used as the plating medium with both Preston and Bolton broth. Figure 1 shows the enrichment protocols as used to compare Preston and Bolton broths (n=40), The former was based on a previous local study (Scates *et al.* 2003) and the latter was as described by Bayliss *et al.* (2000). In addition Preston medium was evaluated with 25g of sample and 225 ml of broth whilst Bolton broth (90ml) was inoculated with 10g of sample. The samples in Bolton broth were enriched for both 24h and 48h in a microaerobic atmosphere (85% N_2, 10% CO_2 and 5% O_2 in a Don Whitley Scientific Mk III anaerobic cabinet (Don Whitley Scientific, Shipley, UK)) to allow the effect of the incubation time on recoveries to be observed. Levels of contamination on mCCDA were assessed by using a standard plating technique and scored according to the apparent level of non-campylobacter growth in each streak (Bayliss *et al*. 2000). Presumptive *Campylobacter* isolates were characterised using a PCR method in order to determine genus (Linton *et al.* 1996) and a multiplex PCR method (Vandamme *et al.* 1997) to speciate *C. jejuni* and *C. coli.*

Local practice has been to incubate the enrichment broth under microaerobic conditions, as well as the selective solid medium. A comparison of microaerobic and aerobic incubation of 10g samples (n=21) in Bolton broth (90ml) was conducted to determine if this practise had any effect on recoveries .

With the selection of the optimal medium and sample size a trial sampling of 100 retail packs was undertaken using two incubation regimes, 37°C, 48h and 37°C, 4h followed by 42°C, 44h.

Figure 1. Comparison of the two methods compared for the isolation of *Campylobacter* spp. from retail packs of raw poultry. All incubations were microaerobic.



Results and discussion

Forty retail packs of poultry were studied to compare recoveries of campylobacters using the manufacturers recommended protocols, Figure 1. Included in the trial was a comparison of the effect of using 90ml and 225ml of both enrichment broths with a 1:9 sample:broth (w/v) ratio. Analysis of variance showed significantly more samples were positive for campylobacters using Bolton broth (n=27) than with Preston (n=24) using the protocols in Figure 1 (p=0.004). This is in agreement with findings of Bayliss *et al.* (2000) who used 25g samples inoculated into 225ml volumes of both enrichment broths. Bolton broth was more efficient at suppressing non-campylobacter flora than Preston broth which, when incubated for 24 and 48 h, yielded 2.4 and 3.6 times more contaminants respectively.

There was no statistically significant difference (p=0.439) between recoveries of campylobacters from 10g samples incubated in 90ml, as opposed to 25g in 225ml, with either medium. Hence the more economical lower volume could be adopted with no significant loss of sensitivity with either Preston or Bolton broth. This result may reflect the relatively high level of contamination of retail poultry with *Campylobacter* spp. (Jorgensen, 2002)

One *Campylobacter* isolate was obtained from each positive sample obtained using the two enrichment media, and two sample weights, and identified, Table 1. Again Bolton broth was seen to be superior as it allowed the detection of a significant number of *C. coli* isolates. Note that mixed cultures of *C. jejuni* and *C. coli* were detected by this procedure indicating that the preliminary picking and re-streaking procedure did not yield a pure culture. This is a frequent observation with this genus in our laboratory, indicating that repeated streaking is essential to ensure that a pure culture is obtained for subsequent studies.

Normally in our laboratory all *Campylobacter* incubations are conducted in a microaerobic atmosphere therefore the effect of microaerobic versus aerobic incubation was investigated. Samples of raw poultry (n=21) were inoculated into Bolton broth, using 10g inoculum, and incubated at 37°C for 4h followed by 44h at 42°C both aerobically and microaerobically. Microaerobic incubation of the enrichment broth was

seen to give the higher recovery of campylobacters with 62% of samples positive, whilst in air 52% of samples were positive. Thus microaerobic incubation of Bolton broth was seen to be the more efficient method .

Table 1. Identification of *Campylobacter* isolates from Preston (n=48) and Bolton broth (n=58) using PCR-based procedures. Bolton broth was superior, as it allowed the isolation of a wider range of species and detected more positive samples

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	Multiplex PCR speciation			
Enrichment medium	*C. jejuni*	*C. coli*	Both *coli* and *jejuni*	Genus only positive
Preston broth (24h)	88%	0%	8%	4%
Bolton broth (48h)	63%	38%	6%	12%

However the incubation temperature of an enrichment broth had been shown to markedly influence the types of campylobacters subsequently isolated (Scates *et al*. 2003), hence incubation at 37^{O}C as well as 42^{O}C could prove beneficial and a trial to compare the two temperatures was designed. This necessitated determining the optimal incubation time for Bolton broth at the lower temperature, and the study was also used to compare the effect of incubation time on recoveries from the broth incubated at 42^{O}C, using the protocol of Figure 1. Samples of raw poultry (n=21) were prepared and streaked out onto mCCDA after 24 and 48h, 37^{O}C, and 42^{O}C. Plates were incubated at the same temperature as the enrichment broths. The 24h incubation time gave higher recoveries, at both temperatures, whilst 48h incubations allowed significantly more non-campylobacters to grow on the mCCDA plates. This is similar to the results seen in previous work with Preston broth where campylobacter recoveries from porcine ileal contents were higher with shorter incubation times (Madden *et al.* 2000).

A survey of 100 samples of retail packs of poultry was then undertaken using the optimised procedures of 10g of sample and 90ml of Bolton broth incubated at 37^{O}C and 42^{O}C for 24h and streaked on mCCDA with the plates being incubated at the same temperature as the enrichment broth. Plates were examined after 24 and 48h. Overall 82% of samples were positive, with incubation at 37^{O}C giving 73% and 42 ^{O}C 75% .

Conclusions

Bolton broth was seen to be better than Preston broth for detecting Campylobacter spp. in retail packs of raw poultry. The former medium also detected significantly more *C. coli* than did Preston broth. The use of a smaller sample size, 10g as opposed to 25g, had no significant effect on recoveries of campylobacters when used at a 1:10 ratio (w/v) with the enrichment broth, but markedly reduced costs. Incubation of Bolton broth for 24h gave better recoveries of *Campylobacter* spp. with fewer contaminants than did 48h.
Incubation of Bolton broth at 37^{O}C and 42^{O}C (with an initial 4h incubation at 37^{O}C), yielded similar number of positive samples but combining the results of both incubations significantly increased the total number of positive samples found.
Overall, therefore, the optimal method in terms of efficiency and cost was the use of 10g sample added to 90ml of Bolton broth and incubated for 24h at either 37^{O}C or 42^{O}C (with an initial 4h incubation at 37^{O}C). The use of both incubation temperatures would be necessary to maximise recoveries.

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IDENTIFICATION OF IMPORTANT SPOILAGE BACTERIA AND FUNGI FROM TWO TYPES OF PORTUGUESE SMOKED DRY SAUSAGES AFTER SHELF-LIFE PERIOD IN MODIFIED ATMOSPHERE PACKAGE

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Background

The microbiology of dry smoked sausages is variable and complex and the rate of spoilage of these meat products can reduce the shelf life and cause substantial financial losses to manufacturing companies. Several studies were conducted to identify the bacterial populations in fermented dry sausages, however, despite increasing interest for modified atmosphere package (MAP), the effect of MAP on the spoilage bacteria and fungi of these meat products is not yet clear. Besides identifying the bacterial and fungal contamination during the manufacture processing, it is further important to identify the spoilage micro-organisms that will remain or proliferate during the storage period in MAP, and which might decrease shelf life and compromise product safety.

Objectives

The purpose of this study was to determine important spoilage bacteria and fungi in two types of Portuguese dry smoked sausages (chouriços) packaged in modified atmosphere (45% CO_2 and 55% N_2), which do not yet show clear spoilage changes at the end of the producer-defined shelf life period (120 days at 20±5ºC).

Materials and methods

Samples studied for this project were "chouriços" type Alentejano (A) and Ribatejano (R) produced by a large factory in Portugal as outlined by Matos et al. (2003). Identification and characterisation of bacterial species were based on phenotypic [cell morphology and fermentation profile according Jensen and Jorgensen (1994) and Jensen et al. (1995)] and genotypic (16S rDNA sequencing) characters according Knarreborg (2002). Identification of fungi was performed according conventional mycological methods based on morphological and physiological characterisation using taxonomic tables. The near-full-length sequences of the isolates have been deposited in GenBank under accession numbers AY587776 - AY587843. The phylogenetic relationship of the different bacterial species was determined by comparative sequence analysis of their 16S rDNA genes.

Results and discussion

A total of 30 fungi isolates were obtained from Alentejano (12) and from Ribatejano (18) types of smoked dry sausage, and the distribution of recovery from the CRB media is given together with the viable counts in Table 1. In Fig. 1 are shown microscopic photographs of five representative mould isolates. Fig. 2. shows the 16S rDNA-based tree reflecting the relationship of bacterial species. A total of 77 bacterial isolates were obtained from Alentejano (35) and from Ribatejano (42) types of sausage. In both types of sausages, and among the mould strains identified, *Penicillium*, *Fusarium* and *Aspergillus* species constituted the predominant mycoflora, with 50%, 16.6% and 16.6% for Alentejano variety and with 61.1%, 11.1% and 16.6% for Ribatejano sausage, respectively. Although contributions from other bacteria than the ones isolated can not be excluded, *Enterococcus faecium* (24.7%), *Bacillus subtilis* (23.4%), *Staphylococcus epidermidis* (14.3%), *B. cereus* (7.8%), *Pediococcus acidilactici* (6.5%) *Bacillus pumilus* (6.5%), *Clostridium bifermentans* (3.9%), *Bacillus licheniformis*, and *Enterococcus faecalis* (2.6%) were found in varieties A and R of Portuguese chouriço after 120 days at 20±5ºC in MAP. Major differences observed between the two varieties of chouriço were the absence of *Pediococcus* species and the presence of high numbers of isolates identified as *Staphylococcus epidermidis* in product A and, the high incidence of *Bacillus* species in sausage type R. Considering that MAP applied to both types of product was constituted

by the same percentage of gases and, the storage conditions, maturation period and smoke treatment were the same, differences found between products may be due to spices formulation in each type of product, the type of natural casings used and, the hygienic quality of the raw meat.

Conclusions

The presence of aerobic *Bacillus* related species and fungi in these MAP chouriços, where the air in the package was replaced by a specific mixture of carbon dioxide and nitrogen (45% CO_2 and 55% N_2), can be explained considering that oxygen is not always completely removed and may also permeate through the packaging material (Vermieren et al., 1999). In fact, the level of residual oxygen in MAP packs may be due to factors such as the oxygen permeability of the packaging material, the ability of the food to trap air, poor heat sealing ability of the packaging material causing air to leak in ineffective evacuation and/or ineffective gas flushing (Smith et al., 1986), the storage period and temperature (Smiddy et al., 2002). Further studies must be carried out to confirm the results obtained in this work in order to establish the spoilage population which might decrease shelf life and compromise product safety.

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Table 1.
Identified fungi, distribution of recovery from CRB media and viable counts obtained for each type of Portuguese dry smoked

Identified fungi[a]	Log CFU g^{-1}	
	Alentejano chouriço 1.77 ± 0.52[b]	Ribatejano chouriço 1.61 ± 0.30[b]
	Number of isolates	
Penicillium terrestres: [Tm15(A), Tm18(A), Tm23(A), Tm01(R), Tm02(R), Tm03(R), Tm06(R), Tm08(R), Tm09(R), Tm21(R), Tm22(R), Tm26(R), Tm27(R)]	3	10
Penicillium sp : [Tm04(A), Tm05(A), Tm19(A), Tm28(R)]	3	1
Fusarium sp: [Tm07(A), Tm20(A), Tm24(R)]	2	1
Fusarium tricinctum: [Tm17(R)]	0	1
Aspergillus glaucus: [Tm30(A), Tm29(A), Tm10(R)]	2	1
Aspergillus versicolor: [Tm11(R), Tm12(R)]	0	2
Rhizopus stolonifer: [Tm25(A)]	1	0
Monilia fruticola: [Tm13(A)]	1	0
Absidia sp: [Tm16(R)]	0	1
Cephalosporium sp [Tm14(R)]	0	1
Total	12	18

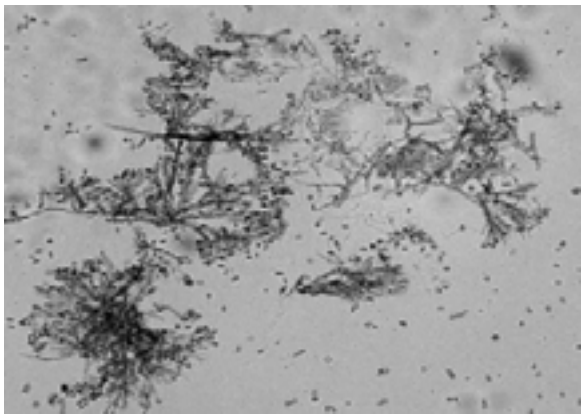
[a] Mould isolates obtained from Alentejano (A) and Ribatejano (R) sausages.
[b] CRB media (Oxoid; CM549), mean value ± standard deviation (n=6).

LEGENDS:

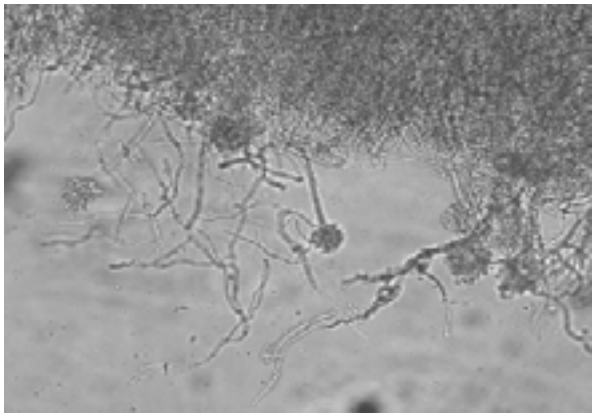
Figure 1. Microscopic photographs of five representative mould isolates: a) *Monilia frutícola* [Tm13(A)]; b) *Aspergillus glaucus* [Tm10(R)]; c) *Fusarium tricinctum* [Tm17(R)]; d) *Fusarium sp* [Tm07(A)]; e) *Penicillium sp* [Tm28(R)].

Figure 2. 16S rDNA-based tree reflecting the relationship of species. The length bar indicates 10% estimated sequence divergence.

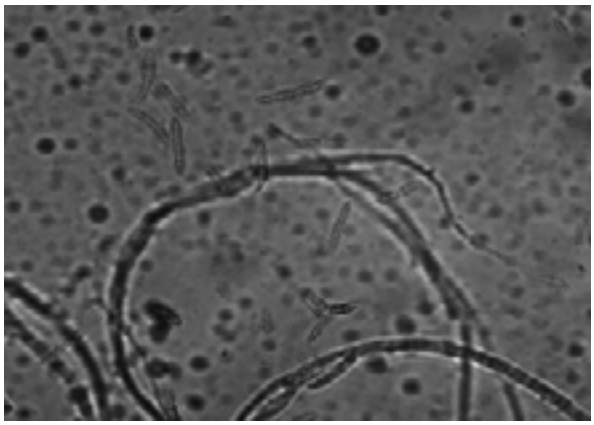
a)



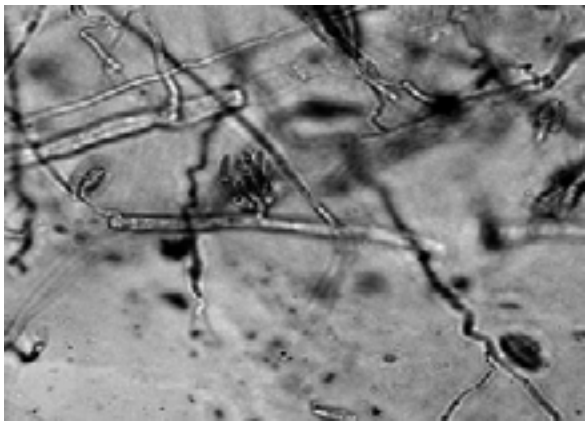
b)



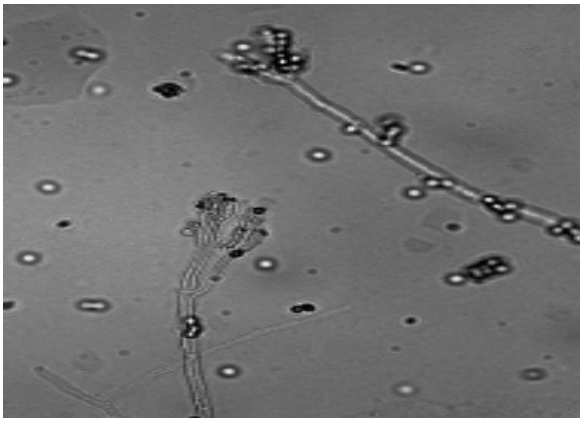
c)

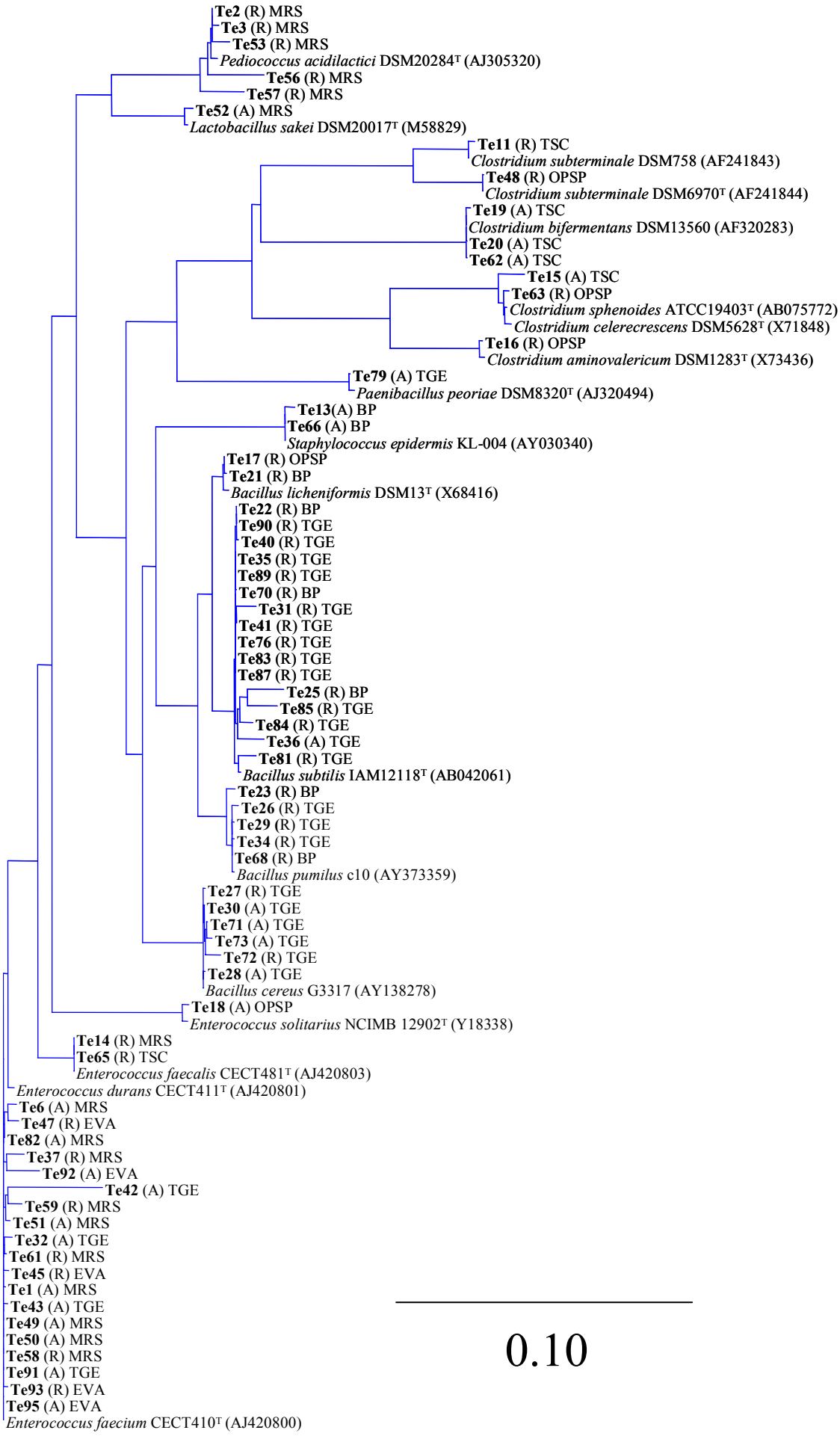


d)



e)




Te2 (R) MRS
Te3 (R) MRS
Te53 (R) MRS
Pediococcus acidilactici DSM20284T (AJ305320)
Te56 (R) MRS
Te57 (R) MRS
Te52 (A) MRS
Lactobacillus sakei DSM20017T (M58829)
Te11 (R) TSC
Clostridium subterminale DSM758 (AF241843)
Te48 (R) OPSP
Clostridium subterminale DSM6970T (AF241844)
Te19 (A) TSC
Clostridium bifermentans DSM13560 (AF320283)
Te20 (A) TSC
Te62 (A) TSC
Te15 (A) TSC
Te63 (R) OPSP
Clostridium sphenoides ATCC19403T (AB075772)
Clostridium celerecrescens DSM5628T (X71848)
Te16 (R) OPSP
Clostridium aminovalericum DSM1283T (X73436)
Te79 (A) TGE
Paenibacillus peoriae DSM8320T (AJ320494)
Te13(A) BP
Te66 (A) BP
Staphylococcus epidermis KL-004 (AY030340)
Te17 (R) OPSP
Te21 (R) BP
Bacillus licheniformis DSM13T (X68416)
Te22 (R) BP
Te90 (R) TGE
Te40 (R) TGE
Te35 (R) TGE
Te89 (R) TGE
Te70 (R) BP
Te31 (R) TGE
Te41 (R) TGE
Te76 (R) TGE
Te83 (R) TGE
Te87 (R) TGE
Te25 (R) BP
Te85 (R) TGE
Te84 (R) TGE
Te36 (A) TGE
Te81 (R) TGE
Bacillus subtilis IAM12118T (AB042061)
Te23 (R) BP
Te26 (R) TGE
Te29 (R) TGE
Te34 (R) TGE
Te68 (R) BP
Bacillus pumilus c10 (AY373359)
Te27 (R) TGE
Te30 (A) TGE
Te71 (A) TGE
Te73 (A) TGE
Te72 (R) TGE
Te28 (A) TGE
Bacillus cereus G3317 (AY138278)
Te18 (A) OPSP
Enterococcus solitarius NCIMB 12902T (Y18338)
Te14 (R) MRS
Te65 (R) TSC
Enterococcus faecalis CECT481T (AJ420803)
Enterococcus durans CECT411T (AJ420801)
Te6 (A) MRS
Te47 (R) EVA
Te82 (A) MRS
Te37 (R) MRS
Te92 (A) EVA
Te42 (A) TGE
Te59 (R) MRS
Te51 (A) MRS
Te32 (A) TGE
Te61 (R) MRS
Te45 (R) EVA
Te1 (A) MRS
Te43 (A) TGE
Te49 (A) MRS
Te50 (A) MRS
Te58 (R) MRS
Te91 (A) TGE
Te93 (R) EVA
Te95 (A) EVA
Enterococcus faecium CECT410T (AJ420800)
0.10

INVESTIGATING OZONE TREATMENT AS A REMEDIAL ACTION FOR MICROBIAL SPOILAGE OF MEAT BY MEASURING VOLATILES USING PROTON-TRANSFER-REACTION MASS-SPECTROMETRY

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Background

An estimated 30% of fresh produce is lost by microbial spoilage from the time of harvest, through handling, storage, processing, transportation, shelving and delivery to the consumer (web1). To preserve food pathogens need to be destroyed or inactivated and the non-pathogenic microorganisms and enzymes responsible for food spoilage need to be eliminated or at least reduced (web2). Several techniques to extend food's shelf-life have been developed over the years for example heating, drying, irradiation and treatment with ozone. All these methods have their advantages, drawbacks and limitations depending on the type of the food, the kind of microorganisms, national regulations and the public demands (like unaltered taste, aroma, colour and vitamin content with no chemical residues after treatment). The use of ozone to treatment of the food using ozone gas meets these requirements quite well.

Ozone is a strong oxidant that kills many microbacterial organisms without leaving any toxic by-products or residues (web3, web4). Ozone has also been used for many years to treat pathogens such as bacteria and algae in water for applications such as drinking water supplies (web4) - ozone should therefore be a useful agent for the destruction of pathogens which are active in microbial spoilage of meat. Despite these advantages the use of ozone in the food industry has not been exploited as extensively since ozone must be manufactured on-site and until recently ozone generators were bulky and expensive (web5). However new developments in the design of small scale in situ ozone generators (using either UV lamps or electrical discharges) now make it practical to develop ozone treatment for food preservation on a commercial scale.

To date there have been only a few studies to quantify the ozone concentrations needed to ameliorate microbial spoilage. To fill the gap in this knowledge the aim of this study is to investigate the influence of ozone on microbial spoilage using the technique of PTR-MS to analyse VOC emissions derived from microbial spoilage in real-time.

RH Dainty's group has shown that it is possible to get information about the spoilage status by chemical measures e.g. analysis of VOCs. The products of microbial metabolism depend on the types of bacteria growing, the substrate on which they are growing and on the storage conditions (air, vacuum or modified atmosphere) (Dainty, 1996). This is confirmed by another study (Mayr *et al.*, 2003) that has shown that the emission of some specific VOCs are characteristic of microbacterial activity, for example emissions detected at mass 63 (dimethylsulphide and diethylsulphide) correlate with total number of aerobic bacteria and enterobacteriaceae respectively in air-packaged beef and ethanol is highly correlated with the bacterial counts of lactic acid bacteria in vacuum packed beef hence monitoring VOC emissions from the food provides a direct methodology for assessing bacterial activity. In contrast to technique of counting bacteria (requiring the incubation period of 1-3 days) detection of VOCs may be performed online and with rapid sampling rates. The use of a PTR-MS system provides an on-line measurement of VOCs with concentrations as low as a few parts per trillion in volume (pptv) and thus may detect bacterial activity when it is just beginning. Another advantage of PTR-MS is that the samples containing the volatile compounds do not need any preparation (pre-sampling, pre-concentration or sample dehydration) before being admitted to the PTR-MS. Thus some problems inherent to sampling in alternative methods used for VOC detection (e.g.: gas chromatography) are avoided, since the food itself is not disturbed and the measured mass spectral profiles closely reflect genuine headspace distributions (Yeretzian *et al.*, 2002). The PTR-MS system and measuring procedure has been described in detail in Refs. (Lindinger *et al.*, 1998) and (Hansel *et al.*, 1995).

Objectives

The purpose of the study was to investigate if ozone treatment affects microbial spoilage of pork.

Materials and methods

Two sets of measurements were performed six months apart. In each case retailed pork cutlets that were air packaged in an oxygen-permeable polyethylene film were bought in a supermarket in Innsbruck on the day when the respective measurements were started. Their expiry date was listed as two (first set of measurements, i.e. Experiment (Exp) 1 and 2) and three (second set, i.e. Exp 3) days after purchase.
Pieces of about the same shape (approx. 35x50x10mm), weight and consistency were cut out of a single cutlet for Exp 1-3, respectively. Each sample was placed into a glass flask (volume V=300ml) with a metal cover containing two gas inlets through which gas could be passed over the meat sample. The control samples were flushed with oxygen/air the others exposed to different concentrations of ozone (see table 1) for ten minutes. All the treated flasks were subsequently stored under identical conditions at room temperature. Measurements of the VOC emissions from the samples were then made at regular intervals over the period of several hours (see table 1). Headspace air was drawn through a heated teflon transfer line into the PTR-MS system for on-line VOC analysis. The mass spectrometric data being collected over a range of masses (m) with m/z=20–150 amu, where z is the charge of the measured ions (in our case z=1), different m being characteristic of different VOCs - in turn a monitor of different microbacterial processes.

Results and discussion

The effect of ozone treatment on the pork's decay behaviour was monitored through the observation of the concentration detected at mass 63 assumed to be dimethylsulphide (DMS) as this signal has been shown to have the largest correlation (up to 99%) with the bacterial contamination of meat (Mayr et al., 2003).
Figure 1 shows the results of the Exp 1-3. After a certain time lag the DMS signal detected from the oxygen treated sample in Exp 1 strongly increased with time whereas the low dose ozone treated sample showed only a slight increase, and the signal of the high dose treated pork piece remained almost constant. The same emission behaviour was found for the first part (t=0-30h) of Exp 2. However, the oxygen treated sample was exposed to a high dose of ozone at t=30h and the DMS concentration was found to strongly decrease, indeed, it took about 9 hours until the initial concentration was reached again. Comparing the results of Exp 1 and 2 one can see the strong influence of the additional ozone treatment on the emissions of the oxygen treated pork samples. The DMS concentrations of the both oxygen treated samples in Exp 1 and 2 were similar before the exposure to ozone at t=30 h. In Exp 1 signals from the non ozone treated sample reached a concentration of $1.3x10^3$ppb at the end of the measurements (t=46h, Fig. 1) whereas in Exp 2 the DMS concentration of the ozone treated sample was only 90ppb at t=46h. The online monitoring in Exp 2 was concluded at t=100h (not shown in Fig.1). The highest DMS signal (with a concentration of 300ppb) of ozone treated meat was reached at t=68h and stayed constant for six hours and was much lower than the highest measured DMS signals from the non ozone treated samples in Exp 1. The trends seen in the first two experiments were confirmed by the results of Exp 3. The DMS signal of the untreated and oxygen treated samples strongly increased with time – less strongly for the oxygen treated pieces. The oxygen treated samples were exposed to ozone after 42 hours with a subsequent decrease in the detected DMS signal and the concentrations remained low until the end of the experiment. The highest ozone exposure resulted in the detected DMS signal showing nearly no increase during the whole measurement time.
The microbiological analysis revealed that microbial counts were very high, independent of the treatment (Mayr *et al.,* submitted).

Conclusions

In the present work we have shown the strong effect of ozone exposure on pork cutlet's emissions, which have been found earlier to be highly correlated to the bacterial contamination, suggesting its usefulness as a remedial action for microbial spoilage to extend food shelf life. Even a later treatment with ozone strongly delayed the bacterial activity. The reduction of VOCs on one hand and the high microbial counts on the other hand indicate that the treatments applied in this study were effective to inhibit and thus reduce physiological activities, but are not necessary effective enough to produce a lethal effect on microorganisms present in meat. Further studies are needed to optimize the use of ozone in order to reduce microbial spoilage of meat.

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Web1: http://www.zentox.com/Ozofood.pdf

Web2: http://ip.cals.cornell.edu/courses/intag402/readings/processingtech.pdf

Web3: http://www.appliedozone.com

Web4: http://www.ecosensors.com/pg4_1applozonetnap_101.html

Web5: http://aggie-horticulture.tamu.edu/extension/newsletters/foodproc/dec01/rrart1.html

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		Treatment				
		untreated	O_2	O_2+high[a] O_3	low[b] O_3	high[a] O_3
Exp 1	no of samples	-	1	-	1	1
	analysis period (h)	-	47	-	47	47
Exp 2	no of samples	-	-	1	1	1
	analysis period (h)	-	-	100 (30)[c]	30	30
Exp 3	no of samples	2[d]	2[d]	2[d]	2[d]	2[d]
	analysis period (h)	46	46	49 (42)[c]	46	46

[a] 1000ppm [b] 100ppm [c] Ozone treatment after (x) hours [d] microbiological analyzed

Table 1: Samples were cut out of a single pork cutlet for Experiment (Exp) 1-3 respectively, treated in the different ways for 10 min and stored under identical conditions at room temperature. The emissions were regularly measured over the given time period. A microbiological analysis was performed for the samples of Exp 3 at the end of the analysis period.

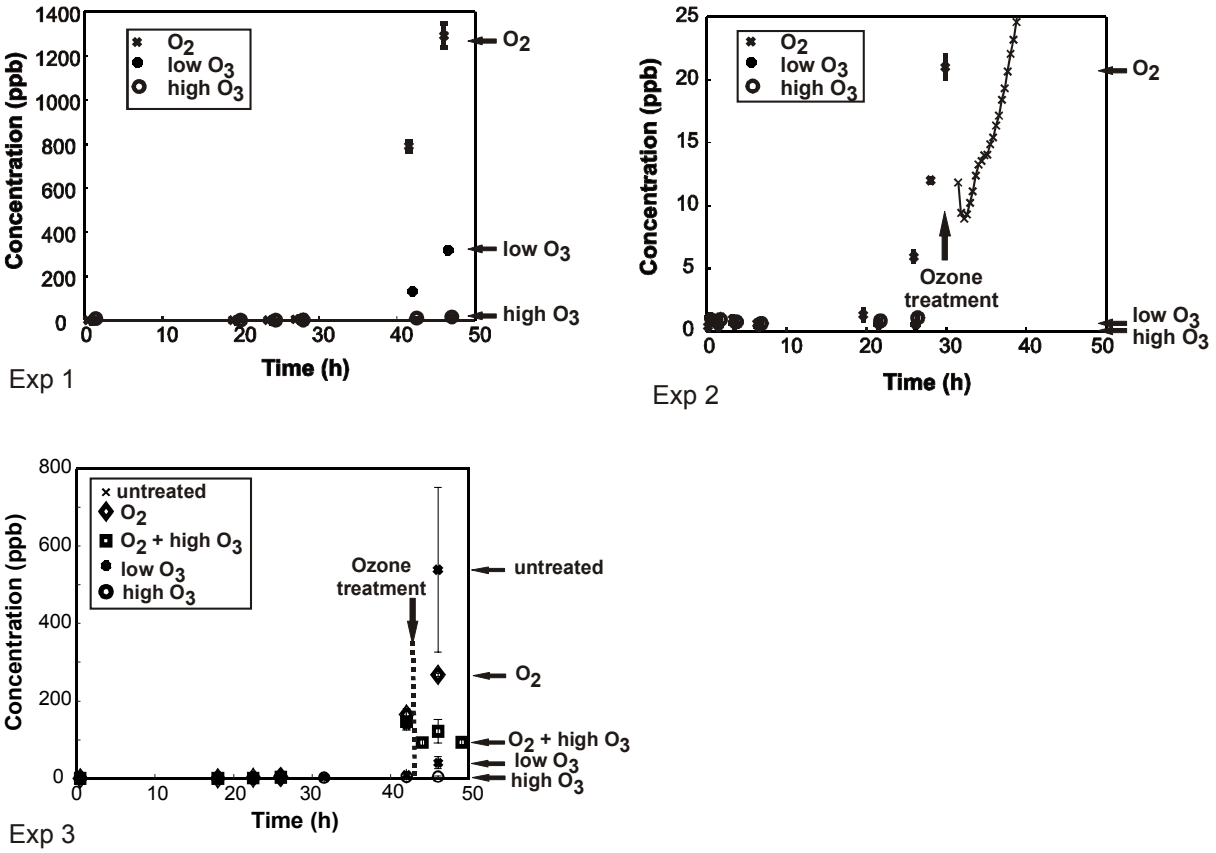

Fig. 1: Concentrations detected by PTR-MS at mass 63 as a function of time emitted by pork samples that were differently treated for 10 min prior to the first measurement at time t=0. These results suggest ozone significantly retards microbial spoilage.

APPLICATION OF LACTATE AND DIACETATE TO IMPROVE THE SHELF-LIFE OF THE BLOOD SAUSAGE "MORCILLA DE BURGOS"

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Background

Morcilla de Burgos is a typical blood sausage from Spain. It is made of a mixture of onion, rice, animal fat (mainly lard), blood and spices. All these ingredients are mixed and stuffed in natural casings and then cooked in water at 94 ºC during 45-60 min, after this cooking step they are air cooled at room temperature. *Morcilla* is sold without packaging in local markets and vacuum packaged in retailing shops. Preservation of *morcilla* is quite difficult, because it is a product with a high water activity (0.984), moisture (around 60%) and pH (up to 6.1) together with a rich nutrient composition (Santos et al., 2003). Vacuum packaged *morcilla* usually lasts around 21 to 30 days depending on the initial contamination and the storage conditions. In general, the spoilage of *Morcilla de Burgos* is due to the growth of heterofermentative lactic acid bacteria that produce changes in the coloration of the casing from black to pink or greenish, blowing of packages and forming ropy slime.
Sodium lactate has been used for several years, in the meat industry because of capability to increase flavour, shelf life and increase the microbiological safety of these products. The antimicrobial effects of lactate are due to their water activity lowering ability and the inhibitory effect of the lactate ion (Koos, 1992; Houstma, 1993). In that sense, they have been successfully applied in the extension of the shelf life of cooked ham by reducing the counts of psychrotrophs, faecal streptococci and enterobacteria and reducing as well the formation of ropy slime (Rondinini et al., 1996). Apart from this role in the extension of the shelf life of meat products, lactate plays and important role inhibiting the growth of foodborne pathogens as *E. coli* O157:H7, *B. cereus*, *L. monocytogenes*, *Clostridium botulinum*, *Staphylococcus aureus*, *Y. enterocolitica* and so on (Bolton, 1998; Shelef, 1994; Miller and Acuff, 1994). One of the problems derivate of using sodium lactate is the salty taste, when it is used in high concentration (> 3%). This problem may be overcome using other lactate derivates as potassium lactate, and adjusting the salt content in the product (Wilmink, 2000). Recent studies have shown the synergic effect of using together lactate derivates and diacetate, against several foodborne pathogens (Mbandi and Shelef, 2001; Glass et al., 2002).

Objectives

The aim of this work was to investigate the effect of several lactate derivates, and the combination of lactate and diacetate, on the extension of the shelf life and microbial quality of the blood sausage *morcilla de Burgos*. The improvement of the shelf life of this product is very important in order to expand the selling market, because *morcilla de Burgos* has a very popular market in Spain as a "tapa". It is also very important to prove that the use of these substances as preservatives do not decrease the sensory properties of the final product.

Materials and methods

Samples: Two trails of four batches of 25 K of *morcilla* with the same composition were elaborated in a typical *morcilla* factory. In batch A we added 3% potassium lactate (Purasal P Hipure. Purac.); in batch B 3% mixture of sodium lactate and potassium lactate (Purasal LITE. Purac.); batch C was considered as control and no lactate derivates was added; and finally batch D was elaborated with 2,5% of a mixture of potassium lactate and diacetate (Purasal P Optiform. Purac.). After the mixtures were made, they were stuffed in beef natural casings and open air cooked at 94ºC during almost 1 hour. After cooking step they were air cooled before being individually vacuum packaged. In that moment, they were brought to the lab in ice boxes to keep them at cool temperature. Packages were kept in a dark place at 4ºC for 32 days, and two packages of each batch were analysed at 0, 5, 10, 15, 20, 27 and 32 days.

Microbial analysis: A slice of 25 g of *morcilla* were sterile weighted, diluted in 225 ml of Ringer solution (Oxoid. Basingstoke, UK), and homogenised before preparing 1/10 serial dilutions. According to the results obtained in a previous work (Santos, 2001), six different microbiological parameters were chosen to evaluate the effectiveness of the treatments:
Total Viable Count plated on PCA agar plates (Oxoid) and incubated at 30ºC during 48 hours.
Enterobacteria were tested in VRBGA agar (Oxoid) incubated at 30ºC during 48 hours.
Pseudomonads were plated on Pseudomonads agar (Oxoid) supplemented with CFC (Cetrimide, Fucidine, Cefaloridine, Oxoid) at 30ºC during 48 hours.
Lactic acid bacteria (LAB) were grown in MRS agar (Biokar Diagnostics, Beauvais, France) and incubated at 30ºC for 48 hours in anaerobic conditions.
Pyschrotrophs were deep plated on PCA agar and plates were incubated at 7ºC during 10 days.
Clostridium perfringens were tested inoculating 1ml of sample dilution in a tube with 20 ml of TSN agar (Tryptone Sulfite Neomycin, Biokar Diagnostics) and incubated at 45ºC during 24-48 hours, in anaerobic conditions. Black colonies were considered as positive.
After taking the sample for microbial analysis, pH was measured in four different points of the *morcilla*, with a penetrating probe.

Sensory analysis: to evaluate if the treatments proposed could introduce some sensory changes in the products, triangle and ranking test were carried out twice for each trial, using a consumer panel (n=30). *Morcilla* were cut in 1.5 cm slices width and microwave heated till 70ºC. Triangle test were only made to evaluate differences between treatments C (control) and D (lactate-K + diacetate), because we thought we obtained the best results with treatment D due to the synergic effect between both preservative substances, according with the literature. The results of this test were analysed in the correspondent tables. Ranking test was made with all four batches of *morcilla* in the same panel session after the triangle test, to evaluate the preference between all four batches. The results were analysed with the aid of Kramer's tables and Friedman test.

Results and discussion

The evolution of pH and the microbiological results during the storage time are shown in table 1. It is possible to observe that pH drops more quickly in batch C than in the others and in 27 days of storage the pH is below 5.00, while in the other batches it keeps above this value. This fact was easily observed; when samples from batch C were opened due to the penetrating sour smell they produce.
On day 0, the same day that *morcilla* were made and packaged, only some colonies grew on PCA agar (TVC). These colonies were irregular in shape and some of them were mucous and they were associated to members of the sporulating genera *Bacillus*, because there are some vegetable ingredients in the composition of *morcilla de Burgos* than can harbour these bacteria, and because after the cooking step it seems that only sporulating bacteria can survive. In the remaining parameters no growth were detected for this sampling day. These results are in accordance with the results obtained by Santos (2001) and Borek et al. (2002) in our lab with same product.
Remarkable rapid growth of bacteria in the product was observed in the first five days, especially in TVC, LAB and Psychrothrophs. This growth could be the quick development of LAB that had favourable conditions to grow. In fact, it is possible to observe that counts of TVC, LAB and Psychrotrophs are quite similar from day 5 to the end of the storage time. Santos described in previous works (2001) the same situation in this product, as well as the static evolution of enterobacteria and pseudomonads during all the storage time. The presence of these microbial populations that appears 24 hours after packaging is due to the post-cooking contamination in the cooling and packaging steps (Santos, 2001; Borek et al., 2002).
LAB is the spoilage dominating population, especially members of the genera *Leuconstoc* and *Weisella* (Santos, 2001). It is in this microbial group where the effects of the different treatments were more significant. In batch C *morcilla*, the spoilage came about around the days 20-25 of the storage time, while in the other treatments spoilage took place later, especially in *morcilla* of batch B; in that case there was a difference of almost two logs in TVC at the end of the storage time. Less effective seems to be the combination of potassium lactate and diacetate. A similar evolution can be seen for psychrotrophs. No growth in TSN agar was detected.

Triangle test done on the second and 7 day of storage showed only a significant difference ($p<0.05$) in one of the four test performed between *morcilla* of batch C and D. Regarding to the ranking test no significant differences were detected on the test days with both statistical methods (Kramer and Friedman).

Conclusions

According to the results found in this study, it seems that the application of a combination of potasium lactate and sodium lactate may have a possitive effect, in the extension of the shelf life of *morcilla de Burgos* this effect could be around 12 days, from 20 to 32 days. Besides this combination does not seem to affect the sensory properties of the product. Something similar happens with treatment A (potassium lactate), however the combination of potassium lactate and diacetate seems to have no effect in improving the shelf life of *morcilla de Burgos*. New studies shoud be done to prove the effectivines of lactate derivates on foodborne pathogens in this product.

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Acknowledgments

We want to express our thanks to the Spanish distributing company Mercadona S.A. for supporting this project, and to the factory Morcillas de Cardeña, where the products were elaborated.

Tables

Table1. Evolution of the main microbial populations in vacuum packaged *morcilla de Burgos* treated with lactate derivates and stored at 4ºC. (log cfu/g).

	Days						
	0	**5**	**10**	**15**	**20**	**27**	**32**
pH							
A	6.39	6.36	5.99	5.92	5.63	5.27	5.08
B	6.35	6.41	6.26	6.10	6.28	5.48	5.23
C	6.33	6.20	5.62	5.60	5.27	4.80	4.82
D	6.12	6.14	5.81	5.75	5.55	5.22	5.01
Total Viable Count							
A	4.30	7.50	8.10	8.15	8.30	9.60	8.50
B	4.16	6.80	7.60	8.00	7.50	9.60	8.70
C	4.30	7.90	8.40	8.40	8.50	8.90	9.60
D	4.25	7.00	8.40	8.20	8.25	8.30	9.70
Enterobacteria							
A	-	2.90	4.00	3.80	3.70	2.20	2.00
B	-	2.70	3.00	4.40	4.80	4.20	3.20
C	-	3.40	4.00	4.60	5.10	5.20	3.80
D	-	3.00	3.20	3.40	3.80	4.00	3.00
Pseudomonads							
A	-	2.00	3.70	4.00	4.30	3.40	3.30
B	-	2.40	4.20	4.90	5.00	3.20	3.20
C	-	4.00	4.30	4.40	4.30	3.50	3.20
D	-	-	-	-	-	3.00	3.00
Lactic Acid Bacteria							
A	-	5.00	6.30	6.65	7.70	6.65	6.54
B	-	4.30	5.70	6.54	6.60	6.74	6.16
C	-	6.20	6.60	7.30	7.20	7.54	8.41
D	-	4.30	6.30	7.30	7.95	7.80	7.66
Psychrotrophs							
A	-	5.00	6.30	6.50	6.40	6.20	6.50
B	-	5.15	6.87	6.20	6.50	6.80	7.00
C	-	6.00	8.00	7.82	8.20	8.00	8.00
D	-	6.40	6.30	7.26	7.00	7.00	7.70

A: 3% of lactate-K
B: 3% of lactate-Na + lactate-K
C: Control
D: 2.5% of lactate-K + diactetate
-: no growth detected

EFFECTS OF HOT WATER AND LACTIC ACID TREATMENT OF PORK CARCASS PRIOR TO COOLING ON MICROBIAL AND ULTIMATE PORK QUALITY

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Background

Prevention of microbial contamination during slaughtering and processing is one of the most critical safety issues of pork. Although the process carried out under ideal conditions, the pork from normal and healthy pigs could present opportunities for contamination with a variety of bacteria including some pathogens. Several intervention strategies have been developed to reduce bacterial numbers on pork carcass surface such as washing with hot water, chlorinated water, organic acids, alone and in combination. The combination of hot water and lactic acid treatment seemed to be the most effective for reducing microbial of beef (Gill and Landers, 2003), pork (Gordon and Bryan, 1995), chicken (Moon et al., 2004) and lamb (James et al., 2000). However, despite the effects of hot temperature organic acids on reducing pathogens, it would be expected to have some deleterious effects on meat quality. Especially pork quality could be affected by hot temperature treatment sensitively, but the information is limited.

Objectives

The effects of hot water and lactic acid treatment on microbial counts of eviscerated pig carcasses prior to cooling and ultimate pork quality at postmortem 24 hrs were investigated.

Materials and methods

Twenty pigs were slaughtered and split. Hot carcasses sides were inoculated with bacterial cocktail by pouring 500 ml of a peptone saline containing E. coli, Salmonella and Listeria. Inoculums were prepared from frozen (-20°C) stock cultures of *Escherichia coli* (ATCC #25922), *Salmonella typhimurium* (ATCC #14028) and *Listeria monocytogenes* (KCTC # 3569). Frozen cultures of *E. coli, S. typhimurium* and *L. monocytogenes* were thawed, and bacteria suspension was inoculated into separate of TSB. After 16 h of incubation at 37°C, suspended in the same volume of 1ml and then pooled together (1,000 ml of *E. coli,* 1,000 ml of *S. typhimurium* and 1,000ml of *L. monocytogenes*) to make a bacterial cocktail. The cocktail (For each specie in 3,000ml, 10^5 or 10^6 CFU/ml) was cooled to 4°C and used to pour upon carcasses. Carcasses were spray washed with hot water and 2% lactic acid at 80°C from a spray nozzle for 10, 20, 30 s, while for the cold spray treatment, the temperature was 15°C and the exposure time 30 s. After spray washing, sampling of the outside of carcasses for bacteriological examination was carried out by swab method. The swab sampling included four sites of the abdominal cavity and three sites of the back. After cooling of the carcasses for 24 hrs at cold room, pork loins were sampled to measure meat color and water-holding capacity. Meat color was measured by using a Minolta Chromameter CR-301, and water holding capacity was calculated by % drip loss (Joo et al., 1999). The data were analysed using statistical analysis systems (SAS. 1999). To evaluate the differences among treatments, data were analysed by analysis of variance and Duncun's multiple range test.

Results and discussion

As expected, total bacterial numbers were significantly reduced by spraying with hot water and 2% lactic acid, but not effectively reduced with spraying of cold lactic acid for 30 s (Table 1). Total plate count (TPC) showed about a 2.79 log unit reduction as a result of hot water spray while about a 1.57 log unit reduction was observed with spraying of cold lactic acid. TPC of pork carcass surface was completely removed by spray washing with 2% lactic acid at 80°C for 30 s. Moreover only 20 s treatment of 2% lactic acid at 80°C also effectively reduced all pathogens. This could be achieved by treatment with hot water for 30 s too. However, spray washing of 2% lactic acid at 15°C for 30 s did not completely eliminate *E. coli, S. typhimurium* and *L. monocytogenes*. Results suggested that a temperature of washing material was more effective in reducing the numbers of pathogens than a kind of washing material. Although spray washing for 30 s with hot temperature water and lactic acid completely eliminated microbial on the surface of carcasses,

pork loin showed undesirable color and water-holding capacity at 24 hrs postmortem (Table 2). The lightness values of pork loin treated hot water and lactic acid were significantly higher than those of cold treatments. Also drip loss % was significantly increased in loins from carcasses that spray washed with water and 2% lactic acid at 80°C for 30 s. After spray washing with hot water for 30 s, pork loins showed pale color, soft texture and exudative surface of meat. The PSE condition of pork loin was probably due to denaturation of proteins by hot temperature spray washing. However, pork loin treated with hot water for 20 s had a normal color and WHC. These results suggested that spray washing with 2% lactic acid at 80°C for 20 s would be the most effective for reducing bacterial numbers on the surface of pork carcass without deterioration of ultimate pork quality.

Conclusions

Spray washing of pork carcass with 2% lactic acid at 80°C for 20 s prior to cooling is the most effective way to reduce microbial including pathogens without any deterioration of pork quality in Korea.

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Table 1. Effects of spray washing with hot water and 2% lactic acid on reducing of microbial numbers

Treatments	Spray washing times (seconds)		
	10	20	30
		Total plate count (log CFU/cm^2)	
Water 15℃	-	-	3.49
80℃	2.57	1.97	0.70
Lactic acid 15℃	2.97	2.32	1.92
80℃	2.08	0.90	0
		E. coli (log CFU/cm^2)	
Water 15℃		-	2.32
80℃	1.99	0.43	0
Lactic acid 15℃	2.04	1.81	1.23
80℃	0.99	0	0
		S. typhimurium (log CFU/cm^2)	
Water 15℃	-	-	2.69
80℃	2.04	1.40	0
Lactic acid 15℃	2.26	1.90	0.60
80℃	1.04	0	0
		L. monocytogenes (log CFU/cm^2)	
Water 15℃	-	-	2.36
80℃	1.92	0.23	0
Lactic acid 15℃	2.08	0.99	0.78
80℃	0.97	0	0

Table 2. Effects of spray washing with hot water and 2% lactic acid on color and water-holding capacity of pork loin

Measurements	Treatments	Spray washing times (seconds)		
		10	20	30
Color (CIE L*)	Water 15℃	-	-	45.41^{B}
	80℃	46.45^{c}	50.97b^{A}	54.85^{aA}
	Lactic acid 15℃	43.39	44.38^{B}	44.42^{B}
	80℃	45.61^{b}	48.18^{bA}	57.92^{aA}
Water-holding capacity (% Drip loss)	Water 15℃	-	-	2.34^{B}
	80℃	2.82^{b}	4.92^{aA}	4.94^{aA}
	Lactic acid 15℃	2.81	2.79^{B}	2.85^{B}
	80℃	2.87^{b}	4.98^{aA}	5.28^{aA}

^{A,B} Means with different superscript in the same column are significantly different ($P<0.05$)
^{a,b} Means with different superscript in the same row are significantly different ($P<0.05$)

LETHAL EFFECTS OF NON-DEHEATED (HOT) MUSTARD FLOUR ON *E. COLI* O157:H7 IN REFRIGERATED NITROGEN PACKAGED GROUND BEEF

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Background

The incidence of *E. coli* O157:H7 which was first reported to cause hemorrhagic colitis and hemolytic uremic syndrome two decades ago (Riley et al. 1983) has been increasing year after year. Consumption of undercooked ground beef has been a common cause of *E. coli* O157:H7 outbreaks (hamburger disease) in North America. United States and Canada have the highest frequencies of foodborne illness caused by *E. coli* O157:H7 in the world. *E. coli* O157:H7 is a "zero-tolerated" pathogen in beef in the U.S., and as a result thousands of tons of ground beef are recalled every year for possible *E. coli* O157:H7 contamination. This has led to considerable financial loss for the meat industry. Canada is the single largest mustard exporter in the world and is one of the world's top five mustard producers. Mustard has been used as a spice or a condiment for centuries throughout the world. Mustard flours when deheated are used as emulsifiers, binders and bulking agents, but non-deheated mustard is used as a spice in seasonings and for flavouring in meat formulations, particularly in sausage and salami manufacture (Cui, 1997). Brown or oriental mustard (*Brassica juncea*) and yellow or white mustard (*Sinapis alba*) contain glucosinolates, which on enzymatic breakdown by myrosinase yield isothiocyanates, cyanides and thiocyanates (Tsao et al. 2002). The heat or flavour of the mustard is due to the presence of isothiocyanates and when used as binders the flour is treated to inactivate the myrosinase. Allyl isothiocyanate, one of the predominant breakdown products of glucosinolates, has been reported to have antimicrobial effects against bacteria including *E. coli* O157:H7 in ground beef, pre-cooked roast beef, alfalfa seeds, lettuce, and 'Asazuke' (a low salt vegetable) (Muthukumarasamy et al. 2003; Ward et al. 1998; Park et al. 2000; Lin et al. 2000; Ogawa et al. 2000). Although pure AIT from natural sources can be used as a food preservative in Japan, its use in food is restricted as a flavouring agent in North America. Moreover, use of pure AIT in food has limitations because of the strong pungent smell associated with volatile AIT which causes eye and nasal irritation and a burning sensation of the skin and tongue. Mustard flour, being a natural source of AIT, may be used as an alternative approach in food systems to eliminate *E. coli* O157:H7.

Objectives

The objective of the research was to study the lethality of mustard flour toward *E. coli* O157:H7 in refrigerated nitrogen packed ground beef. The effect of deheated mustard, which is incapable of producing isothiocyanates due to enzyme inactivation, was compared with non-deheated mustard to understand if isothiocyanates from the flour were responsible for lethal effects on *E. coli* O157:H7. The effect of pure allyl isothiocyanate, on *E. coli* O157:H7 was also studied.

Materials and methods

Five strains of *E. coli* O157:H7, 7128, 7110, 7236 (human isolates) and 7282 and 7283 (hamburger isolates) were kindly donated by Dr. R. Khakria, Laboratory Center for Disease Control, Ottawa, Canada. *E. coli* O157:H7 strains were maintained in tryptic soy broth at 37º C. Inside round beef roasts were obtained from a local supermarket and aseptically ground manually in a meat grinder (9.5 mm plate). The five strain cocktail of *E. coli* O157:H7 prepared in peptone water was inoculated in the meat to reach a level of 3 log CFU/g. Deheated and non-deheated mustard flour at 10 and 20% were added to the ground beef and re-ground to evenly mix the mustard flour. Twenty five grams of meat were placed in Deli*1 bags (Winpak, Winnipeg, Canada), a vacuum was generated, back flushed with nitrogen and the bags were sealed. Samples to study the effect of AIT were prepared by adding 1 ml of pure allyl isothiocyanate (Acros Organics, Geel, Belgium) and a commercial corn oil mixture (AIT: corn oil at a ratio of 7:3) to sterile filter paper inserts and one insert was placed in each bag containing 25g of *E. coli* O157:H7 - inoculated beef before packaging. The packaged ground beef was stored at 4º C. Triplicate samples for each treatment were analysed for *E. coli* O157:H7 and

total bacterial numbers from 0 to 18d at 3d intervals. Eleven grams of beef were mixed with 99ml of peptone water and stomached for 1 min. Serial dilutions were prepared in peptone water and plated with a spiral plater. Sorbitol MacConkey agar supplemented with cefixime-tellurite (CT-SMAC) and tryptic soy agar (TSA) were used for enumerating *E. coli* O157:H7 and total bacteria, respectively. The plates were incubated at 37º C aerobically for 24 and 48h for CT-SMAC and TSA, respectively. In order to determine if the *E. coli* O157:H7 cells were killed or injured as a result of mustard flour or AIT treatment, a resuscitation step on TSA (incubated at 37º C for 3h) was used followed by over-laying with CT-SMAC agar. Survivors on the TSA over-laid CT-SMAC plates indicated recovery of injured *E. coli* O157:H7 cells. All data were analysed by Statistical Analysis System (version 8.1) software. Analysis of variance by the General Linear models procedure and Duncan's multiple range tests were used to find significant differences ($P< 0.05$) among and between treatments.

Results and discussion

Bactericidal effects on *E. coli* O157:H7

The effect of AIT, deheated and non-deheated mustard flour on the viability of *E. coli* O157:H7 in ground beef stored under nitrogen at 4º C are shown in Fig 1. Non-deheated mustard at 20% was most effective in killing *E. coli* O157:H7 compared to deheated mustard at both 10 and 20% or AIT at ca. 1300 ppm. Non-deheated mustard at 20% completely eliminated *E. coli* O157:H7 at an initial level of 3 log CFU/g from ground beef by day 3, whereas AIT at ca. 1300 ppm and non-deheated mustard at 10% required 15 and 18 d respectively to reduce *E. coli* O157:H7 below detectable levels (40 CFU/g). Deheated mustard at both levels did not completely kill *E. coli* O157:H7 in ground beef stored under nitrogen at 4º C even after 18 days. On TSA over-laid with CT-SMAC plates, no recovery of injured cells occurred, indicating that *E. coli* O157:H7 were killed in treatments where no *E. coli* O157:H7 grew on CT-SMAC plates.

The results presented clearly show that non-deheated mustard flour at 20% had strong bactericidal effects against *E. coli* O157:H7 in ground beef stored under nitrogen at 4º C. Non-deheated mustard flour at 10% and AIT were able to completely eliminate *E. coli* O157:H7 but required significantly longer than 20% non-deheated mustard flour. The only difference between the two types of mustard flour used was the presence of active myrosinase in the non-deheated product. Lethal effects of the latter flour were attributed to myrosinase action, causing formation of isothiocyanates in the presence of moisture from the meat. Deheated mustard, with inactive myrosinase, was not significantly lethal to *E. coli* O157:H7. Allyl isothiocyanate is the main component of mustard that might be responsible for bactericidal effects (Isshiki et al. 1992). The average allyl isothiocyanate levels in mustard ranges from 7 to 13 mg/g. The results showed that non-deheated mustard flour at 20% was more effective than AIT (ca. 1300 ppm) in killing *E. coli* O157:H7. The increased lethality of mustard flour may be due to synergistic effects of AIT with other isothiocyanates present in mustard. The bactericidal activity of mustard flour may provide an opportunity for its use in meat and more widely in the food industry as a secondary preservative to control pathogenic microorganisms. This should provide added assurance of safer food for consumers.

Effect on total bacterial numbers

The effect of AIT, deheated and non-deheated mustard flour on the total bacterial numbers in ground beef stored under nitrogen at 4º C is shown in Fig 2. All treatments significantly delayed the growth of total aerobic mesophiles in ground beef stored at 4º C. In the untreated control the total bacterial count was 6.88 log CFU/g by 18d, indicating that the beef could be close to spoilage if untreated with any antimicrobials. Non-deheated mustard flour at 20% and AIT were able to maintain the total bacterial numbers at < 4 log CFU/g by the end of 18d.

Conclusions

Non-deheated mustard flour at 20% was more lethal than deheated flour to *E. coli* O157:H7 in ground beef. Non-deheated mustard flour may be used as a natural antimicrobial in ground beef to eliminate *E. coli* O157:H7 and has potential for similar use in other susceptible foods where mustard flavour is compatible. The use of mustard flour may not only increase the safety of ground beef but also extend its shelf life.

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Fig 1: Viability of *E. coli* O157:H7 in ground beef treated with AIT, deheated and non-deheated mustard flour, stored at 4º C under nitrogen packaging.

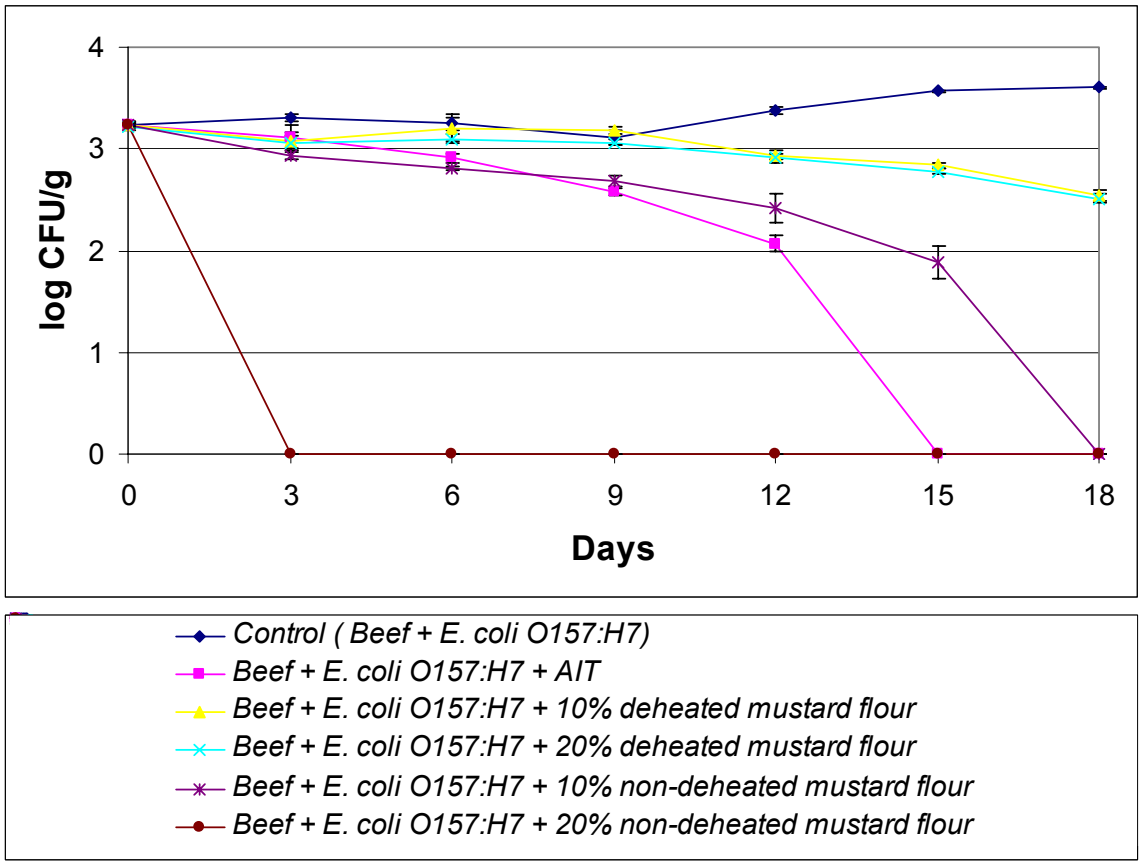
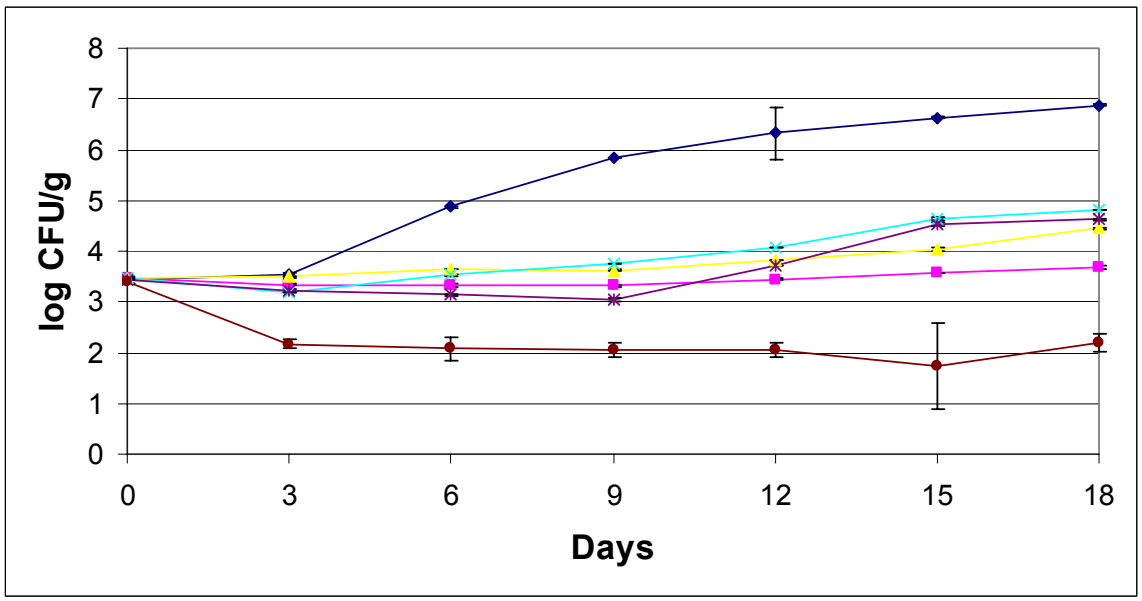


Fig 2: Changes in total number of viable bacteria in ground beef stored at 4º C under nitogen packaging after treatment with AIT, deheated and non-deheated mustard flour.



SHELF LIFE OF MEAT FROM PODOLIAN YOUNG BULLS IN RELATION TO THE AGEING METHOD

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Background

During production, processing, distribution and storage, meat undergoes deterioration from chemical and microbial processes. Meat shelf life is an important parameter since consumers discriminate against meat cuts that have lost their fresh appearance and meat that becomes discoloured is often ground and marketed in reduced-value form. Lipid oxidation is one of the primary mechanism of quality deterioration in meat during display and it is directly related to the formation of precursors of oxymyoglobin oxidation and metmyoglobin (Insausti et al., 2001).
Ageing is an important factor influencing the final perception of meat quality and tenderness is the main parameter affected by this technological factor (Campo et al., 1999). This is particularly relevant for the improvement of meat properties of local breed such as Podolian, which is known to be tasty albeit though (Girolami et al., 1986). Previous works showed significant tenderness enhancement of Podolian meat extending maturation processes (Braghieri et al., 2002; Cifuni et al., 2004).

Objectives

Holding carcasses for prolonged time during ageing may produce chilling and technological losses. Ageing individually the various commercial cuts in vacuum packaging may represent a chance to improve tenderness avoiding this problem. On the other hand Lanari et al. (1989) found that beef aged in vacuum packaging may be darker in colour when removed from package due to the lack of oxygen. Shelf life could be also affected by the ageing method. This study aims to assess the effect of the ageing method (on the carcass or under vacuum packaging) on physical and sensory properties of Podolian meat. Meat shelf life under retail display in relation to the aging method was also evaluated.

Materials and methods

Eight Podolian young bulls, reared according to the traditional local practices (for the first 8 months at pasture and for the following 10 in loose housing conditions with free access to an outside paddock), were used. All the animals were slaughtered at 16÷18 months of age. Mean slaughter weight and dressing percentage were 237 kg and 53%, respectively. *Longissimus dorsi* (LD) and *Semitendinosus* (ST) muscles were removed from the right carcass side 48 h *postmortem*, vacuum-packaged and aged for 5 days at 4°C. The same muscles were taken from the left carcass side previously stored for 7 days at 4°C. Muscles were sliced (2.5 cm thickness), then placed on a polystyrene tray, wrapped in a polyvinylchloride film and displayed for five days at 2°C under 8 h illumination from cool white fluorescent lights (350 lux).
Lipid oxidation was determined by measuring 2-thiobarbituric acid reactive substances (TBARS), according to the method of Salih et al. (1987), at days 0, 2 and 4 and was expressed as mg malondialdehyde (MDA) produced per kg muscle.
Water loss was estimated by weighing the empty pack (Wp) and the pack with meat (Wp+m) on day 0. After storage the meat was removed from the pack and the weight of the pack plus the juice (Wp+j) was recorded. Water loss was expressed as a percentage of the initial weight of the meat (Insausti et al., 2001):

$$\% \text{ water loss: } \frac{(Wp+j) - (Wp)}{(Wp+m) - (Wp)} \times 100$$

CIE L*, a*, b* were measured at day 0, 1, 2, 3, 4 using a Minolta Chromameter (Minolta Co., Ltd, Osaka, Japan).
Microbial count was performed at day 0, 2 and 4 for total viable count (TVC) on Plate Count Agar (Difco) at 30°C for 72 h and *Lactobacillus* spp. on MRS Agar (Difco) at 37°C in anaerobic environment for 72 h.

Warner-Bratzler shear force was measured on LD and ST cores (1.27 cm diameter), cut parallel to the direction of the muscle fibres and sheared by an Instron Universal testing machine (model 1140), equipped with a Warner-Bratzler shearing device.
The sensory analysis (flavour and tenderness) was performed by a trained eight-member panel on LD and ST steaks grilled to an internal temperature of 75°C. Sensory values were normalised standardising each assessor by his standard deviation in order to reduce the effect of the different use of the scale (Cifuni et al., 2004). Data were subjected to analysis of variance for repeated measures with muscle, ageing technique and the interaction muscle x ageing method as repeated factors.

Results and discussion

The ageing method did not markedly affect colour parameters (Fig.1). Conversely other studies (e.g. Boakye and Mittal, 1996) observed that L index significantly increased in LD vacuum aged. In agreement with previous studies (Monin and Ouali, 1992; Torrescano et al., 2003), colour was significantly affected by muscle with higher L (36,32 ± 0,25 vs 36,32 ± 0,25; $P<0.001$) and lower a* (20,44 ± 0,18 vs 21,04 ± 0,18; $P<0.05$) values in ST compared with LD muscle. Muscle function is the most obvious factor accounting for colour differentiation between muscles according to the metabolic types. Intensity of meat colour depends on both the pigment level and surface structure (Monin and Ouali, 1992). Display time affected muscle colour (Fig.1). As also observed by Eikelenboom et al. (2000), for both muscles, redness a* value incresed after 1 day of display and subsequently decresed. The decrease in redness a* values may result from the gradual formation of metmyoglobin on the meat surface, because they have been reported to be negatively correlated (Insausti et al., 1999). It was noterworty that during the same time, there was an increase in TBARS value (Fig. 2). Previously, it was noted that a* redness was negatively correlated with lipid oxidation measured by TBARS values (Anton et al., 1993). According to Chan et al. (1997) the process of myglobin oxidation is a catlyst of lipid oxidation. Colour of fresh meat is a major factor affecting meat acceptability. In red meats, consumers relate the bright red colour to freshness, while discriminating against meat which has turned in brown (Zerby et al., 1999). Lipid oxidation, as indicated by MDA concentration, was not affected by the ageing method. One of the main factors limiting the quality and acceptability of meat products is lipid oxidation. This process leads to discolouration, drip losses, off-odour and off-flavour development and the production of potentially toxic compounds (Gray et al., 1996).
Lipid stability significantly varied between muscles, with higher MDA content in ST than in LD (0,072 ± 0,004 mg/kg meat vs. 0,048 ± 0,004 0,048 ± 0,004, respectively; $P<0.001$). The lower lipid stability observed in *Semitendinosus* muscle may be due to its higher polyunsaturated fatty acid content compared to *Longissimus dorsi* (Cifuni et al., 2004). However, for both muscles and during display the MDA content was well below the threshold value for rancidity of 1- 2 mg/kg of meat (Watts, 1962).
Microbial spoilage of food occurs when total aerobic counts reach 10^7 CFU/g (Nortje and Shaw, 1989). In the present work, TVC increased during display (Fig. 3), as previously reported by Zerby et al. (1999), but not to that level. Bacteria can produce colour changes in beef stored in air due to a reduction in the oxygen concentration at the surface tissue due to microbial respiration (Nortje and Shaw, 1989). TVC ($P<0.001$) and lactic bacteria ($P<0.01$) significantly differed in relation to ageing method with higher values in vacuum ageing method.
Water loss, an indicator of water holding capacity of muscle, may influence consumers choice, when purchasing packaged meat, as too much exudates around the meat is not appealing (Insausti et al., 2001). Drip loss may result from shrinkage of myofibrils *post mortem*, due to pH-fall, denaturation of myosin and formation of actomyosin at the onset of *rigor mortis* (Den Hertog-Meischke et al., 1997). In the present study neither ageing method nor muscle affected water loss (tab. 1).
Although Kannan et al. (2002) reported no effect of ageing method on WBS values of goat meat, in the present study meat aged under vacuum showed higher tenderness, as indicated by lower WBS value ($P<0.01$), compared with meat aged conventionally (tab. 1). No significant differences may be detected for sensory tenderness score between the two maturation method (tab. 1). As previously observed (Torrescano et al. 2003), shear force was significantly affected by muscle, with lower shear values muscle (2.00 ± 0.11 kg vs 2.63 ± 0.11 kg; $P<0.01$) and higher sensory tenderness score (5.90 ± 0.10 vs 5.62 ± 0.10; $P<0.05$) for LD compared with ST muscle. The ageing method and muscle had no effects on flavour of meat (tab. 1).

Conclusions

The ageing method did not markedly affect meat quality and shelf life. This result may encourage to employ vacuum packaging in the ageing of primal cuts from Podolian carcasses avoiding chilling and technological losses that generally occur in traditional maturation method.

Acknowledgments

The research was funded by POP FESR 1999. The authors are grateful to Dr. M. Palazzo, Istituto Zooprofilattico Sperimentale della Puglia e della Basilicata, Potenza for her technical assistance.

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Table 1. Meat characteristics: effect of ageing method (mean ± SE)

	V	C	P
Flavour	6.33 ± 0.09	6.10 ± 0.09	**
Tenderness	5.77 ± 0.10	5.75 ± 0.10	NS
WBS (kg)	2.03 ± 0.11	2.61 ± 0.10	**
Water loss (%)	0.47 ± 0.05	0.50 ± 0.05	NS

Fig. 1. Redness (a*) of beef during meat display at 2°C

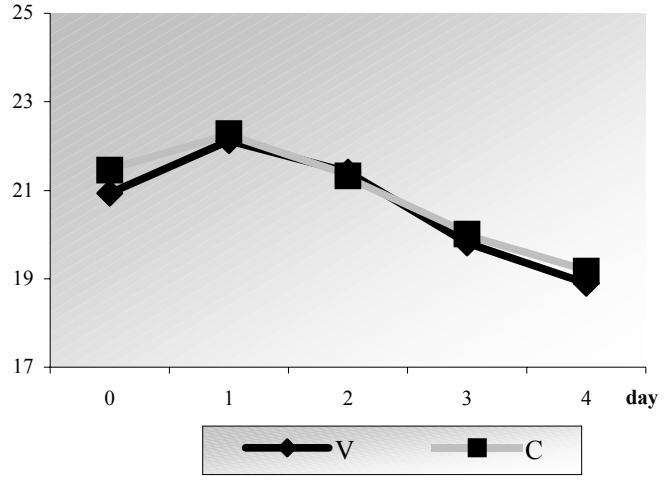


Fig. 2. Levels of 2-TBA values (mg MDA/kg product) during meat display at 2°C

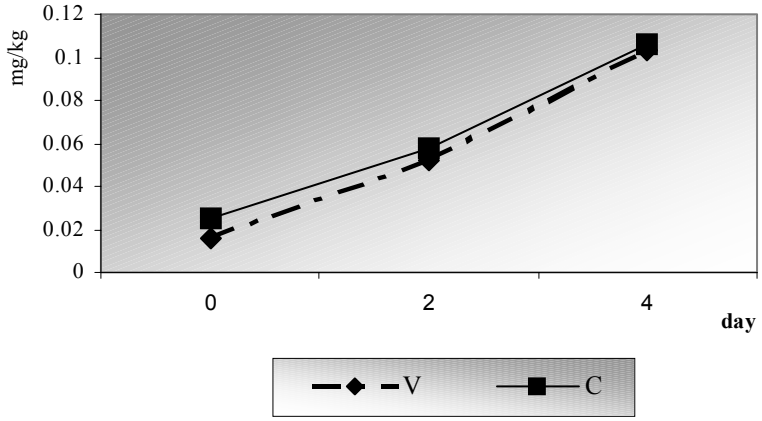
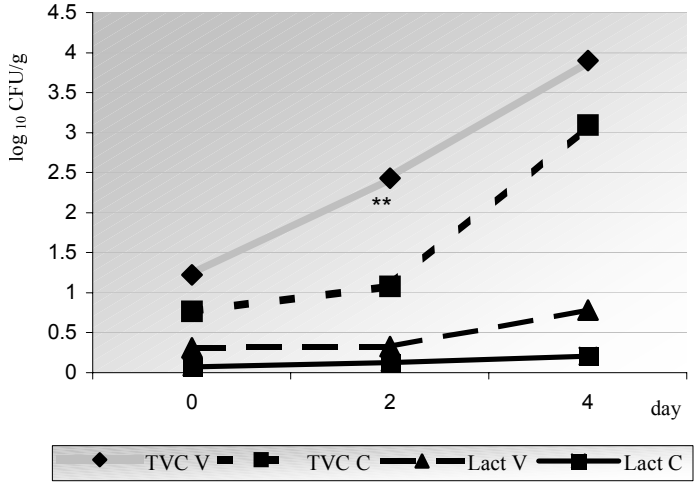


Fig.3. Microbial counts of beef: effect of ageing method



OPTIMUM PROCESS CONDITIONS FOR SINGLE-SIDED PAN-FRYING OF HAMBURGERS FOR SAFETY

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Background

Hamburger (meat patty) is one of the most popular foods in North America and also spread all over the world. However, such a simple food has ever led to numerous foodborne diseases. Hamburger disease is caused by *E. coli* O157:H7. FDA has recommended a minimum target cooking temperature of 68.3ºC with 16 s holding, for foodservice operations to enhance food safety (FDA 1999). However, it is difficult to implement these standards in a restaurant or at home due to the complexities and accuracy in measuring the internal temperatures, and the non-homogenous composition of the patties.

Objectives

To improve the quality of cooked patties while ensuring food safety, it is necessary to systematically study the influences of different cooking conditions, and optimize the single-sided pan-frying with turnings for frozen and unfrozen patties.

Modelling

During recent years, several models of patty cooking have been proposed (Ikediala *et al*. 1996; Chen *et al*., 1999; Zorrilla and Singh 2003; Pan *et al*. 2000). To develop the models, for pan-frying, heat was considered to be transferred to the patty surface by convection and conduction from the hot plate, and through the patty by conduction. Moisture and fat transfers were assumed to be due to the capillary flow due to patty shrinkage and pressure increase at increased temperature. The predicted temperature at each node in the patty was used to calculate the destruction or survival rate of microorganisms. The destruction rate of microorganism was represented by first-order kinetics and the decimal reduction time differential equation model. The simulation program was written in MATLAB-simulink and executed on a PC.

Materials and methods

The patties were bought as a batch. The frozen patties were individually packed in plastic bags to prevent moisture loss, and stored in a freezer at -8°C. To obtain unfrozen patties, the stored patties were thawed at a 4°C overnight. Patties were pan-fried using a combo health grill. A proportional controller through a solid state relay controlled frying temperature. Frying time was up to 10 min depending on patty initial temperature and pan temperature. For unfrozen patty, the first flipping (turning) was after 120 s, the second at 240 s and the third at 360 s with total time of 480 s. For frozen patty, these times were 240 s, 360 s, 480 s and 600 s, respectively. Water content was measured using AOAC (1984) method #24.002 with four replications. Fat content was measured using AOAC (1984) method #24.005, i.e. Soxhlet method. The temperature histories at the pan surface, and geometric centre and top and bottom layers of the selected patties were measured using high temperature rated copper-constantan thermocouple probes with diameter of 0.25 mm.

Results and discussion

Experimental validations: Predicted and experimental beef patty temperatures at 3 locations in a patty were compared (Fig. 1) and agreement is good. The temperature of the patty bottom rises quickly at early stage. The prediction errors for the bottom, centre, and top temperatures range from 2.33ºC to 5.28ºC. The errors for centre temperature predictions are smaller than those for bottom and top temperature predictions. Good

fit between the measured and predicted moisture contents (r^2 = 0.986, slope = 0.915, and intercept = 0.026 for the linear trendline) was observed. The root mean squared errors (*RMSE*) between experimental and predicted data were calculated. The prediction errors of average moisture contents for frozen and unfrozen patties were 0.1032 d.b. and 0.1695 d.b respectively. Good fit between the measured and predicted fat contents (r^2 = 0.989, slope = 1.023, and intercept = -0.029 for the linear trendline) was also observed. The fat content had a tendency to converge at equilibrium fat content gradually when increasing the cooking time or heating temperature. *RMSE* of fat contents for two cookings were 0.0778 and 0.0638 d.b.

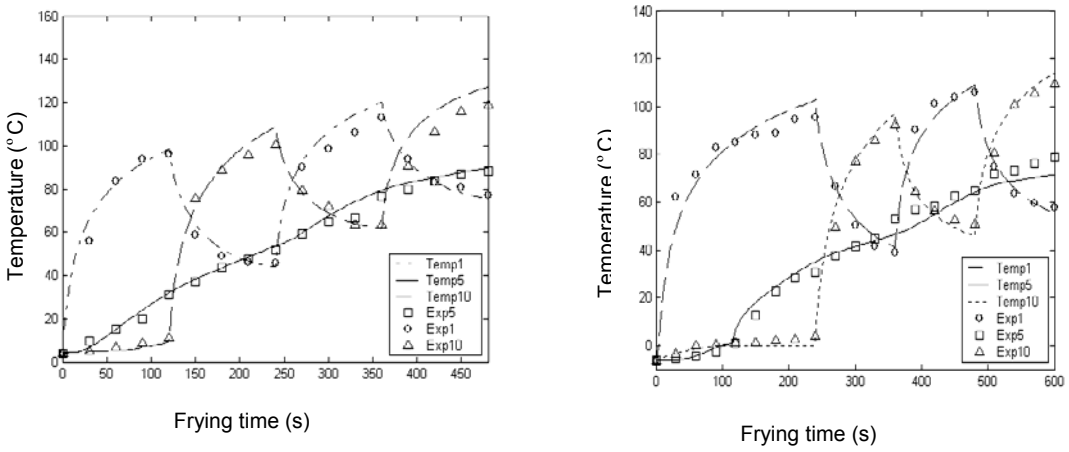


Fig. 1 Temperatures for unfrozen (left) and frozen (right) patties with overturned 3 times at 160°C pan temperature (Temp1, 5 and 10 are predicted temperatures of node1, 5 and 10, respectively. Exp1, 5 and 10 are measured temperatures of these nodes)

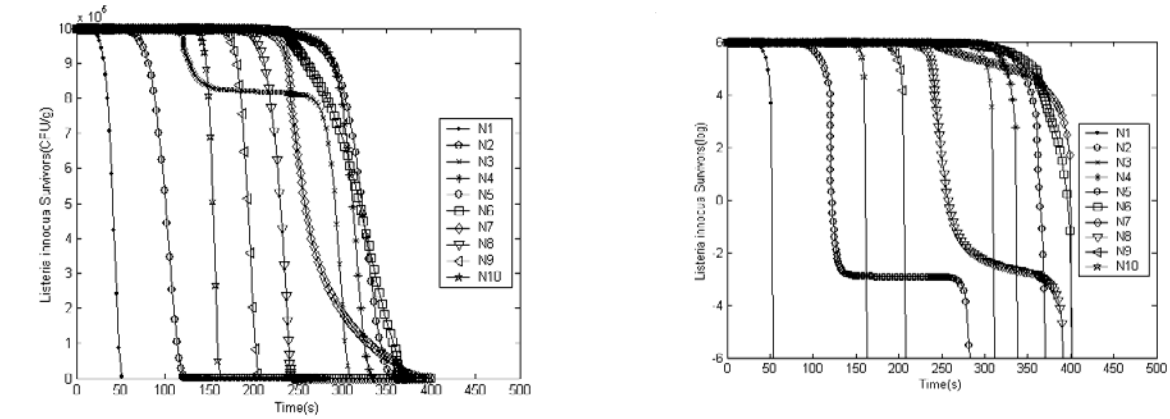
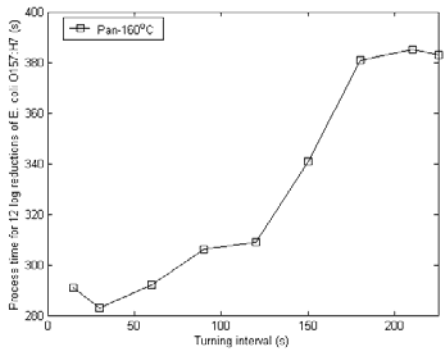


Fig. 2 Predicted *Listeria innocua* inactivation at different locations during pan-frying for frozen (left) and unfrozen (right) patties

Effect of different turning intervals: The results show significant differences from 0.25 min to 3.75 min of turning intervals (Fig. 3). The maximum difference of process time was up to 102 s. When increasing the length of each interval, the time showed an ascending tendency and leveled off at about 3.5 min. The minimum time was 283 s at a turning interval of 0.5 min. Compared to the time at 1 min intervals, the difference was just 9 s. If the turning interval is too large, it could result in quality deterioration of the patty. Thus, for unfrozen patties, 1 min turning interval may be more practical.

Effect of initial temperatures (Tpi) of the patty: With increasing Tpi, the process time is reduced at a constant heating temperature (Fig. 4). When frying frozen patties, process time decreased slowly with the increase in Tpi at 160°C and 180°C pan temperatures. At 140°C pan temperature, the process time reduced faster. When the patties were thawed before frying (Tpi = 0 to 20°C), the time reduced gradually with the increase in Tpi. The process time for the unfrozen patty is considerably less than that for the frozen. At Tpi -20°C, the process time for 140°C pan temperature was almost twice as long than that for 180°C pan temperature.

Effect of patty thickness (Xpi): More time is needed to complete 12 log reductions of *E. coli* O157:H7 when increasing the Xpi. With decrease in the Xpi from 11 mm to 8 mm, the process time reduced considerably. At pan temperature of 140°C, the process time for an 11 mm thick patty was >3 times longer than that for 8 mm Xpi. A higher turn-over frequency is a means to reduce the process time for thicker patties. At 140°C and 160°C pan temperatures, increasing the number of overturns from 3 to 5 reduced the required process time by 1102 s and 200 s, respectively. At 180°C pan temperature, basically there was no difference between the process times for 3 and 5 overturns. This suggests that for low pan temperatures, increasing overturns effectively reduces the process time.



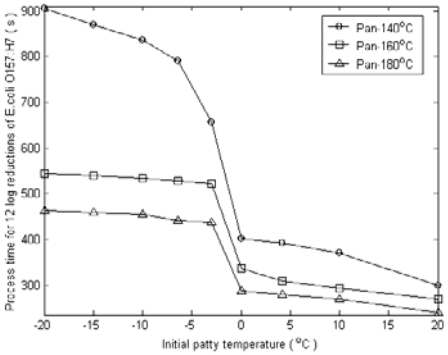
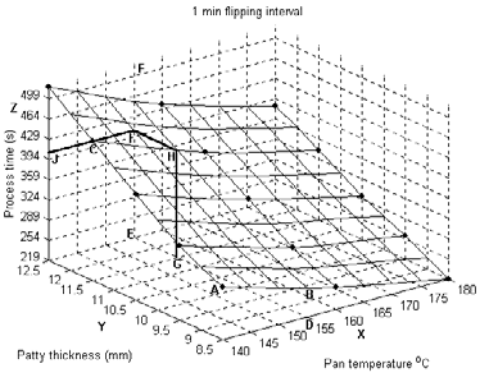


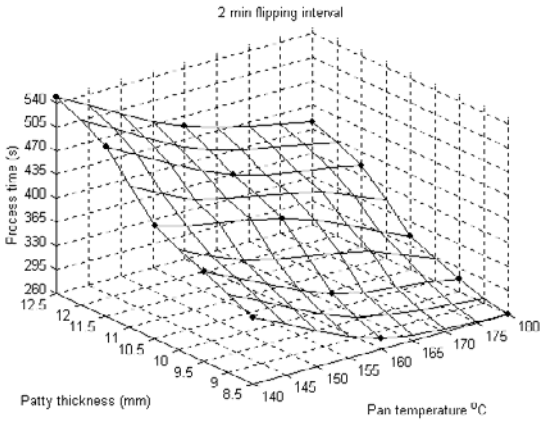
Fig. 3 Effect of different turning intervals on process time for 12 log inactivations of *E. coli* O157:H7 at 160°C pan temperature for (Tpi = 4.2°C) single-sided pan-frying

Fig. 4 (right) Process time at various initial patty temperatures and pan temperatures for with 3 overturns (Tpi < 0°C)

Effect of Various D Values of *E. coli* O157:H7: The heat resistance for each bacterium is affected by intrinsic and extrinsic factors, such as meat species, muscle types, product formulations, and other factors (Murphy *et al*., 2002). Increasing the fat content of hamburger increased the D values of microorganisms. More time was needed when increasing the D value of *E. coli* O157:H7. At 160°C and 180°C pan temperatures, the process time increased very slowly with increase in D. When comparing the results at 140°C pan temperature, the difference of the process time was considerable (182 s) with D value from 4840 s to 6280 s. Thus, a small variation of D value of *E. coli* O157:H7 has less effect on the process time at higher heating temperatures.

Three-dimensional plots for the process time: The three-dimensional plots present the relationship of pan temperature, patty size (thickness), and the process time for 12 log reductions of *E. coli* O157:H7 (Fig. 5).





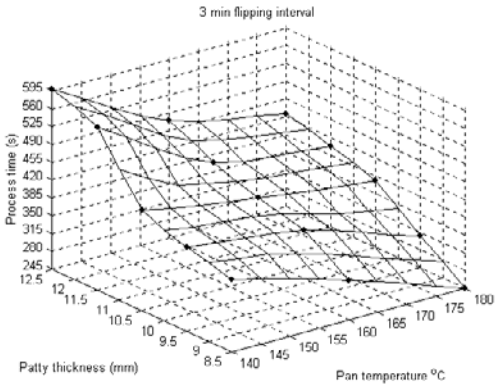


Fig. 5 Three-dimensional plots for single-sided pan-frying to determine process time

Conclusions

Good agreements were obtained between observed and simulated temperature profiles, and moisture and fat losses. The increase in heating temperature and initial patty temperature resulted in an increased patty centre temperature and higher rates of moisture and fat losses, but decreased the process time for 12 log reductions of *E. coli* O157:H7. The optimal turning interval of 1 min at 160ºC pan temperature was obtained. The process time for the unfrozen patty is considerably lower than that for the frozen to overcome the latent heat of fusion. With decrease in the patty thickness or increasing the number of overturns, the process time decreased considerably. A small variation of D value had less effect on process time at higher heating temperatures, but had significant difference for lower heating temperatures.

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MICROBIOLOGICAL, CHEMICAL AND PHYSICAL CHANGES IN ITALIAN "LONZA"

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Background

"Lonza" is a traditional Italian meat product, mainly produced in Central Italy, obtained by dry-curing of pork loin; this product is sometimes called "Lonzino". It was originally produced by peasants from heavy Italian pork during Winter, kept in dry rooms for dry-curing and maturation and consumed in summer and autumn. At present, chilled pork loins are usually sold as raw meat, and a minor part of production is used for curing. In modern factories, the production process starts by trimming pork loins in order to remove external fat, aponeurosis, and tendons. Soon after trimming, loins are seasoned with salt, flavoured and pickled at refrigeration temperature for one week. Then, loins are stuffed into cellulose casings, which are tied up and left to dry in dry-curing chambers, maintained at a relative humidity of 55 to 75 % and a temperature of 25°C to 18°C for about one week. Dry-cured loins are placed for maturation in chambers maintained at a relative humidity of 70 to 90 % and a temperature of about 14°C for 30-40 days. The present manufacturing process is considerably shorter than the original one, due to the lower fat content of raw materials.
Although Lonza is very appreciated in the European market, no data are available on the microbiological, chemical and physical changes which take place during the manufacturing process of this product.

Objectives

The aim of the present work is to point out the features of Italian Lonza, considering microbiological, chemical and physical changes throughout its manufacturing process. In order to obtain more details on the evolution of qualitative parameters, surface and core samples were collected and analysed.

Materials and methods

Sampling. Lonza samples were collected from two production cycles in the same factory. Sampling was carried out at different process times (days): 0 (raw pork loin), 1(start salting, after tumbling), 5 (salting), 8 (end salting), 8,5 (start dry-curing), 13 (dry-curing), 15 (end dry-curing), 20-22-27-29-33-35 and 45 (maturation). Analyses were performed on slices taken from the central portion of the product, collecting samples from the core (inner) and the surface (outer).
Microbiological analyses. Core and surface samples (10 g) were homogenized in a Stomacher lab-blender in 90 ml physiological solution. Decimal dilutions of the suspension were prepared in physiological solution, plated, and incubated as follows: mesophilic aerobic count (PCA, Oxoid: 30°C x 48 h), lactic acid bacteria (MRS Broth, Oxoid: 37°C x 72 h, anaerobiosis), micrococci (MSA, Oxoid: 37°C x 24 h), *Staphylococcus aureus* and coagulase-negative staphylococci (BPA + Egg Yolk Tellurite Emulsion, Oxoid: 37°C x 48 h), total yeasts and moulds (Sabouraud + 150 ppm chloramphenicol, Oxoid: 25°C x 48 h and 5 d, respectively).
Chemical and physical analyses. pH values were measured using a Mettler Toledo MP 220 pHmeter on aqueous dispersions (1:10) of the samples. Moisture was determined using the AOAC (1980) method. Water activity (a_w) was measured using a dew-point hygrometer Aqualab CX2 (Decagon Devices). Hunter L*, a*, b* values were determined by Minolta CM-508d colorimeter and chromaticity index (a*/b*) was calculated. Texture was evaluated using an Instron Universal Testing Machine mod. 5542, equipped with a 500 N load cell; penetration test was carried out by using a punch (diameter: 10 mm) at a cross-head speed of 0.83 mm s^{-1}. The maximum penetration force was considered as hardness index.

Results and discussion

Figure 1a shows the evolution of lactic acid bacteria (LAB) during the manufacturing process. After tumbling (24 hours), changes in LAB numbers were similar to those observed for mesophilic aerobic count (data not shown), but LAB increased more rapidly in core samples; this behaviour might be attributed to the stimulating effect of a lower redox potential inside the product. LAB count increased sharply throughout dry-curing, due to the inhibitory effect of the low a_w on competitive microbial populations. A slight decrease was observed in the second half of the maturation period (after 30 days), possibly due to the a_w decrease; similar results in surface and core samples were described by Vilar et al. (2000) in dry-cured lacón.

This salting effect is confirmed by the evolution of micrococci and coagulase-negative staphylococci (CNS), described in Figures 1b and 1c, respectively. Micrococci, and particularly CNS, are the microorganisms which are usually isolated in the greater proportion in dry-cured meat products (Carrascosa *et al.* 1992; García *et al.* 1995), due to their salt-tolerance, as well as to their resistance to low a_w and high osmotic pressure conditions. The salt effect on micrococci became evident during dry-curing, leading to 9 Log CFU g^{-1} in surface samples and 7 Log CFU g^{-1} in core samples throughout maturation. *Staphylococcus aureus* was not detected on the surface and inside the product during the manufacturing process.

In Figures 1d and 1e, the evolution of yeasts and moulds is shown. Yeasts numbers were generally higher in surface samples at the beginning of maturation (20 days). At the same time, temperature and relative humidity conditions during maturation contributed to increase the moulds count, up to 8.5 Log CFU g^{-1}. Moulds and yeasts have been reported as dominant organisms on the surface of different types of dry-cured ham throughout maturation (Nuñez et al 1996). In fact, it is known that they may contribute to ripening by means of their proteolytic and lipolytic activity.

Moisture and a_w (Table 1) decreased progressively during processing, as a consequence of air-drying and water-binding effect of salt on meat. As expected, these changes were more significant in the outer zone of the product. After salting, a deep decrease of pH occurred, possibly due to LAB growth, reaching the lowest value at the end of dry-curing. pH increase during maturation could be related to the activity of endogenous and microbial enzymes; this evolution is particularly evident in surface samples, where moulds are dominant (Figure 1e).

Lightness (L*) decreased throughout the process, whilst the chromaticity index (a*/b*) increased. These results could be attributed to both water loss and the progressive formation of meat pigments, in the presence of a source of nitric oxide, resulting in the typical and desidered color of dry-cured meat products.

Lastly, texture changes were observed in the product during the manufacturing period (Table 1). A marked decrease of hardness was detected in the samples taken after dry-curing, possibly related to the endogenous and microbial proteolytic activity (Parreno *et al.* 1994; Rodríguez *et al.* 1998). However, protein denaturation and oxydation, as well as water loss, caused a progressive hardness increase during maturation, more evident in the outer zone.

Conclusions

The prevalence of coagulase-negative staphylococci, micrococci, and lactic acid bacteria in the final product show a correct evolution of microbial populations during the manufacturing process of Italian Lonza. Final a_w and moisture were similar to those observed in other traditional dry-cured meat products. Colour and texture changed as a consequence of the processes occurring during maturation, greatly contributing to the product quality. The combination of salt and nitrites as preservatives, competitive lactic acid bacteria, a lowered pH and a_w, can be considered efficient hurdles, contributing to the quality of this traditional product, as well as to its microbiological and chemical stability.

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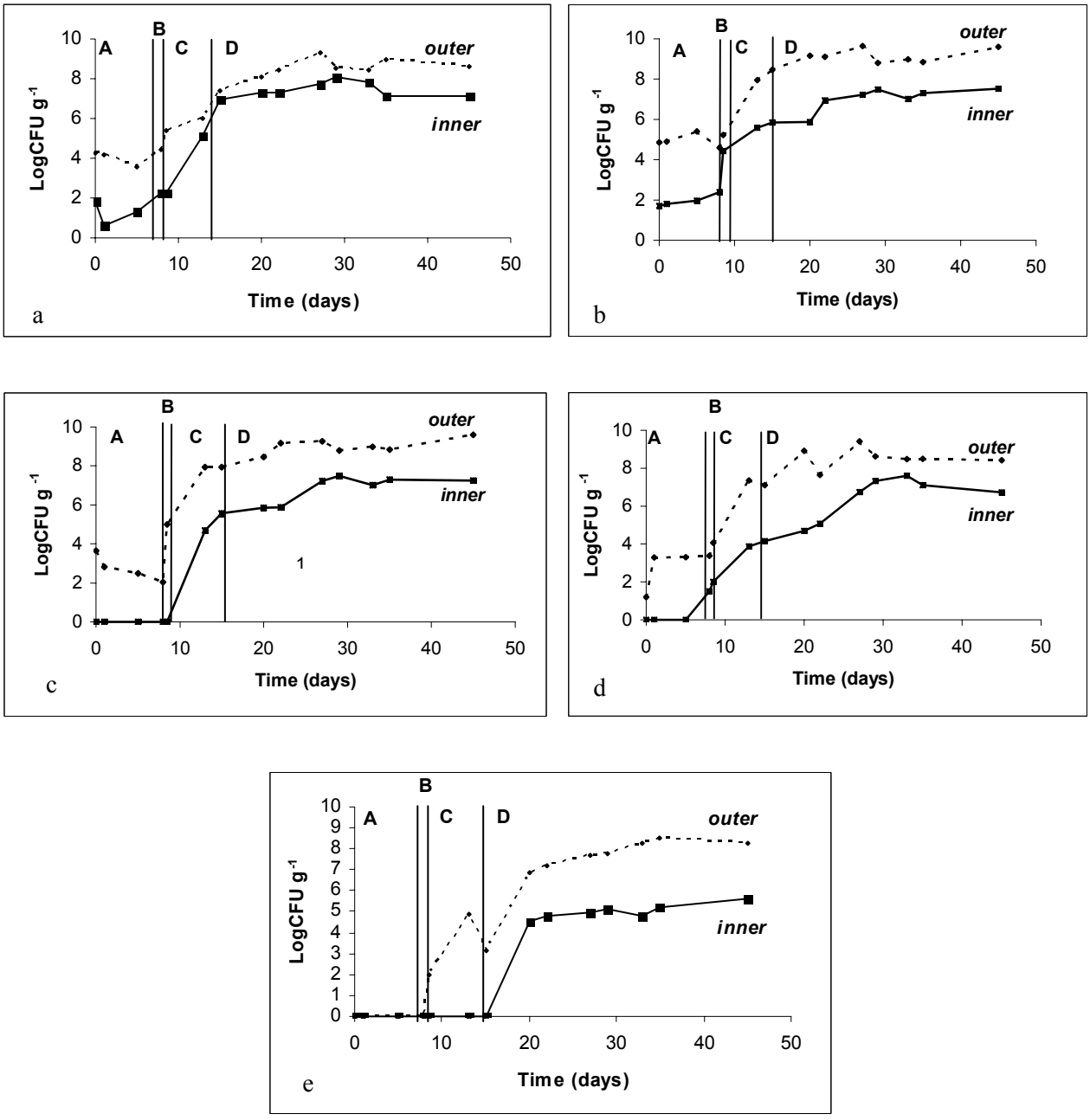

Figure 1: Evolution of LAB (a), micrococci (b), coagulase-negative staphylococci (c), yeasts (d) and moulds (e) during the manufacturing process of Italian Lonza (process steps: A: salting; B: stuffing; C; dry-curing; D: maturation).

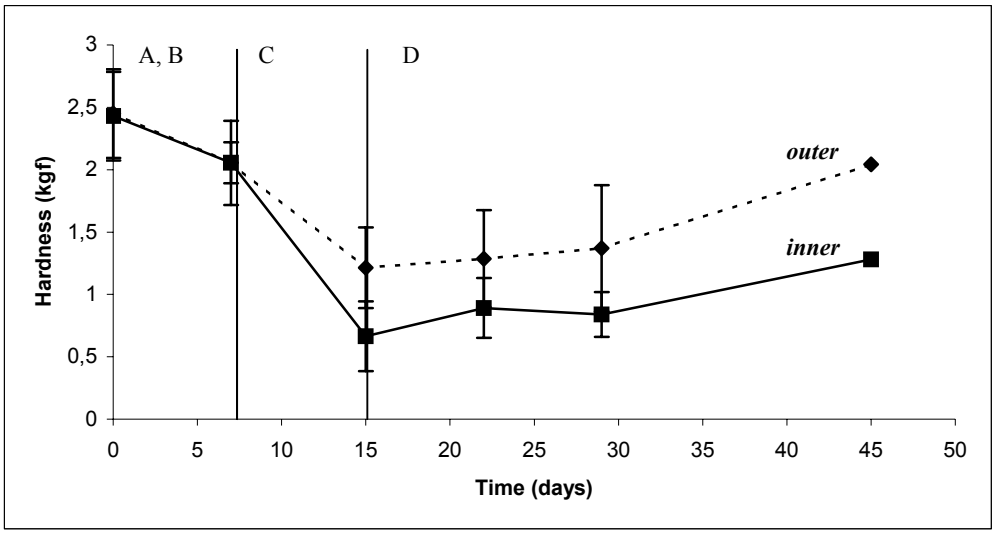

Figure 2: Textural changes of Italian Lonza (inner and outer zone) during the manufacturing process (process steps: A: salting; B: stuffing; C; dry-curing; D: maturation).

		Process steps					
		Raw meat	***Salting***	***Curing***	***Maturation***		
		days					
Parameters	***Zone***	0	7	14	**21**	**35**	**45**
Moisture	inner		70,20	64,87	69,18	66,13	63,64
	outer		67,18	55,54	62,34	55,68	49,94
a_w	inner	0,99	0,98	0,96	0,96	0,94	0,94
	outer	0,99	0,98	0,94	0,94	0,93	0,91
pH	inner	5,62	5,72	5,42	5,67	5,56	5,89
	outer	5,59	5,67	5,47	6,17	6,19	6,71
Colour(*) L*	inner	48,7	44,92	45,23	42,67	42,88	40,81
a*/b*		0,23	0,83	1,01	0,83	1,08	1,21

Table 1: Moisture, a_w, pH and colour parameters of Italian Lonza at different process steps.

Acknowledgements

This study was financially supported by the Italian Ministry for Productive Activities (Tecnolonza Research Project, FIT E01/0229).

MICROBIOLOGICAL CONDITIONS OF SHEEP CARCASSES FROM HIGH-CAPACITY SLAUGHTERHOUSE: A PRELIMINARY STUDY.

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Background

A rigorous attention in the practice of slaughtering is, in meat production, of crucial importance in the prevention of the microbiological contamination of the carcasses aimed to assure both meat quality and safety of public health. In order to allow the risk assessment in question and the determination of the measures to be taken, the slaughtering process analysis must be completed by collecting specific control data of the slaughter house on the microbiological condition of the carcasses. The ovine slaughtering shows some features, compared to other species, that condition the initial level of carcasses contamination and of meat derived from them, such as the presence of critical process stages (removal of the fleece).
The Committee Decision 2001/47/ECC of 8 June 2001, "which lays down rules for regular controls on general hygienic conditions carried out by the operators in the factories, according to the EEC rule 64 433 on health conditions for the production and circulation of fresh meat and according to EEC rule 71 118 related to health problems in fresh meat exchanges of yard birds", established guidelines for microbiological controls in slaughter houses on the basis of HACCP methods.

Objectives

The purpose of this study has been the microbiological monitoring of adult ovine and lambs carcasses and of processing surface (Total Viable Count-TVC, *Enterobacteriaceae*-EC and detection of *Salmonella*, *E.Coli O157*, *Listeria monocytogenes*), in five high-capacity slaughterhouses of Abruzzo and Molise.

Materials and methods

In five high-capacity slaughterhouses (named 1,2,3,4,5), the processing hygienic conditions of 30 ovine carcasses (10 adult and 20 lambs) have been estimated.
In adult ovines, on each side of the carcass, samples have been collected with the swabbing method on the following sampling sites: flank, thorax lateral, brisket and breast, using a 100 cm^2 template.
The difference, regarding 2001/471/EEC Decision, is in testing in different way the fore side and back side. The four swabs of the fore side (brisket and breast), dipped in 50 ml of Maximum Recovery Diluent-MRD (Oxoid), have formed a single sample; and the procedure was the same for the back side (flank and thorax lateral).
For lambs the non-destructive method has been always used. There was one sampling site for each shoulder and thigh of both sides of every carcass, using template of 25 cm^2, as stated in 2001/471/EEC Decision.
In this case too the difference, related to the original 2001/471/EEC Decision, is in testing apart the fore side and the back side.
The four swabs of the fore side, dipped in 50 ml of MRD, were a single sample; and the procedure was the same for the back side. The samples, transported to laboratory in sterile way at the temperature of +4°C, have been immediately analyzed.
For the TVC and *Enterobacteriaceae*, the methods foreseen in the 2001/471/EEC Decision have been applied. The presence of the pathogenic microorganisms (*Salmonella, E.coli O157, Listeria monocytogenes*) has been analyzed by sponge-bag (PBI International).
Each sponge, rehydrated with 10 ml of MRD, has been scoured on the entire carcass and transported at refrigeration temperature to laboratory, adding 90 ml of the same diluent. After they have been homogenised with peristaltic Stomaker, pre-enrichment, enrichment and VIDAS screening have been carried out.
Salmonella: according to the official method AFNOR V08-052; AFNOR BIO 12/10-09/02.
Listeria monocytogenes: according to the official method AFNOR BIO 12/9-07/02.
E. coli O157: according to the official method AFNOR BIO 12/8-07100.

All suspected colonies from selective media were screened and identified by biochemical systems (API-Biomerieux).
Regarding surfaces, the samples have been performed after cleaning and sanitization operations and however immediately before operation starts. In case of visible dirt, cleaning should be considered as unacceptable without any further microbiological evaluation. For wet areas dry cotton swabs have been used. Samples have been collected with cotton swabs moistened with 1 ml of diluent, as reported in 2001/4717ECC Decision, from a surface area of 20 cm^2.
According to the Decision TVC and *Enterobacteriaceae* concentrations have been assessed.
The presence of *Salmonella*, *Listeria monocytogenes*and *E.coli O157* have been evaluated as previously reported.
Statistical analysis: colony count results were transformed into log values and depicted in box plots. The mean values, obtained by the set of samples coming from fore and back side, were analysed by the variance analysis (ANOVA) and regression analysis.

Results and discussion

The histogram reported in Fig. 1 and related to TVC values, shows the behaviour of mean values as a function of the two sampling sites.
The statistical analysis, carried out for TVC, shows that no statistical difference is visible among the different slaughterhouses, adult ovine and lambs and the mean values of surface and carcasses. The medium values of the fore side and back side instead, showed a positive coefficient of regression, but due to the low number of sample and presence of some anomalous data, the correlation coefficient is not very high (0,56).
The higher contaminated side was the back. In literature, it has been reported that bacterial count on carcasses processed according to the conventional dressing, show the back side as the most contaminated area, moreover the areas of highest contamination are the sites where cuts are made through the skin (Bell et al., 1993; Bell et al., 1996; Vanderlinde et al., 1999).
All of the TVC mean values are within the acceptable range of the 471/2001/ECC Decision (<3,5 log ufc/cm^2) like those described in literature on ovine carcasses, after sampling with non-destructive method (Gill et al., 1998; Vanderlinde et al., 1999; Duffy et al., 2001; Phillips et al., 2001; Hedges et al., 2002; Reid et al., 2002; Mazzette et al., 2003; Zweifel et al., 2003).
The TVC values were always higher than the other species, particularly equine and bovine (Vergara et al., 2002; Sarli et al., 2003; Splendiani et al., 2003; Reid et al., 2003). This results have been explained by the presence of the fleece, which is the most important factor of contamination. Hadley et al. (1997) have observed that carcasses derived from ovine with dirty fleeces have been shown to carry up to 1000 times more microorganisms than carcasses derived from visually cleaner animals. Moreover the lower values of TVC found in the bovine, are due to the fact that large areas of the beef carcass surface are not touched by workers and their equipments, whereas the surface of smaller sheep carcass is handled or touched by equipment during dressing (Gill et al., 1998).
The levels of *Enterobacteriaceae* was not detectable as already found in previous surveys (Colavita et al., 2003; Splendiani et al., 2003).
All the examined carcasses showed negative results regarding *Salmonella, Listeria monocytogenes* and *E.coli O157.*
Regarding surfaces (Fig. 2), in three of the five plants, although the optimal microbiological values of carcasses, the reported values over the terms of acceptability show difficulty in correct SSOPs management (sanitation standard operating procedures) carried out by workers.

Conclusions

Our findings show that the back side has a highest microbial contamination because its area is more manipulated during the dressing.
The lack of the correspondence between the mean values of carcasses and surfaces, evidences the necessity of a better management of sanitization operations. As the hygienic peculiarities of the working environment have a basic role in food industries, it could be useful that the plant is degraded in class A, for a single unacceptable finding.

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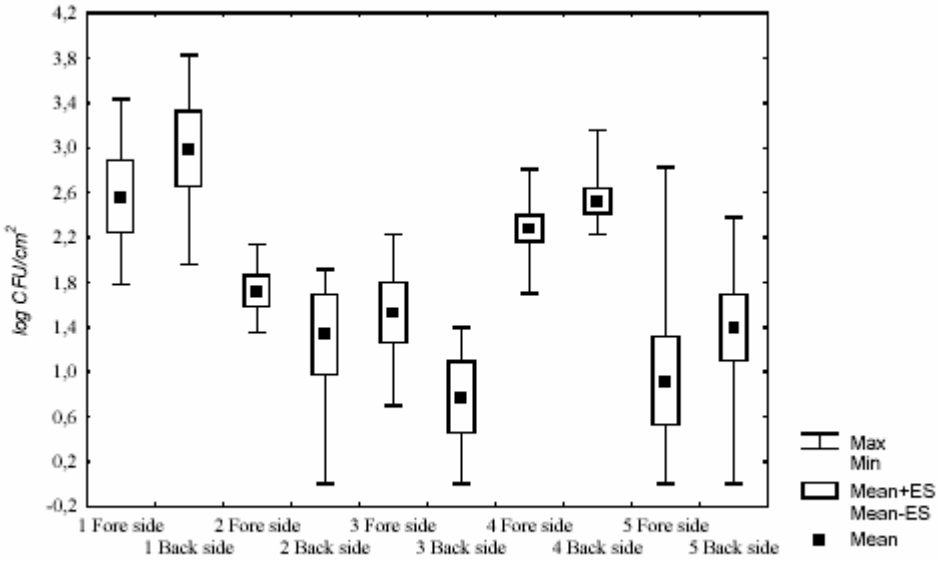

Fig. 1 show the TVC mean values of fore and back side in the five slaughterhouses.

Slaughterhouse	Acceptable range 0-10/cm^2	Unacceptable >10/cm^2
1	X	
2	X	
3		X
4		X
5		X

Fig. 2 show the result of surfaces value in the five slaughterhouse in according to Decision 2001/471/ECC

INFLUENCE OF INJECTION, PACKAGING AND STORAGE CONDITIONS ON THE MICROBIAL AND COLOUR STABILITY OF BEEF AND BISON STEAKS

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Background

Maintaining quality and appearance is essential during the distribution and merchandising of perishable food products like meat. The only criterion consumers have at the point of purchase when selecting meat cuts is visual appearance. To prevent product shrinkage and to delay the onset of its deterioration, optimum storage temperature and appropriate packaging are necessary for retail display. There are many packaging choices available; however, knowledge of the physical characteristics of the materials and systems in question, along with other factors that influence the keeping quality of meat, is essential when selecting the appropriate packaging. Vacuum (VP) and modified atmosphere packagings (MAP, *i.e.*, with one or more gases) are being used by the industry to design different conditions so as to maximize the shelf-life of meat and to promote desired product attributes. The most commonly used gases for packaging of meat are carbon dioxide (CO_2), nitrogen (N_2) and oxygen (O_2). The O_2 in the package headspace enhances meat colour and extends the stability of oxymyoglobin, while CO_2 restricts the growth of aerobic spoilage bacteria (Jeremiah, 2001).

Objectives

The objective of this study was to assess the influence of injection, packaging and storage conditions on the microbial, oxidative and colour stability of beef and bison steaks.

Materials and methods

Fresh beef and bison loins (*longissimus lumborum*, LL - 4 each) were procured from local sources. Each LL was divided into two sections. One section was injected with brine containing NaCl and sodium tripolyphosphate (0.5% and 0.3%, respectively in the finished product) to achieve 20% extension by weight, while the other section was kept as a non-injected control. Then, each loin section was divided into as many steaks (1" thick) as possible. These steaks were randomly allocated to storage atmospheres (MAP and VP), storage temperatures (-1 and +4°C) and storage interval subgroups (overnight, 1 and 2 weeks; and 1, 2 and 3 weeks for MAP and VP, respectively). The steaks (n=48) for VP treatment were individually packaged in ethylene/vinyl acetate copolymer polyvinylidene-chloride (PVDC) laminate bags. After making 2 holes through the over-wrap film for free exchange of gases, the steaks (48) for MAP were transferred to Cryovac B series bags, the headspace was evacuated, filled with a mixture of 70% O_2/30% CO_2 and then sealed. After removal from the main packaging following the designated storage intervals, pH, 2-thiobarbituric acid reactive substances (TBARS), purge during storage and total aerobic plate counts were determined. Colour (L*, a* and b*) was measured using a HunterLab Miniscan XE colorimeter. The change in colour was calculated for each L*, a* and b* values; *i.e.*, ΔL^* ($L_x^* - L_0^*$), Δa^* ($a_x^* - a_0^*$) and Δb^* ($b_x^* - b_0^*$), where L_0^*, a_0^*, b_0^* were at time zero and L_x^*, a_x^*, b_x^* were at time x. For each type of meat (beef and bison), data were analyzed as a split-plot with two replications. Storage temperatures of -1 and +4°C were the main plot treatment. Sub-plot treatments included a factorial combination of 2 injection treatments, and 6 packaging-storage treatments. Least-squares means were calculated for all main effects or interactions that were represented by a significant *F*-test.

Results and discussion

As expected, the pH of injected steaks was significantly ($p<0.05$) higher than non-injected samples (Table 1). The pH of beef steaks remained constant in MAP but decreased in the third week in VP. For injected bison, the pH decreased from 5.74 to 5.49 over time in vacuum-packed systems but did not change significantly ($p<0.05$) in the non-injected counterpart. At the higher storage temperature, pH of the meat decreased with time. Owing to a high O_2 concentration and subsequent increased lipid oxidation, MAP beef steaks stored for 1 or 2 weeks exhibited higher TBARS values compared to VP steaks. In bison meat, lipid

oxidation was also significantly influenced by different packaging treatments; however, their effect on TBARS was strongly dependant on injection and temperature of storage, as indicated by the packaging *x* injection and packaging *x* temperature interactions (p=0.000 and p=0.007, respectively). TBARS values for steaks stored under MAP for 1 week were higher than those stored under vacuum, while TBARS values for steaks stored under MAP-OV were the lowest (p<0.01). In the present study, the interactions between injection and temperature were probably due to the magnification of the TBARS differences among packaging treatments within non-injected steaks and those stored at the higher temperature. Both beef and bison showed significant injection *x* storage treatments interactions for purge lost during storage. Except for MAP steaks stored overnight, injected steaks had significantly lower fluid loss compared to non-injected samples. Regardless of the injection treatment, steaks stored under vacuum had significantly higher fluid loss in comparison to MAP treatments. The differences in purge among the storage treatments were greater in the non-injected than in the injected meat.

Storage temperature has a major influence on the microbial quality of meat products. As expected, microbial loads of steaks that had been injected and the ones stored at +4°C were higher than non-injected steaks and those stored at -1°C, respectively (Fig. 1). Modified atmospheres around steaks delayed the time to spoilage, which was evident from the data obtained on microbial load in the present study. Aerobic counts for steaks stored under MAP were lower than those stored under VP; nevertheless, counts from both packaging atmospheres were in the acceptable range. In the present study, a mixture of 70% O_2 and 30% CO_2 was employed. The CO_2 in the package atmosphere restricts the growth of aerobic spoilage bacteria, while the elevated O_2 concentration enhances meat colour and extends the stability of oxymyoglobin (Jeremiah, 2001). Aerobic counts increased gradually with storage, but generally colour deterioration occurred in advance of significant changes in aerobic plate counts.

In the present study, bison meat bloomed quickly with a dark red colour, but it tended to loose its brightness readily on storage compared to beef. As expected, steaks stored at the lower temperature (-1°C) held better colour than those stored at the higher temperature (+4°C) (Fig. 2). It has been reported that a low storage temperature promotes oxygen penetration into meat surfaces and increases the solubility of oxygen in tissue fluids, which increases the depth of the oxymyoglobin layer on meat surfaces (Hood, 1984). At the time of storage, non-injected beef steaks had higher a* values than injected ones. However, after 3 weeks of storage marination had no significant (p>0.05) affect on the colour of beef steaks. While for bison steaks, the change in a* values during storage due to brine injection was dependant on storage time and type of packaging. There was a positive influence on the colour of steaks under MAP treatment after overnight and 1 week of storage, whereas the colour of steaks deteriorated (*i.e.*, decreased a* values) significantly (p<0.05) after vacuum packaging irrespective of the storage time. These changes in a* values were more pronounced in injected than non-injected bison steaks (Fig. 3).

The largest increase in a* value of beef and bison steaks was observed during overnight storage under MAP. A slight improvement of the colour was also seen in beef steaks stored under MAP for 1 week. The a* value remained constant in beef steaks held under MAP for 2 weeks and under vacuum for 1 week but decreased significantly after 2 and 3 weeks of storage under VP. Bison steaks held under MAP for 1 week retained a bright red colour only in injected treatments and those stored at the lower temperature. Regardless of storage temperature and injection, bison steaks held under MAP for 2 weeks were totally discoloured, so rendered as unacceptable and were discarded from further assessments.

Conclusions

Beef LL steaks were able to retain their bright red colour longer than bison steaks. However, the deterioration in colour of bison steaks occurred in advance of significant changes in aerobic plate counts. Bison appears to develop higher TBARS on storage, and this might have an influence on the resulting rapid loss of redness with bison. Storage at -1°C provided greater colour stability and longer storage life for both beef and bison meat. MAP is an excellent option for short-term storage due to its positive effects on meat colour, but for longer storage, VP may be necessary. Storing meat under vacuum and then placing it under MAP just before retail display might be another option to increase shelf-life.

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Table 1. Effects of marination, packaging and storage on pH, TBARS and purge of beef and bison steaks.

		pH		TBARS (mg malon-aldehyde eq./kg)		Purge (%)	
		Beef	Bison	Beef	Bison	Beef	Bison
Temperature							
-1		5.51	5.55	0.565	0.957	3.47	4.65
+4		5.39	5.43	0.553	1.164	3.71	5.10
p-value		*0.349*	*0.223*	*0.929*	*0.173*	*0.365*	*0.283*
Injection							
INJ		5.59a	5.66a	0.553	0.572b	2.69b	3.55b
NO		5.31b	5.32b	0.565	1.183a	3.86a	4.32a
p-value		*0.000*	*0.000*	*0.866*	*0.000*	*0.000*	*0.000*
Storage							
MAP-OV		5.48a	5.53ab	0.487bc	0.411b	0.97a	1.23a
MAP1		5.49a	5.55a	0.688b	2.529a	2.47b	2.99b
MAP2		5.45a		1.142a		2.65b	
VP1		5.53a	5.54a	0.362c	0.546bc	4.98c	5.54c
VP2		5.42ab	5.44b	0.390c	0.684c	5.73cd	6.32cd
VP3		5.32b	5.41b	0.410c	0.681c	6.20d	7.19d
p-value		*0.045*	*0.035*	*0.000*	*0.000*	*0.000*	*0.000*
Interactions							
INJ	MAP-OV		5.72a		0.265e	1.00h	1.03h
INJ	MAP1		5.78a		1.304b	2.03g	2.45g
INJ	MAP2					2.06g	
INJ	VP1		5.74a		0.464de	3.95de	4.68e
INJ	VP2		5.56b		0.516d	4.59cd	5.01de
INJ	VP3		5.49b		0.481d	4.98c	6.06cd
NO	MAP-OV		5.33c		0.575d	0.93h	0.93h
NO	MAP1		5.31c		4.404a	3.01f	3.64f
NO	MAP2					3.39ef	
NO	VP1		5.33c		0.632cd	6.29b	6.56bc
NO	VP2		5.33c		0.871c	7.14ab	7.96ab
NO	VP3		5.32c		0.908c	7.72a	8.54a
p-value		*0.372*	*0.017*	*0.736*	*0.000*	*0.004*	*0.000*
-1	MAP-OV				0.394c		
-1	MAP1				1.935b		
-1	VP1				0.578bc		
-1	VP2				0.703b		
-1	VP3				0.741b		
+4	MAP-OV				0.429c		
+4	MAP1				3.242a		
+4	VP1				0.514bc		
+4	VP2				0.666bc		
+4	VP3				0.623bc		
p-value		*0.117*	*0.061*	*0.749*	*0.007*	*0.968*	*0.585*

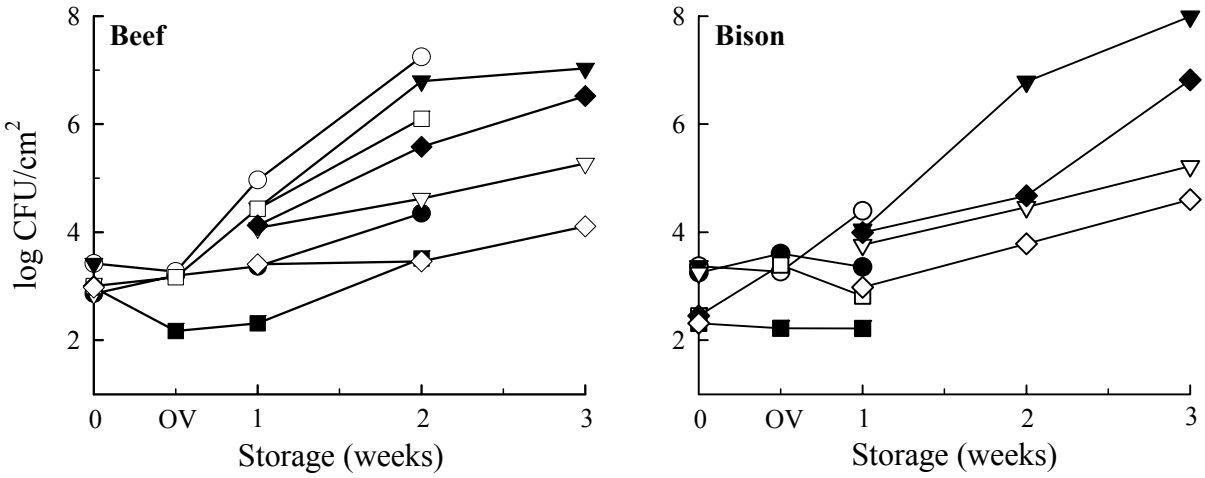

Fig. 1. Total aerobic plate count in beef and bison steaks.

● Inj, -1°C, MAP; ○ Inj, 4°C, MAP; ▽ Inj, -1°C, VP; ▼ Inj, 4°C, VP;
■ Non, -1°C, MAP; □ Non, 4°C, MAP; ◇ Non, -1°C, VP; ◆ Non, 4°C, VP.

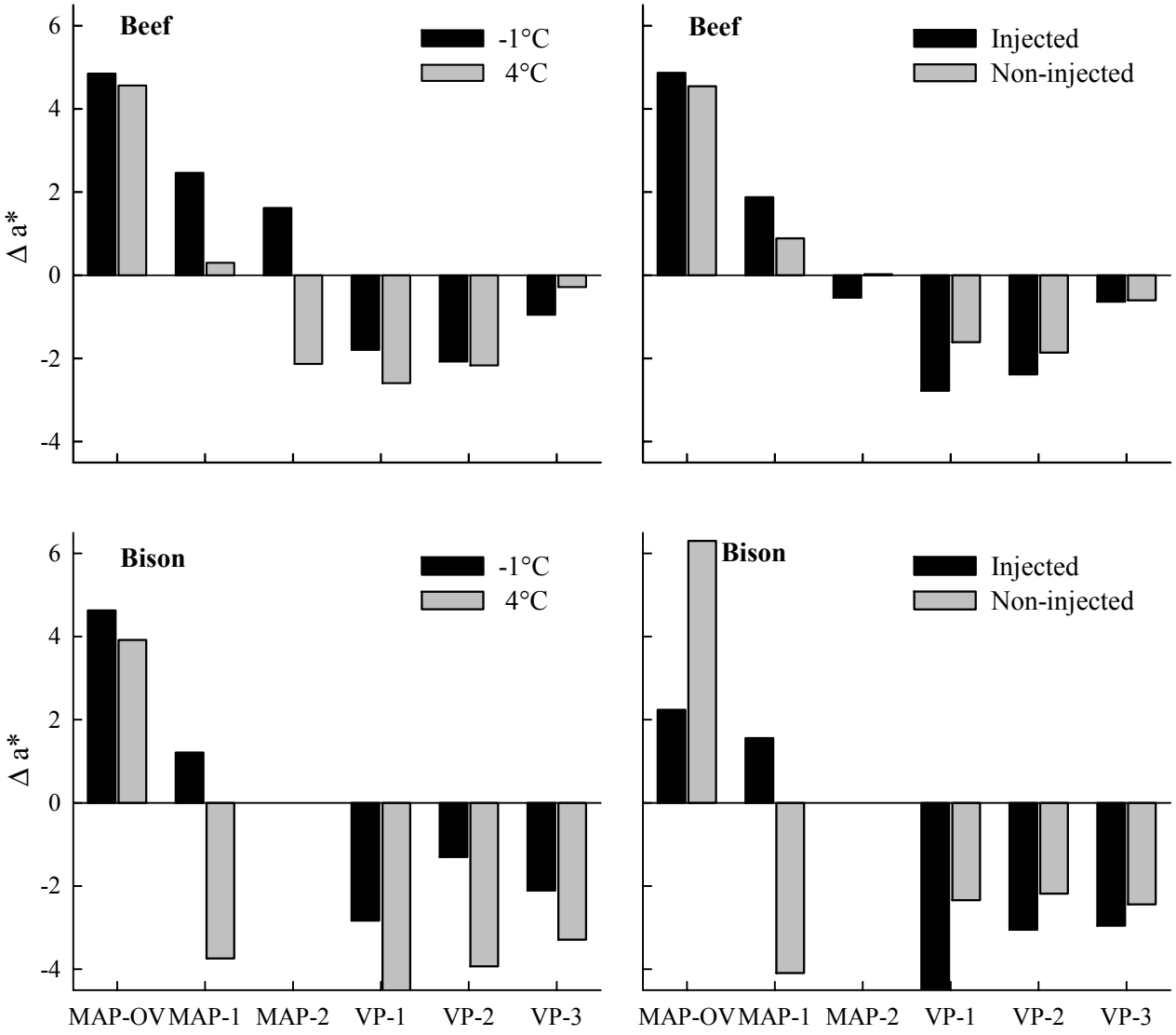

Fig. 2. Effect of temperature & packaging on change in a* during storage of steaks.

Fig. 3. Effect of injection treatment & packaging on change in a* during storage of steaks.

EFFECT OF MILD CHEMICAL AND PHYSICAL HURDLES ON THE SHELF LIFE OF VACUUM-PACKED PORTIONS OF ITALIAN MORTADELLA SAUSAGES

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Background

In a good manufacturing process of cooked meat products the curing and the cooking operations could be sufficient to guarantee their shelf life throughout the cool storage, nevertheless before the trade, the product is shared and packaged again causing a superficial re-contamination, which often compromises the microbiological safety (Pizza et al, 1981). Therefore these products need a mild treatment, limiting the chemico-physical damage: besides to the method already proposed by our laboratory to control this critical point (superficial pasteurisation of the samples at 70°-80°C for a few minutes), an interesting technique is the application of high isostatic pressures (HP): this "cold-pasteurisation" technique should limit heat-induced damages, with a better preservation of sensory and nutritional food characteristics. However, studies performed on meat showed that immediately after treatment, there occur oxidation phenomena (Cheftel and Culioli, 1997), above all colour changes (Carlez et al., 1995; Defaye and Ledward, 1995; Goutefongea et al, 1995; Cheftel, 1992) and an increase in lipid oxidation rate (Chea and Ledward, 1996).
In order to improve the stability to oxidation, the substrate can be enriched with natural ingredients having antioxidant activity, such as tomato powder and rosemary extract. In the first case the antioxidant activity of lycopene is due to an increase in the defences against the attack of singlet oxygen, to a decrease of the free radicals formed and to the inhibition of the oxidations induced by pro-oxidant metal ion (Lavelli et al., 2000; Lavelli et al, 1999; Oshima et al, 1996)). The second choice utilizes some substances, which are able to end the radical reactions and to block oxygen-reacting species (McCarthy et al., 2001).
In our Institute we confirmed the effect of tomato powder addition to traditional formulations on the shelf life of mortadella slices vacuum-packed in plastic trays and subjected to HP (Bergamaschi et al., 2002).
Moreover, the use of natural ingredients and HP could be a good tool in order to reduce or to eliminate from cooked meat processing an additive such as sodium nitrite, which is judged by consumer a risk factor for human health, even though its bacteriostatic (Castellani and Niven, 1955) and antioxidant activity are well-known (Morrissey, 1985).

Objectives

The aim of this work was to evaluate the single and combined effects of mild chemical and physical hurdles, based on both conventional and emerging technologies, on microbiological safety and oxidative stability of vacuum-packed portions of typical Italian mortadella sausages throughout the storage at 0°C.

Materials and methods

Experimental design

The experimental design is based on 4 formulations x 3 pasteurisation treatments arrangement in an unbalanced randomized block design: the results, grouped on the basis of chemical and physical treatments are reported as means and standard deviations, calculated at each storage step, by means of ANOVA analysis, SPSS 12.0 statistical package.
In a manufacturing firm four different mortadella formulations were prepared with the same raw meats, using the following curing agents: a) with 150ppm of sodium nitrite (mix **0**); b) without sodium nitrite (mix **1**); c) without sodium nitrite and with tomato powder (mix **2**); d) without sodium nitrite and with rosemary extra ct (mix **3**). All products were portioned and vacuum-packed in multilayer plastic trays, therefore they were divided into three lots: the first did not undergo any treatment after packaging (standard, **STD**), the second was treated by dipping in hot water (80°C) for four minutes (**T**), and the third was subjected to the action of isostatic high pressure at a level of 600 MPa for five min at 15°C by means of a ABBQFP6-Sweden

pilot plant, available in our Institute (**HP**). All the samples were thermostated at 0°C and at discrete storage times, they were controlled for bacteriological, chemical and sensorial characteristics.

Microbiological analyses

The following bacteriological analyses were carried out on all samples: total aerobic count after incubation at 30°C for 72h in TS Agar; lactic acid bacteria after incubation at 30°C for 48h in MRS Agar.
A load of $5*10^6$ cfu/g was considered as being the threshold value for sample spoilage.

Chemical and sensorial analyses

The analyses were carried out on the ground lean component of the products. The pH was measured by means of a HI 9321 MICROPROCESSOR pH-meter (HANNA INSTRUMENTS) on the minced sample. The oxidative state of the lipid component of the samples was evaluated by measuring thiobarbituric acid reacting substances (TBARS) according to the method of Tarladgis and Watt (1981), adapted to meat. The D- and L-lactic acids were measured by means of an enzymatic kit (cod 1112821 BOEHRINGER, Mannheim, Germany) on the extract of 5g of sample according to the official SSICA method. Just after the opening of the packaging, the perception of off-flavour was evaluated by a 7-8 members experienced panel, using the following scores: 0=extremely poor, 1=poor, 2=satisfactory, 3=fair, 4=good, 5=very good.

Results and discussion

Table 1 shows that all samples mainly were spoiled by lactic acid bacteria (LAB), moreover bacterial growth was not significantly different among the formulations, except for mix 0, in which the values of sodium nitrite additions and the residue (5-6 ppm) were sufficient to significantly inhibit total aerobic flora outgrowth.
After 15-20 days for **STD** samples, LAB concentration quickly reached high values (10^6-10^7 cfu/g), while the shelf-life of **T** and **HP** samples could be prolonged to two months (Figure 1). **HP** treatment was more effective than **T** treatment and sodium nitrite-HP combination better warranted the microbiological safety of product for all the period of observation.
Since no sugar was added in the mix, LAB grew by using other metabolic ways, throughout the storage of all samples, the D-lactic acid values not changing: consequently, the pH values didn't drop significantly (Figure 2); moreover the sum of L- and D-lactic acid remained constant, since L-form was changed into D- isomer.
The formulations had a different oxidative stability (Figure 3): in fact, throughout the storage, the TBARS concentrations were below 0.2 ppm of malonaldehyde (MDA) in all samples with sodium nitrite (mix **0**), while these values quickly increased in the absence of sodium nitrite. The rosemary extract had a greater antioxidant activity than tomato powder, but, on the whole, the results were not satisfactory, since these ingredients individually didn't allow a good preservation of the product. This confirms that sodium nitrite couldn't be entirely replaced, without changing the typical sensorial characteristics of this product belonging to the ancient Italian tradition: even at these low concentrations of natural ingredients, the panelists underlined a roast fragrance in samples with rosemary extract and an agreeable pink colour in samples with tomato powder.
The **STD** samples were slightly more oxidized than **HP** and **T** samples, with values not significantly different ($p<0.01$). The flavour was well preserved by sodium nitrite in **HP** samples and less in **T** products; moreover it quickly dropped in samples in function of both chemical and physical hurdles (Figure 4).

Conclusions

Both the thermal treatment (T) and high isostatic pressure (HP) reduce the initial microbial load and stabilize the samples, throughout the storage, but HP is more effective than T treatment. Consequently the HP samples slowly spoil and the flavour keeps good for a longer time. Moreover these physical hurdles don't preserve the product against the oxidation.
The chemical hurdles have no effect on microbial stability, except for sodium nitrite addition that also reduces the oxidation of the samples; the rosemary extract has a slight antioxidant effect, while even low concentrations of tomato powder demonstrate a slight antioxidant effect in the case of abuse storage (not published data). Consequently traditional formulations can be revised by decreasing the initial concentration of nitrite through mild thermal or pressure based hurdles and the addition of moderate concentrations of not conventional natural ingredients. No thermal resistant or pressure resistant outgrowth has been observed, if the storage temperature is strictly controlled.

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Table 1. Results of the microbiological analyses (cfu/g) of all non-treated products (mix 0,1,2,3), stored at 0°C until 66 days.

Sample		Storage time (days)					
		0	**10**	**24**	**41**	**52**	**66**
STD 0	**Total aerobic count**	3.54E+03	1.44E+04	6.30E+03	5.64E+06	3.00E+05	1.35E+07
	Lactic acid bacteria	51	< 3	7.20E+02	5.13E+06	3.00E+05	6.60E+06
STD 1	**Total aerobic count**	9.00E+02	4.80E+02	3.06E+06	1.17E+06	2.17E+07	1.29E+07
	Lactic acid bacteria	27	90	1.14E+04	1.38E+06	1.24E+08	1.38E+07
STD 2	**Total aerobic count**	4.23E+03	2.43E+03	2.07E+05	6.18E+07	1.19E+08	1.20E+08
	Lactic acid bacteria	3.60E+02	1.80E+02	3.48E+03	2.55E+07	1.19E+08	8.82E+07
STD 3	**Total aerobic count**	7.50E+02	6.90E+02	8.40E+05	7.80E+06	1.26E+07	1.89E+08
	Lactic acid bacteria	4.80E+02	< 3	9.00E+02	6.57E+06	6.00E+06	3.00E+07

Figure 1. Evolution of lactic acid bacteria (LAB) on the 0 (**a**), 1 (**b**), 2 (**c**), 3 (**d**) samples, non-treated STD (♦), subjected to pasteurisation T (■) and to high isostatic pressure HP (▲) after packaging.

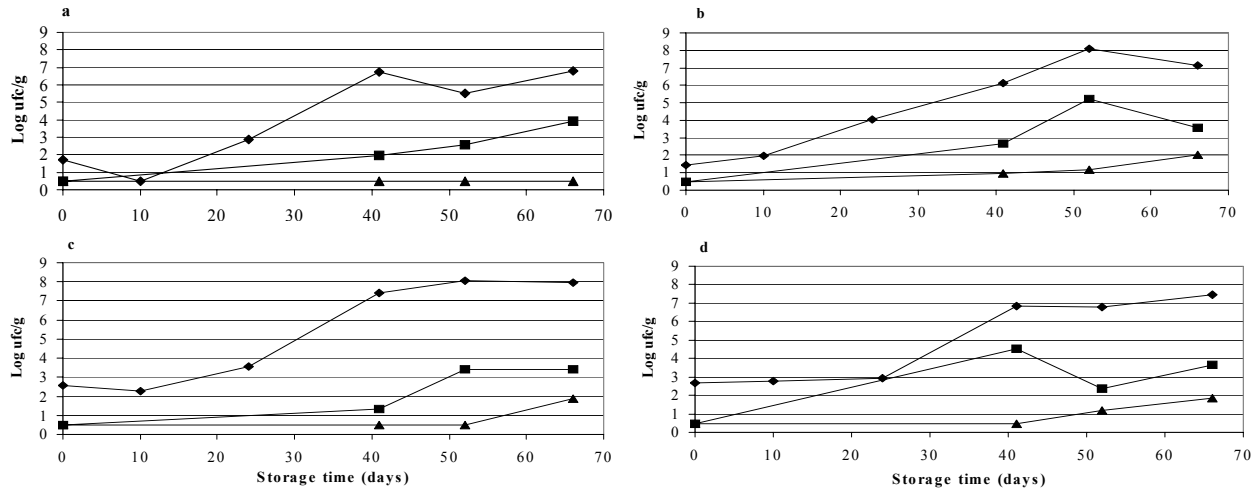


Figure 2. pH values of the 0 (**a**), 1 (**b**), 2 (**c**), 3 (**d**) samples, non-treated STD (♦), subjected to pasteurisation T (■) and to high isostatic pressure HP (▲) after packaging.

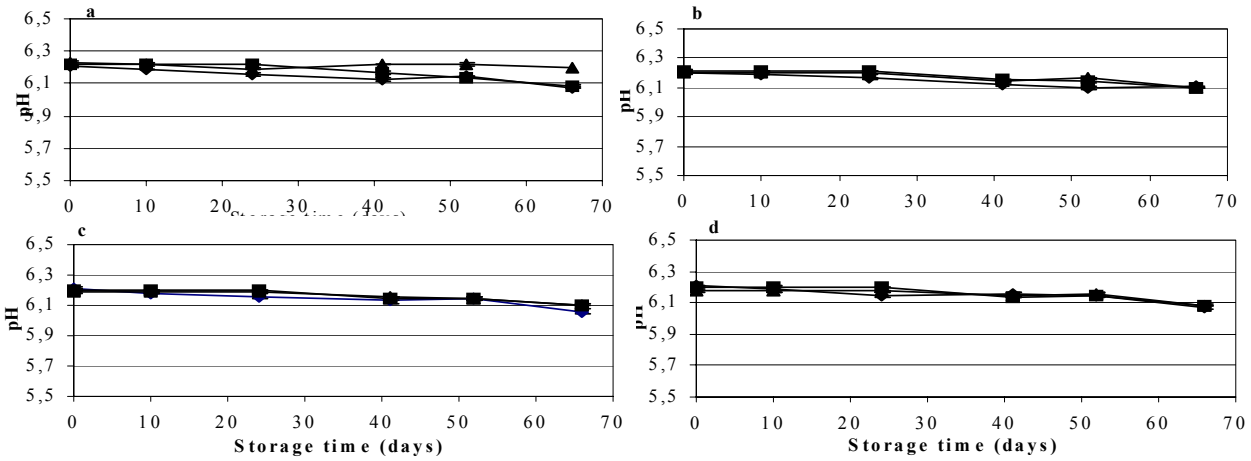


Figure 3. TBA values of the non-treated STD (**a**), subjected to pasteurization T (**b**) and to high pressure HP (**c**) samples.

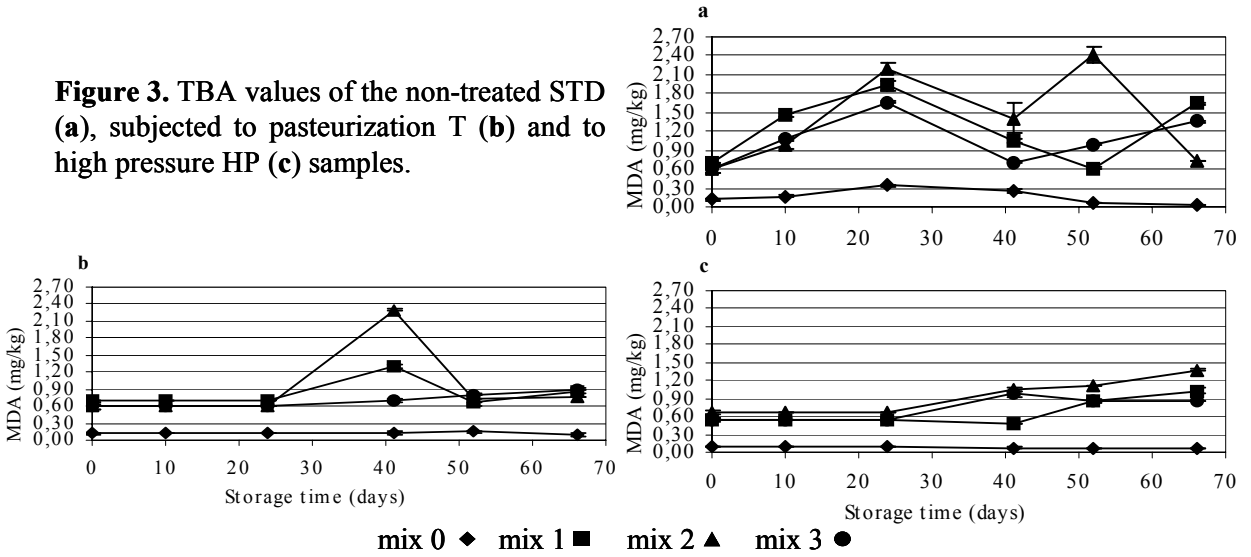
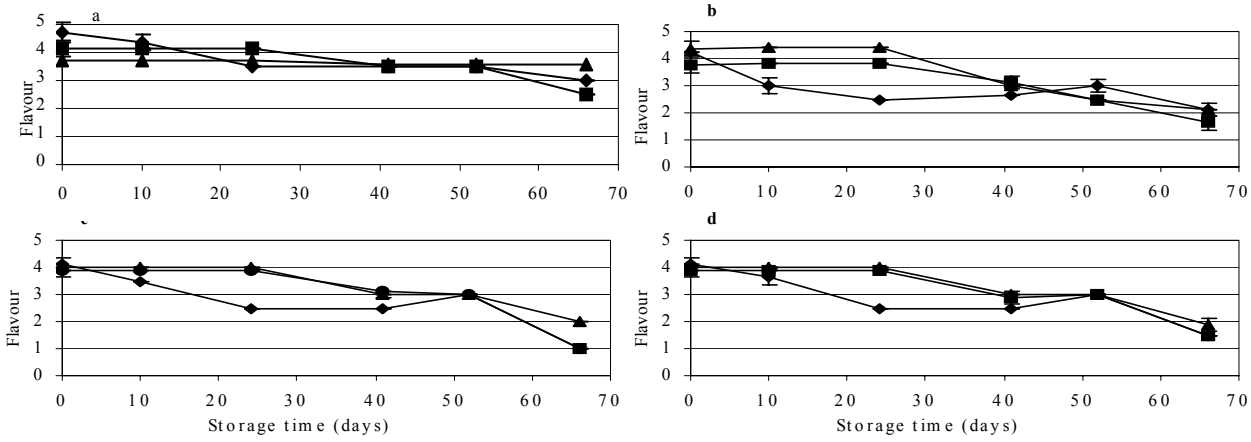


Figure 4. Sensory evaluation (flavour) of the 0 (**a**), 1 (**b**), 2 (**c**), 3 (**d**) samples, non-treated STD (♦), subjected to pasteurisation T (■) and to high isostatic pressure HP (▲) after packaging.



A PROPOSAL ON SETTING PERFORMANCE OBJECTIVES FOR CAMPYLOBACTER AND EHEC IN MEAT INDUSTRY

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Background

Food Safety Objective (FSO) is the maximum level of a hazard, which still provides an appropriate level of protection in a food at the time of consumption. Governmental risk managers should evaluate the need for an FSO for a certain hazard and food combination. However, risk managers in food industry usually encounter the challenging task to implement these FSOs to HACCP (Hazard Analysis, Critical Control Points) - and GHP (Good Hygiene Practice)-plans so that FSOs are really met. A practical tool in this work, Performance Objective (PO) is the maximum level of a hazard in any specified food at a specified time of production before the consumption in order to ensure the achievement of an FSO. In the Proposed Draft by Codex Alimentarius (2004) on Microbiological risk management it is suggested that PO could be less stringent than the FSO for those food items, which are supposed to be cooked by the consumer. Nevertheless, if these food items can be a source of cross-contamination the PO and FSO could be the very same according to the Codex Alimentarius proposal (2004).

In year 2002 the National Public Health Institute in Finland reported 3738 cases of campylobacteriosis and 17 cases of EHEC infections (Enterohaemorrhagic E.coli O 157:H7) (KTL 2003). In most of these cases the connection with the causative food item was missing. According to Finnish strategy for zoonosis (MMM 2004) the progress of risk assessment for both Campylobacter and EHEC are among the national priorities in Finland. The parameters used are the number of reported human cases for Campylobacter and EHEC as well as the number of EHEC –isolates in cattle and the prevalence of Campylobacter in poultry. The aim is to lower the number of reported human cases and to decrease Campylobacter among poultry and to keep the existing low number of EHEC isolates in cattle.

There is a great seasonal variation of Campylobacter in Finnish poultry. About 10% of the herds are infected with Campylobacteria during summer months. From November to May infected herds are uncommon. According to Lahti et al. (2001) EHEC was isolated from 1,3 % of the 1448 bovine faeces samples taken at Finnish slaughterhouses in year 1997. In 2003 EHEC was isolated from 0,4% of the samples (EELA 2004).

Objectives

The purpose of this paper is to propose a model that could be used in establishing performance objectives for meat industry.

Materials and methods

There is a simple mathematical model, which illustrates the relationship between maximum level of a microbiological hazard in food, (Food Safety Objective, FSO), the initial level of the hazard in food (H_0), the reduction of hazard during process ($\sum R$) and the total increase in the level of hazard during distribution and the shelf-life ($\sum I$). In this model; $H_0 - \sum R + \sum I \leq FSO$, $H_0 \leq FSO + \sum R - \sum I$ the parameters are expressed in $\log_{10}$ units. (Stewart et al. 2003).

The model is practical for food items, which are ready-to eat and the reduction of hazard during process ($\sum R$) means the effect of heating or cooking. However campylobacter and EHEC are hazards in non-heated meat products, which are expected to be cooked at home.

Results and discussion

Campylobacter and poultry

The setting of Performance objective, PO is accomplished in two steps by estimating the initial safety level and by comparing this estimate to known initial levels of Campylobacter in food.

I Estimation of the safety level by identified tolerable intake

The Food Safety Objectives should be based on real risk assessment. Since there are no official Microbiological Food Safety Objectives for Campylobacter in poultry, the safety level of Campylobacter in poultry is estimated according to the identification on minimum infective dose.

The amount of Campylobacteria in a portion of cooked poultry should not exceed its tolerable intake. When the known infective dose is 500 cfu (NFA 2003) and it is considered as the tolerable intake, a 100 g portion of cooked poultry should not contain more than 500 campylobacterial cells. Thus the level of hazard should not exceed 5 cfu/g (0,7 $\log_{10}$). However, the safety level is estimated to be one thousandth of the tolerable intake, that is 0,005 cfu/g (-2,3 $\log_{10}$).

The model $H_0 - \Sigma R + \Sigma I \leq FSO$ is used for the situation at home kitchen. Supposing the temperature has been below 32°C during transportation and storage and no growth has taken place neither during the transportation nor during the storage at home kitchen, thus the H_0 for the home kitchen is the performance objective, PO for the industry. The reduction of hazard during home cooking is estimated to be as low as 3,5D, ($\Sigma R = 3,5$). The estimate of the safety level –2,3 $\log_{10}$ is used in this model instead of the FSO. ΣI is zero.

$H_0 \leq FSO + \Sigma R - \Sigma I$, when FSO = safety level = -2,3; $\Sigma R = 3,5$: $\Sigma I = 0$; $H_0 = PO$

$PO \leq$ safety level $+ \Sigma R - \Sigma I$

$\Leftrightarrow$

$PO \leq -2,3 + 3,5-0$

$\Leftrightarrow$

$PO \leq 1,2$

The performance objective of 1,2 $\log_{10}$ means that the level of Campylobacteria in 1 g of raw poultry could be 15 cfu/g before cooking at home. This level of hazard is the initial level of hazard before cooking and it is also the level of hazard, which is not supposed to be exceeded at the end of the industrial processing of raw poultry.

II Comparing the performance objective to the identified levels of Campylobacteria in poultry.

According to the Finnish National Food Agency (2003) in 1999 Campylobacter was found in 4,1% of the analysed poultry samples and the levels of Campylobacter were less than 90 MPN/kg, excluding two samples, which had levels of 200 MPN and 10500MPN/kg. If these results obtained by most probable number method are considered comparable to figures of cfu/g, it can be concluded that the highest levels of Campylobacter in poultry are 0,2cfu/g (-0,7 $\log_{10}$) and 10,5 cfu/g (1,02 $\log_{10}$). According to this published information on levels of Campylobacter, the PO of 1,2 $\log_{10}$ is not exceeded in the industry.

The levels of Campylobacter in poultry vary from country to country and between establishments as well. When comparing results, the analysing method used must be paid attention to. According to Stern and Robach (2003) the level of Campylobacter in broiler carcasses was 3.03 $\log_{10}$ (2,10-4.59) per postchilled carcass. Studies were made by rinsing method.

EHEC and food

When setting the Performance objective, PO is accomplished in two steps by estimating the safety initial level and by comparing this estimation to known initial levels of EHEC in food.

I Estimation of the safety level by identified tolerable intake

The Food Safety Objectives should be based on real risk assessment. Since there are no official Microbiological Food Safety Objectives for EHEC, the safety level of EHEC is estimated according to the knowledge on minimum infective dose.

The amount of EHEC in a portion of food like beef should not exceed the tolerable intake. According to the National Food Agency, the known infective dose is only a few cells. If the figure of 5 cfu is considered as the tolerable intake, dose of 100 g should not contain more EHEC cells than 5. Thus the level of hazard should not exceed 0,05 cfu/g (-1,3 $\log_{10}$). However, the safety level is estimated to be one thousandth of the tolerable intake, that is 0,00005 cfu/g (-4,3 $\log_{10}$).

The model $H_0 - \sum R + \sum I \leq FSO$ is used for the situation at home kitchen. The growth of EHEC is dependent on the temperature and is characteristic for the food. Supposing the temperature during transportation from industry to the retail shop has been less than 10 degree C, no growth has taken place. The growth during transportation from retail shop and during home storage can be estimated by predictive modelling programme (USDA PMP6). Supposing the transportation temperature is 18 degree C, the time is 2 h, the pH of the food is 6,9 and water activity 0,997, the growth during transportation that can be estimated is 0,13 $\log_{10}$. The growth during storage can be estimated by the same way. If this storage time is of 3 days at 10 degree C, the growth during storage is 1,11 $\log_{10}$ and $\sum I_0$ is 1,24 $\log_{10}$.

The initial level H_0 for the home kitchen is the performance objective PO and the $\sum I_0$.

$H_0 \leq MFSO + \Sigma R - \Sigma I$, when FSO = safety level = -4,3; ΣR =3,5: ΣI = 0; $H_0 = PO + \sum I_0$

$PO + \sum I_0 \leq$ safety level + $\Sigma R - \Sigma I$

$\Leftrightarrow$

$PO + 1{,}24 \leq -4{,}3 + 3{,}5$

$\Leftrightarrow$

$PO \leq -4{,}3 + 3{,}5 - 1{,}24$

$\Leftrightarrow$

$PO \leq -2{,}04$

The performance objective of –2,04 $\log_{10}$ means that 1 g of food could have EHEC level of 0,0092 cfu/g at the end of the processing in the industry level. The in proper storage of food at 10 ° C for 3 days at home is included in the estimation.

II Comparing the performance objective to known levels of EHEC

It's very rare to know the number of EHEC-bacteria in a food. Therefore reliable comparisons between estimated PO and real levels are not possible.

The estimated performance objectives are linked to the effect of home cooking. If the reduction effect of cooking at home is not taken into consideration at all, ΣR is zero and the performance objectives according the model would be much more stringent.

Conclusions

According to the model used in this paper, the estimated performance objective of 1,2 $\log_{10}$ for the Campylobacteria in poultry is not exceeded in the industry. If poultry meat is heated and cross-contamination is avoided at home kitchen, poultry should not be a risk and a source of Campylobacter infections.

However, a lot of research is needed before more exact estimations can be given. More information on the actual levels of Campylobacteria in poultry is needed and quantitative methods should be favoured instead of qualitative analysis. Instead of estimation of safety level used in this paper, proper national Food Safety Objective, FSO should be assessed for Campylobacteria in poultry.

The performance objective of –2,04 $\log_{10}$ for EHEC in food is estimated in this paper. However the quantitative information of EHEC levels does not exit and the practical achievement of this theoretical performance objective is not evaluated.

Both estimated performance objectives are dependent on the effect of cooking at home.

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QUANTITATIVE RISK ASSESSMENT OF PATHOGENIC *YERSINIA* AND *LISTERIA MONOCYTOGENES* CONTAMINATION PROCESS ALONG PRODUCTION STAGES OF ORGANIC AND TRADITIONAL PORK MEAT

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Background

Consumers are becoming increasingly aware of the quality of meat and meat products as well as animal welfare and production issues. Consequently, interest on organic production where animals have lower animal density and possibilities for expressing normal behaviour has grown. In general, consumers also expect products from this kind of system to be of a higher microbiological quality compared to products from conventional production systems. Unfortunately, only limited amount of information on differences of microbiological food safety risks in organic and conventional farming is available (Muukka *et al*. 2003, Williams & Hammitt 2001). In order to obtain a reliable data for public discussions as well as for farmers planning to start the organic production, a study was launched for pig production. Examples of quantitative risk assessments can be found e.g. in Coleman *et al*. 2003, Hartnett *et al.* 2001, Rasmussen *et al.* 2001 and Nauta *et al.* 2000.

Objectives

The goal of this study is to create a risk assessment model in order to assess risks in different parts of the pork production chain for three microbiological pathogens (*Yersinia enterocolitica*, *Yersinia pseudotuberculosis* and *Listeria monocytogenes*) in organic vs. conventional production systems (CAC/GL-30 1999).

Materials and methods

Three groups of pig farms were chosen for the study:

- 5 organic farms,
- 5 conventional farms with similar production capacity as organic farms, and
- 5 conventional large meat producing farms

In every farm, pigs are sampled at farm level as well as in slaughterhouses. In addition, product samples from organic and conventionally produced meat will be taken. In addition to that, other relevant data on production farms, risk factors, behaviour of these three pathogens in environment, meat production volumes etc. are gathered for the risk assessment model. The model will cover the chain from animals intended for slaughter up to the meat cuts sent for retail level.

The infection status of a pig is analysed at two points by microbiological testing: at the farm from living pigs and after slaughter. While the first measurement is a single faecal test, the second measurement consists of several different tests taken at the slaughterhouse. The two tests are taken from the same individuals which allows detailed studies of the change in infection status over production chain.

A probability model is constructed to describe the probability of infection at the first testing and the transition probability from this initial infection status to the second infection status at the slaughterhouse. This model can further extended to describe the third measurement which is taken from meat cut samples. However, the third measurement no longer represents individual specific data. The model then describes the population level only, i.e. the probability of certain meat sample result given the prevalence in certain pig population at the slaughter stage testing. The model can either describe the apparent prevalence at all observation points in two different production systems (Figure 1), or it can be used to estimate the true prevalence by accounting for the test sensitivity for each type of test. It can also be used to simulate the effect of possible interventions, if needed (Gelman *et al.* 2004, Clough *et al.* 2003, Cox 2002, Congdon 2001)

Preliminary results and discussion

The results can be used as point estimates or as Bayesian probability distributions of likely parameter values, given data. The joint distribution can be exploited when simulating the production process for making predictions under the current situation and under alternative scenarios concerning changes in the production (proportion of organic vs. conventional can change, small farms quit, all farms will be of the highest/lowest risk index group, etc.) We have already do simple experiment with limited amount of data, so the process is still going on. It will be possible to start final model simulation runs and compare both production systems when all of the data collection is finish. Simulation runs and reporting the result of the model will be done by the end of the year 2005.

Acknowledgments

We are gratefully acknowledged to M.Sc. Arja Peltomäki from the Finnish Association for Organic Farming, food industry and farmers. This work was supported by the Agriculture Development Foundation of MAKERA/ Ministry of Agriculture and Forestry.

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Figure 1

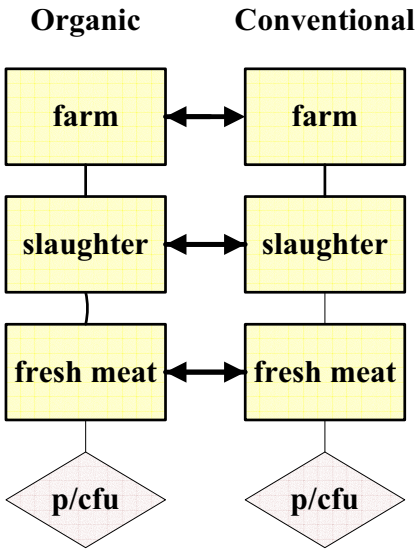

FIGURE 1. Skeleton of mathematic model

EFFECT OF MODIFIED ATMOSPHERES PACKAGING ON THE HYGIENIC AND ORGANOLEPTIC QUALITY OF "CECINA DE LEON" KEPT FOR EXTENDED STORAGE

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Background

Spanish "Cecina" is a salted, dried, and smoked beef meat product manufactured traditionally in the province of Leon (north-western Spain). The preparation method is similar to that used in dry-cured ham manufacture. The final product has a typical red colour, smoked flavour and a characteristic slight salty taste.
During the last years many conservation procedures have been developed, such as vacuum packaging, packaging in CO_2/N_2 atmosphere, etc., in order to increase the shelf-life period of meat products to guarantee its sanitary and organoleptic quality. However, no studies have been carried out on packaging of "Cecina de Leon".

Objectives

The aim of the present study was to investigate the shelf life of commercial "Cecina de Leon" packaged in CO_2/N_2 atmosphere and vacuum under refrigeration for an extended storage period.

Materials and methods

Samples. The study was carried out on 12 pieces of "Cecina de Leon" provided by Protected Geographical Indication (PGI) "Cecina de Leon". Each piece was divided in 5 portions: one portion was used for initial analyses and the other portions were packaged.
Packaging and storage. The "Cecina" portions were individually packaged either: (a) in plastic bags (polyamide/polyethylene) which were subjected to vacuum (VP) and sealed using a packer TECNOS.CVP mod: A-300 or (b) in polystyrene rigid trays which, after gassing with a mixture of 20/80% CO_2/N_2 (MAP), were closed by heat-sealing with a packer TECNOVAC mod: Linvac 400 with a high barrier film. Packages had a headspace volume ratio of 1:1. The gas content of each pack was controlled using a Servomex model 1450 B3 gas analyzer. The packages were stored at 6ºC. Four packs of each treatment were opened for subsequent analysis after 15, 30, 60, 90, 150 and 210 days of storage. The entire experiment was replicated twice.
Microbiological analysis: 10 g of each sample were taken aseptically and were homogenised with 90 ml of tryptone water for 2 min in a PK 400 Masticator. Serial decimal dilutions were made in sterile tryptone water and plated onto growth in duplicate. The samples were analysed for aerobic mesophilic bacteria (Plate Count Agar (Scharlau, Spain) at 30 ºC for 72 h), psychrotrophic bacteria (Plate Count Agar (Scharlau, Spain) at 7 ºC for 10 days), anaerobic bacteria (Schaedler Agar (Scharlau, Spain) at 37 ºC for 48 h), enterobacteria (Violet Red Bile Glucose Agar (VRBGA, Scharlau, Spain) at 37 ºC for 24h), enterococci (Slanetz Bartley Agar (Scharlau, Spain) at 37 ºC for 24 h), pseudomonas (Pseudomonad Agar (Oxoid, Spain) supplemented with Cetrimide, Fucidine and Cephaloridine (CFC, Oxoid, Spain) at 30 ºC for 48 h), lactic acid bacteria (LAB) (MRS Agar (Scharlau, Spain) at 30 ºC 72 h), micrococci (MSA (Scharlau, Spain) at 37 ºC for 48 h), yeasts and molds (Agar OGYEA (Oxoid, Spain) supplemented with Oxytetracicline (Oxoid, Spain) at 25 ºC for 5 days).
Physicochemical analysis: Water activity (a_w) was measured by CX2 AQUA LAB equipment. The pH values were determined by puncture with a pH meter equipped with a glass electrode. Objective measurement of colour was performed at the surface of "Cecina" portions using a reflectance spectrophotometer (Minolta CM-2002). Colour coordinates were determined in the CIE-LAB system and the results were expressed as lightness (L*), redness (a*) and yellowness (b*). Instrumental Texture Profile Analysis (TPA) (Breene, 1975) was performed with a TA-XT2 Texture Analyzer (Stable Micro Systems Ltd.). Six cubes of "Cecina" (1x1x1 cm) were compressed twice with a cylindred probe of 1 cm in diameter, at 1 mm/min speed and the

level of compression was 60% of the thickness of the sample (1 cm). The test was accomplished always at room temperature and the parameters determined was: hardness, springiness, cohesiveness and chewiness.
Sensory evaluation was carried out on "Cecina" slices after each storage time by an experienced 8-member sensory panel. The sensory attributes (colour, odour, flavour, hardness, juiciness and overall acceptance) were scored using 5-points scales, 5 denoted extremely high and 1 denoted extremely low.
Statistical analysis of data was carried out by one-way analysis of variance, and means were separated by Tukey- honest significant difference test at 5% level (Statistica software package).

Results and discussion

Figure 1 shows results of the microbiological analysis, except enterobacteria and enterococci counts, which were always $<10^2$ cfu/g. Aerobic mesophilics and anaerobes counts were similar to psychrotrophs counts. Psychrotrophs numbers in VP remained constant for up to 210 days. However, a significant increase ($p<0.05$) was noted in MAP after 210 days of storage ($>10^7$ cfu/g). Among the undesiderable microbial strains, Pseudomonas were subjected to a significant growth inhibition under 20/80% CO_2/N_2. This fading is caused by the bacteriostatic effect of CO_2. The typical microflora of "Cecina" (LAB, yeasts and molds and micrococci) remained constant for up to 210 days in VP but a significant increase ($p<0.05$) was noted at the end of storage in MAP.

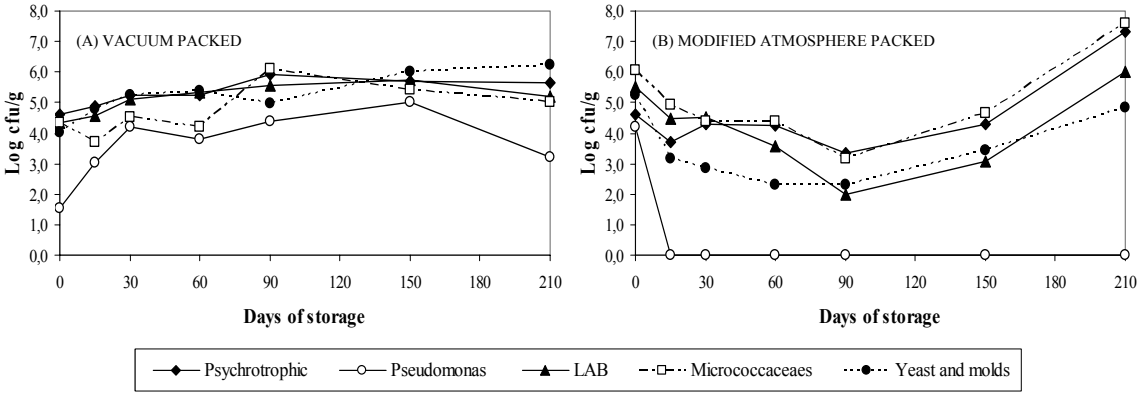

Figure 1: Evolution of microbiological parameters of "Cecina" packed under vacuum (A) and under 20/80% CO_2/ N_2 (B) during storage.

Regarding pH and a_w values, these parameters remained constant during storage. No differences in pH and a_w were found from VP and MAP samples. The pH values in all samples were in the range 5.8-6.1 and the a_w values were typical of the intermediate moisture foods (0.878-0.903).
Results of colour measurement are shown in Figure 2. No differences ($p>0.05$) in lightness (L*) and yellowness (b*) values were found between treatments at any given storage times. With regard to redness (a*), which has been used as an indicator of colour stability in meat and meat products, a pronounced initial fading was seen within the first 15 days for VP samples and within the second month for MAP samples. Presence of white film on surface of "Cecina" portions is a probable explication for discolouration. The later appearance of white film in MAP samples can be attributed to presence CO_2. Arnau *et al*., (1988) found that CO_2 delayed white film formation in Iberian ham packed in modified atmosphere

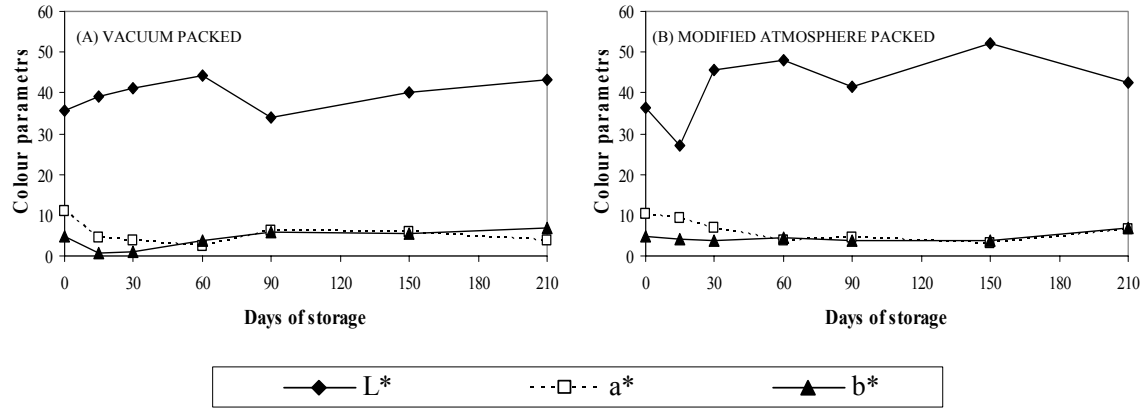

Figure 2: Evolution of colour parameters of "Cecina" packed under vacuum (A) and 20/80% CO_2/N_2 (B) during storage.

In relation to the texture parameters, no differences ($p>0.05$) were found between MAP and VP samples during the studied period. Values (means ± SD) found for different parameters were: hardness=2413.7±486.2 g; springiness=0.368±0.036 mm; cohesiveness=0.395±0.017; chewiness=375.09±111.76 g mm. Taking into account the normal variability found for these parameters, all values were considered normal for this type of product (García et al., 2004).

Table 1: Sensory parameters (mean ± S.D.) of "Cecina" slices packaged under different conditions during storage.

Parameter	Packaging	0 days	15 days	30 days	60 days	90 days	150 days	210 days
Colour	Vacuum	$^{a}_{B}$4.8 ± 0.2	$^{a}_{A}$4.5 ± 0.5	$^{a}_{B}$4.8 ± 0.5	$^{a}_{A}$4.4 ± 0.5	$^{a}_{A}$4.4 ± 0.5	$^{a}_{A}$4.4 ± 0.5	$^{a}_{A}$4.6 ± 0.5
	20/80	$^{a}_{A}$4.3 ± 0.2	$^{a}_{A}$4.4 ± 0.5	$^{a}_{A}$4.1 ± 0.4	$^{a}_{A}$4.3 ± 0.5	$^{a}_{A}$4.3 ± 0.7	$^{a}_{A}$4.3 ± 0.5	$^{a}_{A}$4.3 ± 0.5
Odour	Vacuum	$^{d}_{B}$5.0 ± 0.0	$^{bcd}_{A}$4.5 ± 0.5	$^{cd}_{A}$4.6± 0.5	$^{abc}_{A}$ 4.1±0.4	^{abc}3.9$_{A}$ ± 0.6	$^{ab}_{A}$3.8± 0.5	$^{a}_{A}$3.6 ± 0.5
	20/80	$^{b}_{A}$4.2 ± 0.3	$^{b}_{A}$4.1 ± 0.8	$^{b}_{A}$4.1 ± 0.6	$^{b}_{A}$3.9 ± 0.4	4.0 $^{b}_{A}$ ± 0.8	$^{ab}_{A}$3.4 ± 0.7	* $^{a}_{A}$2.9 ± 0.3
Flavour	Vacuum	$^{b}_{B}$5.0 ± 0.0	$^{ab}_{A}$ 4.4 ± 0.5	$^{b}_{B}$ 4.5 ± 0.5	$^{ab}_{A}$ 4.4 ± 0.5	$^{a}_{A}$3.8 ± 0.5	$^{a}_{B}$3.8 ± 0.5	$^{a}_{B}$3.8 ± 0.5
	20/80	$^{c}_{A}$ 4.1 ± 0.3	$^{bc}_{A}$ 3.8 ± 0.5	$^{bc}_{A}$ 3.8 ± 0.5	$^{c}_{A}$ 3.9 ± 0.6	$^{c}_{A}$ 4.0 ± 0.5	$^{ab}_{A}$ 3.0 ± 0.5	* $^{a}_{A}$ 2.8 ± 0.5
Hardness	Vacuum	$^{b}_{A}$ 5.0 ± 0.0	$^{ab}_{A}$ 4.5 ± 0.5	$^{ab}_{A}$ 4.4 ± 0.5	$^{a}_{A}$ 4.1 ± 0.4	$^{a}_{A}$ 4.0 ± 0.8	$^{a}_{A}$ 3.9 ± 0.6	$^{a}_{B}$ 3.8 ± 0.4
	20/80	$^{b}_{A}$ 4.6 ± 0.1	$^{b}_{A}$ 4.3 ± 0.5	$^{b}_{A}$ 4.3 ± 0.9	$^{b}_{A}$ 4.3 ± 0.7	$^{b}_{A}$ 4.1 ± 0.8	$^{ab}_{A}$ 3.9 ± 0.8	* $^{a}_{A}$ 2.9 ± 0.4
Juiciness	Vacuum	$^{b}_{A}$ 5.0 ± 0.0	$^{ab}_{A}$ 4.6 ± 0.5	$^{ab}_{A}$ 4.5 ± 0.5	$^{a}_{A}$ 4. ± 0.4	$^{a}_{A}$ 4.1 ± 0.8	$^{a}_{A}$ 4.1 ± 0.6	$^{a}_{A}$ 3.9 ± 0.2
	20/80	4 $^{b}_{A}$.6 ± 0.2	$^{ab}_{A}$ 4.4 ± 0.5	$^{ab}_{A}$ 4.3 ± 0.5	$^{ab}_{A}$ 4.1 ± 0.8	$^{ab}_{A}$ 4.1 ± 0.6	$^{ab}_{A}$ 3.9 ± 0.6	$^{a}_{A}$ 3.5 ± 0.5
Acceptance	Vacuum	$^{d}_{B}$ 5.0 ± 0.0	$^{bcd}_{A}$ 4.4 ± 0.4	$^{d}_{B}$ 4.6 ± 0.3	$^{cd}_{B}$ 4.5 ± 0.5	$^{a}_{A}$ 3.7 ± 0.5	$^{abc}_{A}$ 3.9± 0.4	$^{ab}_{B}$ 3.8 ± 0.4
	20/80	$^{b}_{A}$ 4.1 ± 0.1	$^{b}_{A}$ 3.9 ± 0.6	$^{b}_{A}$ 3.9 ± 0.4	$^{b}_{A}$ 3.9 ± 0.5	$^{b}_{A}$ 4.0 ± 0.5	$^{ab}_{A}$ 3.4 ± 0.6	$^{a}_{A}$ 3.1 ± 0.3

Means with different small letters indicate significant differences between storage times (Tukey test: $p<0.05$).
Means with different capital letters indicate significant differences between packaged treatments (Tukey test: $p<0.05$).
* Values below 3 means that "Cecina" was not accepted.

Table 1 shows the results of the sensory evaluation of "Cecina". According to panellists, MAP samples were slightly less acceptable (3.1) than VP samples (3.8) at the end of storage. As panellists evaluated odour, flavour and hardness in MAP samples below 3, it could be considered that colour and juiciness were the main factors which influenced the panellist in the evaluation of overall acceptance. This agrees with the conclusion of Ruiz *et. al.* (2002) who reported a relationship between juiciness and acceptance in Iberian ham. The sensory evaluation of colour did not confirm the instrumental results since sensory analysis was carried out on "Cecina" slices instead of portions.

Conclusions

On the basis of the results reported, in microbiological and sensory terms the shelf life of chilled vacuum packed "Cecina" in the conditions given in this study is 210 days, and the shelf life of chilled modified atmosphere packed (20/80% CO_2/N_2) "Cecina" is 150 days (based mainly on microbiological counts).

Acknowledgments:

This research was supported by the ITA project (2002/000999). Rubio, B and Molinero, C gratefully acknowledges of a grant from Junta de Castilla y Leon-Spain.

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SENSORY AND MICROBIOLOGICAL PROPERTIES OF DRIED HAMS TREATED WITH HIGH HYDROSTATIC PRESSURE (HP)

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Background

Expansion of ready to eat (RTE) foods in the last decade has urged the need to ensure microbial safety while maintaining sensory and nutritional qualities. Among innovative mild technologies high hydrostatic processing (HP) is a promising technique for the treatment of those products that are not heat treated, such as dried hams and sausages.

Objectives

Aim of the present study was to evaluate the effect of HP treatment on the sensory and physical (instrumental colour assessment) properties of dry cured hams. The study was conducted after a preliminary investigation indicated the best conditions (pressure, time) enabling inactivation of *Listeria monocytogenes* (LM).

Materials and methods

HP equipment

HP treatments were performed by a pilot plant developed by Avure Technologies (Vasteras – Sweden). It consisted of a vertical vessel with a total capacity of 35 litres and enabling a T range of 4-90°C and max pressure of 600 MPa.

All HP treatments were performed at 600 MPa with different holding times (3, 6 and 9 minutes) at 25 °C.

Survival of Listeria monocytogenes in HP treated hams

The trials were carried out on ham slices (14 month of maturing, a_w=0.92) inoculated with *Listeria monocytogenes*, then vacuum-packed and finally treated at 600 MPa for three holding times: 3, 6 and 9 minutes (3 independent treatments). Microbial analyses were carried out at 24 hours after treatment.

1. *Inoculum preparation and inoculation procedure*

   The inoculum was prepared from a mixture of strains (Scott A and LM38, LM39, LM51 and LM134) previously isolated from meat products. The samples (N=140) were slices of dried hams (a_w= 0.92) of 25 g weight. They were partly inoculated (N= 100) at a concentration of about 10^4 cfu/g , while the remaining 40 slices were used as control. All slices were vacuum packaged for HP treatment.

2. *Microbiological analysis*

   a. *Detection of Listeria monocytogenes.* In order to determine the presence/absence of LM in 25 g of product, the ISO 11290-1 method was applied both as such and by using the ALOA (Biolife) medium (37° C for 24/48 hours) for isolation and differentiation of Listeria colonies. To quantify the contamination level (n. of Listeria/g), direct plate as count (ISO 11290-2) and/or the MPN as proposed by USDA were used. In both methods, the ALOA medium (Biolife) was used.

   b. *Total aerobic microbial count.* Triptone soy agar was used as medium (OXOID) and count was determined after incubation at 30°C for 72 hours determined.

Sensory evaluation

Descriptive attribute analysis of hams after HP treatment was conducted by a 8-member trained panel. Descriptors were rated on an intensity scale ranging 0-9. Descriptors were uniformity and intensity of colour, matured taste, salty taste, off-flavour, fibrousness on chewing, firmness.

Results and discussion

Effect of HP treatment of Listeria monocytogenes survival.

Surviving data after treatments are reported in table 1 as positive packages and surviving Listeria cells. Increasing the treatment duration (from 3 to 9 minutes) decreased viable LM cells. After 3 minutes at 600

MPa, all packages were found positive; after 6 minutes 9 out of 25 packages were positive and after 9 minutes LM was present only in one package. Results are consistent with those of other researchers who reported inactivation of fresh meat and dry-cured ham of 4-5 log cycles and 2-3 cycles respectively (1-4).
HP effects on typical microflora of dry-cured hams were also evaluated (fig.1). Microflora was reduced at all treatment times (mainly Staphylococcus strains), with 2.6, 4 and 4.5 decimal reductions after 3, 6 and 9 treatment times, respectively.
It is known that the effect of HP treatment on bacterial inactivation is influenced by several factors such as microbial morphological and structural characteristics (Gram positives more resistant than Gram negatives, cocci more resistant than rods) and physico-chemical properties of food-matrix (5). Our findings indicate that HP had more effect on LM than on typical microflora of dry-cured ham.

Sensory quality assessment of HP-treated dried hams: effects of maturing time and storage after treatment.
A trained sensory panel was asked to judge dry cured hams of different maturing age (14 and 18 months) after HP treatment. Mean scores of sensory attributes are reported in table 2, showing that colour, salty taste and firmness were mainly affected. Colour intensity decrease was less evident at 18 months, with HP treatment not significant ($P<0.05$) compared with treated samples. The matured taste did not change significantly after treatment, whereas salty taste was more intense in treated samples for both classes of age, with a marked increase in the less matured ones. Major changes in lean meat structure were also reported in terms of increased fibrousness on chewing and firmness (resistance to compression). Data indicate that sensory properties were less affected in the more aged hams, which was likely due to the relative lower impact of pressure on more dehydrated meat; this conclusion is in agreement with similar observations from other Authors working on meat substrates and other than dried hams (6).
Lighness (L*) increased after HP treatment, whereas redness (a*) decreased and yellow (b*) increased significantly (table 3). Therefore the a*/b* ratio was lower in all treated samples while the *hue* index, being inversely correlated with redness, increased. These changes were consistent with the sensory test data (table 2), suggesting that discoloration occurred as result of treatment.
In order to assess whether cold storage of HP treated hams were susceptible to change of sensory quality traits, hams were analysed at different times during one month of storage at 4°C. Results are graphically reported in fig.2 for sensory colour intensity, showing an increase of scores after 7 days of storage. It is concluded that cold storage of hams after treatment is a means to reduce the impairing effect of pressure on meat colour affecting dried hams immediately after the HP processing.

Conclusions

High hyperbaric pressure treatment allows reduction of *Listeria monocytogenes* to negligible levels in dry cured ham. Treatment affects colour (discoloration) and saltiness (enhanced perception) in such a way that changes are inversely related to the age of the ham.

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Table 1. Effect of HP treatment at 600 MPa on *L. monocytogenes* at inoculum level of 4.64 ± 0.58 log cfu/g.

Treatment time (minutes)	Packages treated	Packages positive after treatment (LM in 25 g)	Sample N°	Average value	Range (p=0.95)
3	25	25	14	<0.3 MPN	0.0-0.94
			11	23.2 ufc	2.6-43.8
6	25	9	9	0.47 MPN	0.0-0.97
9	50	1	1	<0.3 MPN	0.0-0.94

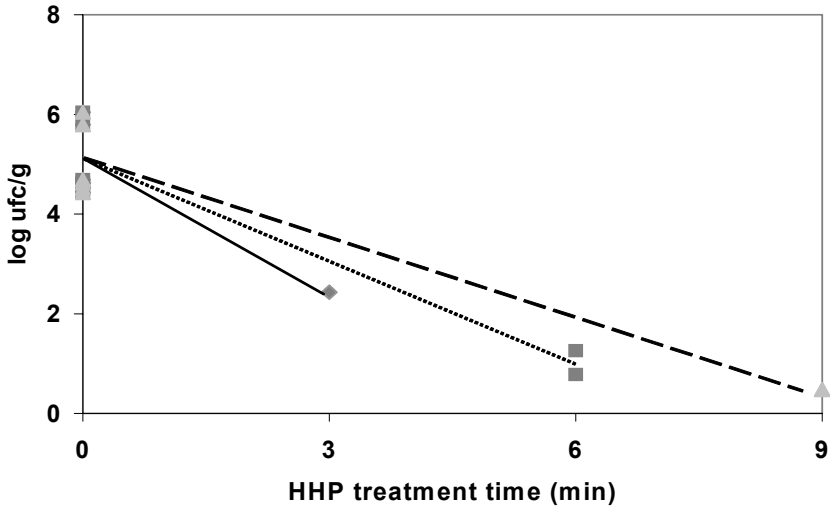

Figure 1. Total aerobic count changes after HP treatment for three different times.

Table 2. Mean scores (scale 0-9) of sensory attributes of HP-treated hams (14 and 18 months of maturing) and corresponding control samples. Means within a row with different letters differ significantly (P<0.05). Higher scores denote more intense perception of attributes.

	Dry-cured hams			
	14 months		**18 months**	
	untreated	**HP-treated**	**untreated**	**HP-treated**
Colour uniformity	6.7 [a]	6.1 [b]	6.7 [a]	6.4 [ab]
Colour intensity	6.7 [a]	5.2 [b]	6.9 [a]	6.4 [a]
Aged taste	6.3 [bc]	6.1 [c]	6.7 [a]	6.5 [ab]
Salty taste	6.0 [b]	6.9 [a]	6.3 [b]	6.8 [a]
Fibrousness	1.9 [c]	4.3 [a]	1.8 [c]	3.7 [b]
Firmness	4.6 [b]	6.3 [a]	5.1 [b]	6.6 [a]

Table 3. Instrumental colour measurements of HP-treated hams (14 and 18 months of maturing) and corresponding control samples. Means within a row with different letters differ significantly (P<0.05).

	Dry-cured hams			
	14 months aged		**18 months aged**	
	untreated	**HP treated**	**untreated**	**HP treated**
L*	42.8 [b]	46.9 [a]	41.7 [b]	45.8 [a]
a*	10.1 [a]	9.6 [b]	10.3 [a]	9.9 [ab]
b*	9.6 [b]	11.2 [a]	9.6 [b]	11.6 [a]
Chroma*	14.0 [c]	14.8 [ab]	14.1 [bc]	15.4 [a]
hue	43.3 [b]	49.5 [a]	43.2 [b]	49.4 [a]
a*/b*	1.07 [a]	0.86 [b]	1.07 [a]	0.86 [b]

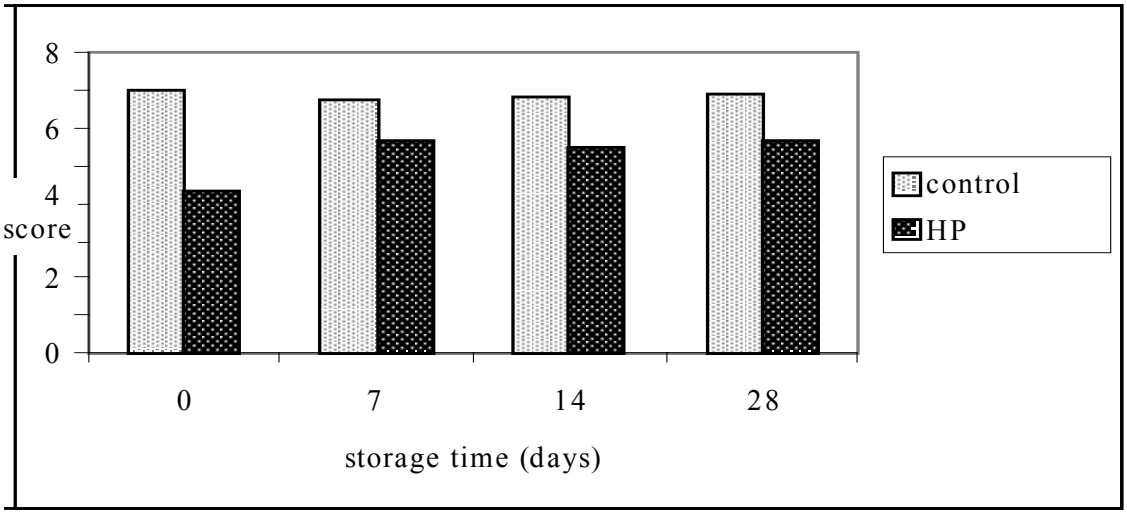

Figure 2. Sensory colour intensity of HP-treated hams and correspondig control samples at several times of cold storage.

MANUFACTURING PROCESS DEVELOPMENT ON A READY TO HEAT AND EAT PORTUGUESE TRADITIONAL PRODUCT: "MARANHOS"

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Background

Cooked meat products are generally marketed after having been heat-processed within the production facility. The application of heat to these products as a finishing production step, and before they are sliced and/or packaged, is necessary to make them easily digestible, to inactivate enzymes (e.g. lipasas) and microorganisms, and to give them a specific colour, taste and consistency (Hammer, 1991). In recent years there has been a tremendous growth in the chilled food market, with a wide variety of products available to the consumer through retail and catering outlets (HMSO, 1993). Safety is the prime consideration and food manufacturers must ensure that products pose a minimum hazard to the consumer. The safety required has to be achieved both by preventing the growth of pathogens during production and by reducing the remaining contamination to the lowest possible level (Barbuti & Parolari, 2002).

"Maranhos" is a goat meat sausage, traditionally home-made in Beira Baixa, a region in the centre of Portugal, where it is a very popular dish. It is a product with low price raw materials, easily perishable, with a reasonable composition and energetic value and low salt content. Although no pathogenic flora is usually present in the product, research on and counts of hygienic indexes are higher than desirable. Therefore, the microbiological quality of the product is low (Salavessa & Barreto, 2003).

Objectives

Research on manufacture of this type of product has been extremely limited, therefore this work aims to study the effect of pre-cooking on both the chemical and microbiological characteristics of "Maranhos".

Materials and methods

Three batches of this meat product were prepared in three different times, according to a traditional recipe and technological procedures used by a local manufacturer. "Maranhos" were prepared from raw ground adult goat meat mixed with rice and seasoned with salt, peppermint (*Mentha* sp.) and white wine. All the ingredients were then stuffed into natural casings, small bags especially made from the goat gastric compartments, and boiled for 75 minutes in a pan.
The water temperature and the temperature in the slowest heating point (SHP) of the product were measured during the cooking process with a CTF9008 Precision Digital Thermometer & F_0- Value Computer, ELLAB.
Samples of four uncooked and four cooked "Maranhos" were collected from each batch and immediately transported under cooling conditions to the laboratory.
Moisture content was determined by dissection until constant weight at 105ºC (Martins & Patarata, 1993). Ashes were determined by weighing the mineral residue after incineration at 550-600ºC (Martins & Patarata, 1993). Fat content was determined by the Soxhlet method (Martins & Patarata, 1993). Protein content was determined by the Kjeldhal method (Martins & Patarata, 1993). Carbohydrate content was estimated by exclusion of moisture, ashes, fat and protein contents. Energetic value was determined according to the classical conversion factors of Atwater (Martins & Patarata, 1993). pH was measured with a pH-meter HI9023-HANNA INSTRUMENTS. Water activity (a_w) was measured with the ROTRONIC HYGROSKOP DT, with the measure cell WA-14TH at 25ºC of constant temperature. Salt content was determined by the current method (NP-1845, 1982). Free fatty acids (FFA) were measured by extraction by chloroform and titration with 0.1N sodium hydroxide using phenolphthalein (Person, 1970). Peroxide value (PV) was determined by extraction by chloroform and titration with 0.01N sodium tiossulphat using starch as an indicator (Hungarian Standards, 1973). Tiobarbituric acid (TBA) was determined by spectrophotometer UV/Visible PHARMACIA BIOTEC Ultrospec®2000 with λ=538 nm (Martins & Patarata, 1993). Total basic volatile nitrogen (TVN) was determined by Conway cells method (Martins & Patarata, 1993).

For the microbiological analysis a 25 g sample of each sausage was aseptically transferred to a sterile plastic bag and pummelled in a stomacher LAB BLENDER-400 with 225 ml of buffered peptone water (DIFCO). Decimal dilutions of suspension were prepared using triptone salt solution (SCHARLAU) and plated in duplicate on different growth media. The following media and incubation conditions were used: total aerobic in Plate Count Agar (SCHARLAU) at 30ºC for 2 days; total psicrophiles counts in Plate Count agar (SCHARLAU) at 7ºC for 10 days; total anaerobes count in Anaerobic Agar acc. to Brewer (MERCK) inside an anaerobic jar at 7ºC for 10 days; total termophiles counts in Plate Count agar (SCHARLAU) at 42ºC for 2 days; moulds and yeasts counts in Cooke Rose Bengal agar with clorophenicol (OXOID) at 25ºC for 5 days; *Enterobacteriacea* counts in Violet Red Bile agar (OXOID) at 37ºC for 2 days; lactic acid bacteria counts on Man Rogosa Sharpe agar (OXOID) at 30ºC for 3 days; *E. coli* research with Kovacs reagent in Briliant Green broth (SCHARLAU) and Peptone water (DIFCO) at 45ºC for 2 days; *Clostrdium* sulphite reducers spoors research in Sulfadyzine Polimyxine Sulphite agar (MERCK) at 45ºC for 2 days; *S. aureus* research by the cuagulase test after isolation of suspicious colonies in Baird Parker agar (OXOID) and then Brain Heart Infusion (DIFCO) at 37ºC for 1day; *Salmonella* research by biochemical test API 20E (BIOMÉRIEUX) after isolation of suspicious colonies; and *Listeria monocytogenes* research by biochemical test API *Listeria* (BIOMÉRIEUX) after isolation of suspicious colonies.

Means of uncooked and cooked product were subjected to one-way ANOVA analysis of Statgraphics v. 7.0.

Results and discussion

Heat treatment is a critical operation, which takes part in most manufacturing processes of meat products, that controls not only microrganism growth but also affects taste, flavour, colour and texture of end products, allowing for sensorial features required by consumers (Botelho *et.al.,* 2003). The evolution of temperature values in SHP during the cooking process of the product is shown on the time/temperature chart (Fig. 1), the product reached temperatures above 90 °C for a range of time longer than 10 minutes, which are expected to cause a 6 log reduction of all vegetative pathogens present including spores of psychrotrophic *Clostridium botulinum*. However, heat resistant spores and pre-formed toxins may persist. That is, the heated product should be cooled as quickly as possible through temperatures that minimize the risk of spore germination and outgrowth. The cooling time will vary from product to product, but as a guideline, it should not exceed 4 hours (HMSO, 1993). Tables 1, 2, 3 and 4 summarise the results as mean, minimum and maximum values, standard deviation (SD), coefficient of percentage variation (CV%) and treatment significance (P) on the physicochemical and microbiological analyses.

Mean values for chemical composition of the uncooked product were the following: moisture 59.30%, protein 12.33%, fat 10.36%, carbohydrates 16.22% and ashes 1.79% and the mean energetic value was 871.25 kj/100g. Mean values for chemical composition of the cooked product were the following: moisture 60.58%, protein 14.08%, fat 7.50%, carbohydrates 16.50% and ashes 1.35% and the mean energetic value was 797.00 kj/100g. The differences observed between uncooked and cooked product in protein, fat and ash contents were very significant ($p<0.01$). The decrease in fat content could be explained by fat losses that occur during the cooking process, with a consequent increase in protein content in the centesimal composition of the cooked product. Fat melts at 37-40ºC, free fat may therefore escape from a product mixture at quite low temperatures unless held in an effective matrix (Ranken, 2000).

No significant differences between uncooked and cooked product were observed in the degradation indexes analysed. Mean values for uncooked and cooked product were: for FFA 3.67% and 3.85% as oleic acid, for PV 15.87 and 13.27 mequiv/kg of extracted fat, for TBA 0.28 and 0.37 mg of malonaldehyde/kg of analysed product and for TVN 25.91 and 21.81 mg/100g.

pH and a_w mean values were of 5.90 and 0.937 in the uncooked product and of 6.22 and 0.939 in the cooked product, which means that in both cases “Maranhos” is an easily perishable meat product. The differences observed in the pH value, which increased slightly after cooking, were very significant. A slight increase in the pH value is expected to happen when sodium salts are added to meat products because of their slight alkaline nature (Wirth, 1992). Highly significant differences in salt content were observed with a decrease from 1.07% in the uncooked product to 0.74% in the cooked one. These differences are due to salt diffusion into the water used to boil the product.

Highly significant differences were found in all the microbiological counts, and very significant differences for the *Enterobacteriacea* counts. The mean value for the aerobic plate count in the uncooked product was of 7.06 $\log_{10}$ cfu/g and after cooking the value decreased to 3.94 $\log_{10}$ cfu/g. Termophiles also reduced the accounts from an initial 4.34 to 2.93 $\log_{10}$ cfu/g, psicrophiles reduced from 7.05 to 2.93 $\log_{10}$ cfu/g, anaerobes reduced from 6.35 to 2.22 $\log_{10}$ cfu/g, moulds and yeasts reduced respectively from 2.24 and 4.92 to 1.11 and 2.24 $\log_{10}$ cfu/g, *Enterobacteriacea* reduces from 5.34 to 3.66 $\log_{10}$ cfu/g and the number of lactic acid bacteria reduced from 5.04 to 2.32 $\log_{10}$ cfu/g. *Staphylococcus aures* coagulase positive was present up to 0.1g in the cooked product. Research on the cooked product for *E.coli* and *Clostridium* sulphite reducers' spoors (absent in 1 g), and for *Salmonella* and *Listeria monocytogenes* (absent in 25 g) was always negative.

Conclusions

The ingredients hygienic quality is low therefore the raw product is heavily contaminated with vegetative microorganisms. Sometimes pathogens can be present. The shelf life of the raw product is very short and, therefore, it can be considered a dangerous product because it may be a source of cross contamination to other foods.

The heat processing of the product improves its hygienic standards and eliminates vegetative pathogens but not all spore forms. However, it cannot be overestimated because there is risk of recontamination during assembly that may present a food safety hazard. On the other hand, the heat treatment is probably too long, which results in fat losses and production yields.

Shelf life of the heat-processed product will depend on the control of all the above-mentioned factors. The growth of pathogenic flora can be controlled by product formulation, packaging systems and chill storage conditions. Modifications based on technology and combined preservative factors should be researched in order to develop a hurdle technology capable of preventing microbiological and chemical spoilage as well as the risk of food borne diseases to the consumer.

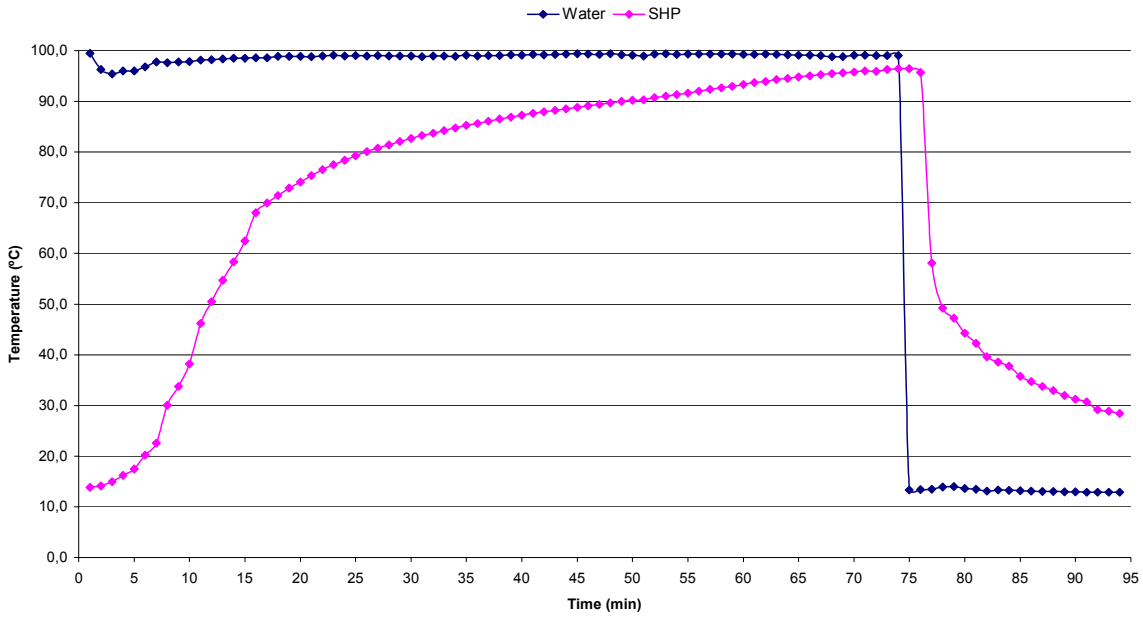

Figure 1. Temperature evolution in water and SHP of the product.

Table 1. Chemical composition**.**

	Uncooked					Cooked					
	Mean	Min	Max	SD	CV%	Mean	Min	Max	SD	CV%	P
Moisture (%)	59.30	47.69	64.51	4.79	8	60.58	56.01	64.93	2.52	4	n.s.
Protein (%)	12.33	10.53	14.94	1.04	8	14.08	11.10	16.15	1.45	10	**
Fat (%)	10.36	7.48	17.47	3.06	30	7.50	5.93	9.29	1.08	14	**
Carbohydrates (%)	16.22	12.72	22.06	2.87	18	16.50	12.96	20.73	1.90	12	n.s.
Ash (%)	1.79	1.37	2.51	0.34	19	1.35	1.03	1.75	0.23	17	**
Energetic value (kj/100g)	871.25	745.46	1210.68	138.54	16	797.00	696.66	904.68	57.32	7	n.s.

n.s.: not significant (p>0.05), *p<0.05, **p<0.01, ***p<0.001

Table 2. Degradation indexes**.**

	Uncooked					Cooked					
	Mean	Min	Max	SD	CV%	Mean	Min	Max	SD	CV%	P
FFA	3.67	1.59	7.93	2.11	58	3.85	1.26	8.66	2.38	62	n.s
PV	15.87	3.51	29.53	9.58	61	13.27	1.1	29.49	8.57	65	n.s
TBA	0.28	0.13	0.65	0.17	59	0.37	0.07	1.03	0.32	86	n.s
TVN	25.91	16.54	33.04	4.21	16	21.81	10.56	28.31	5.29	24	n.s

n.s.: not significant (p>0.05), *p<0.05, **p<0.01, ***p<0.001
FFA: % as oleic acid; PV: mequiv/kg of extracted fat; TBA: mg of malonaldehyde/kg; TVN: mg/100 g

Table 3. pH, aw and salt content**.**

	Uncooked					Cooked					
	Mean	Min	Max	SD	CV%	Mean	Min	Max	SD	CV%	P
pH	5.90	5.60	6.48	0.27	5	6.22	5.99	6.40	0.15	2	**
a_w	0.937	0.929	0.949	0.01	1	0.939	0.928	0.955	0.01	1	n.s.
NaCl (%)	1.07	0.81	1.44	0.19	18	0.74	0.46	1.09	0.19	26	***

n.s.: not significant (p>0.05), *p<0.05, **p<0.01, ***p<0.001

Table 4. Microbiological counts (log ufc/g).

	Uncooked					Cooked					
	Mean	Min	Max	SD	CV%	Mean	Min	Max	SD	CV%	P
Aerobic	7.06	6.41	7.57	0.33	5	3.94	2.66	4.79	0.69	18	***
Termophiles	4.34	3.70	4.85	0.34	8	2.93	1.98	4.09	0.58	20	***
Psicrophiles	7.05	6.62	7.51	0.25	4	3.59	2.09	4.72	0.82	23	***
Anaerobes	6.35	5.97	6.59	0.20	3	2.22	1.00	3.50	0.64	29	***
Moulds	2.24	1.00	2.94	0.61	27	1.11	1.00	2.00	0.28	25	***
Yeasts	4.92	4.23	5.60	0.43	9	2.24	1.00	4.53	1.13	50	***
Enterobacteracea	5.34	4.68	5.70	0.33	6	3.66	1.00	5.36	1.54	42	**
Lactic acid bacteria	5.04	3.63	6.18	0.87	17	2.32	1.00	4.58	1.19	51	***

n.s.: not significant (p>0.05), *p<0.05, **p<0.01, ***p<0.001

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CHEMICAL CHANGES IN THE LIPID FRACTION OF TRADITIONAL DRY FERMENTED SAUSAGE "PAINHO DE PORTALEGRE" DURING RIPENING

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Background

In manufacture of dry fermented sausages, the drying/ripening stage is of decisive importance to the final product sensory properties. During this process, the occurrence of complex physicochemical, biochemical and microbial phenomena affect proteins and lipids, modifying their structural integrity and physical characteristics (Gray & Pearson, 1984; Melgar *et al*., 1990). Different formulations are adopted according to cultural traditions of the geographical regions where they are produced but generally lipids represent the major fraction. The specificity of the flavour is then greatly dependent on the intrinsic composition of raw materials and on the hydrolytic and oxidative changes operated on that substrate. This is influenced by a wide range of factors namely, the relationships of environmental temperature/relative humidity/ventilation, type of ingredients/spices and duration of ripening.

Objectives

The present study was undergone to evaluate the chemical composition of "Painho de Portalegre", a traditional dry fermented sausage, and the variations occurred during the ripening period.

Materials and methods

Preparation of Painho de Portalegre and sampling – Chilled chunks of pork lean and fat were ground to obtain mincing sizes of about 2 cm (hole plate grinder), mixed together (Sample 1) and added of paprika paste, NaCl and garlic paste. After a holding period of 3 days at 0-2˚C (sample 2), the mixture was stuffed in natural casings (rectal portion of pig intestine) and transferred to a traditional drying/smoking house (firewood burning inside) until an a_w value of 0.88-0.85 was reached. Depending on the prevalent atmospheric conditions, this period varied between 30 to 40 days. Samples 3, 4, 5, 6 and 7 were taken after 6, 15, 20, 30 and 40 days of the ripening process. After processing the product was transferred to a room temperature environment where they were stored up to day 60 and then packaged under vacuum (BB4L plastic bags) up to 180 days. During the storage period samples 8, 9 and 10 were taken at days 55, 120 and 180.

Physicochemical analysis – pH was determined using a Metrohm 654 pH meter, according to the method described in NP 3441 (1990). Water activity evaluation was carried out with Rotronic Hygrolab using a probe AwVC-DIO. Moisture was determined using the method described in NP 1614 (2002). Total lipids were extracted by the procedure of Folch *et al* (1957). Protein was determined through total nitrogen using the Kjeldahl method. Sodium chloride content was determined following the procedure described in NP 1845 (1982).

Determination of free fatty acids – Lipids were extracted from Paínho samples with chloroform/methanol (2:1) according to the method of Folch (1957). FFA were purified from neutral lipids using an anionic exchange resin (Amberlyst A-26). An aliquot of 500 mg of lipids was dissolved in 30 ml of a mixture of acetone/methanol (2/1) as described by Alasnier *et al.* (2000) and Needs. The mixture with the lipid fraction was mixed with 750 mg of the exchange resin, was added with 1 ml of the internal standard solution (20µl of heptanoic acid/ml acetone/methanol) and shaken for 60 min. The resin was washed five times with 5 ml of acetone/methanol mixture for removing of neutral lipids. After drying for 1 h at ambient temperature, the resin with adsorbed FFA was placed in a reaction vial for FFA methylation with BF_3 in methanol (14%) as described by

Partidário (1998). The methyl esters were separated and quantified by gas chromatographic analysis, on a TRACE GC, series 2000 instrument (Thermo Quest, USA), using a DB-23 (50% cyanopropyl-

methylpolysiloxane) fused silica column (60 m long, 0,25 mm i.d., 0,25µm film thickness), supplied by J&W Scientific, Folsom, USA.
For the determination of fatty acid composition of triacylglycerols, extracted lipids were dissolved in isooctane and transesterified with a methanolic solution of KOH (0,1N). Methyl esters were separated and quantified as just described.

Results and discussion

Table 1 shows the mean, standard deviation, maximum, and minimum values found out for some general parameters, which are important to the characterisation of centesimal composition of "Painho de Portalegre". For a similar water availability (mean a_w=0.87), this traditional dry fermented sausage presented some variation in protein/fat relationship among producers, from a minimum of 0.30 up to a maximum value of 0.62. Sodium chloride content, an important factor influencing the ripening process evolution, in particular the rate and extension of the lipid autoxidation process, also showed variations of about 2%, between 3% and 5% approximately. The continuing decrease of pH, which likely affects the preservation ability, the physical aspect and the taste characteristics of the product, is closely associated to microbial development along the processing stages. The degree of variation verified in this study is enough expressive to differentiate the final products into acid (pH< 5.0) or moderately acid (pH>5.5).

Table 1 – Physico-chemical parameters of "Painho de Portalegre" composition.

	Protein (%)	**Moisture (%)**	**Fat (%)**	**pH**	**aW**	**NaCl (%)**	**Acidity** (ml NaOH 0,1N)
Mean	20.44	31.12	43.45	5.26	0.87	3.95	4.34
SD	2.77	3.52	4.63	0.31	0.02	0.56	0.39
Min	16.00	25.14	38.15	4.74	0.85	3.15	3.79
Max	23.80	37.01	51.85	5.61	0.89	4.90	4.73

Results for the fatty acids composition of pork fat used in "Painho de Portalegre" production showed a saturated/unsaturated fatty acids relationship lower than the usual profile referred for this type of product (Ordónez *et al*., 1999) (Table 2). This difference (0.48 vs 0.69) is basically due to the lower presence of C16 (-4.2%) and C18 (-4.6%) elements and a significantly higher concentration of oleic acid (+9.4%) and could be ascribable to the rearing and feeding procedures applied to the animal production system.
Raw material analysis showed already important amounts of FFA, reaching about 707 mg/100g of fat (Table 2). Among these compounds, C18:1 (9), C16 and C18 predominated, with values of 276.5 mg, 144.7 mg and 112.9 mg/100g of fat, respectively, keeping that order all over the ripening process. Linoleic acid appeared in 4th place with 80.6 mg/100g fat, and was the only detected polyunsaturated fatty acid. All the other elements appeared at much lower concentrations. At this early processing stage, MUFA slightly predominate over SFA, making a SFA/MUFA relationship of 0.93. During the standing of spiced raw materials in the refrigerator (flavour up taking and natural flora selection purposes) FFA formation was just slightly increased (+5.7%). However, a remarkable change occurred in the FFA profile, due a the significant rise in SFA (497.5 mg vs 301.9 mg) and, on the contrary, a sound reduction in MUFA and PUFA concentration, approximately –40% in both classes. As a consequence, the SFA/MUFA relationship more than doubled (0.93 vs 2.44). The evolution of C16/C16:1 and C18/C18:1 quotients clearly shows that the relative extension of that saturation mechanism affected mostly the former (6x vs 1,6x). However the possible impact over the product sensory quality coming from oxidation of oleic fatty acid
should also be emphasised due to its much higher concentration in the raw material prior the chilling holding stage (303 mg- C18:1 (9)+(11) vs 18.6 mg-C16:1 (7)+(9)).

Table 2- Fatty Acid (FA) profile and Free Fatty Acid (FFA)content of "Painho de Portalegre" along the ripening phase.

	FA profile (g/100g FA)	FFA (mg/100g fat)									
		S1	**S2**	**S3**	**S4**	**S5**	**S6**	**S7**	**S8**	**S9**	**S10**
C8	0.29										
C10	0.07										
C10:1	0.12										
C12	0.08	2.77	1.67	1.79	2.30	2.24	2.81	4.22	4.20	8.79	10.15
iC14		4.08	2.66		1.29		3.32	2.29	0.97		
C14	1.21	11.41	10.01	14.47	21.40	24.56	32.16	41.70	56.23	94.79	115.73
C14:1		2.30			2.23		2.99	1.12	1.36		
C15	0.05	0.63	2.48	0.95	1.21	1.96	2.46	1.75	2.77	3.23	3.53
iC16		17.74	19.86		1.24		9.53	3.84	1.96	3.13	1.70
C16	20.82	144.68	224.44	264.90	342.86	398.19	514.36	668.36	801.37	1221.38	1408.58
C16:1 t			0.90		1.09		4.12				
C16: 1 (7)	0.40	1.82	1.22	3.28	7.71	6.66	7.95	10.44	13.98	21.63	18.80
C16: 1 (9)	2.79	16.78	9.80	18.98	45.25	37.16	51.85	73.22	113.92	158.22	164.40
C17	0.31	3.10	2.91	3.46	6.73	9.61	9.75	10.69	10.75	15.72	15.33
C17:1	0.35	1.34		1.39	4.88	7.12	5.52	7.13	10.55	3.77	15.70
iC18		4.60	2.57								
C18	9.34	112.90	230.33	235.60	293.29	355.08	410.08	516.24	507.55	616.31	543.33
C18:1 t	0.36		1.05			0.82	13.57	10.11	19.24	28.40	15.74
C18: 1 (9)	47.86	276.49	168.16	333.22	758.21	658.56	887.50	1121.06	1736.59	2181.83	2180.19
C18: 1 (11)	3.30	26.53	20.37	35.81	88.26	77.84	99.95	124.35	200.69	308.44	265.41
C18:2	8.50	80.60	45.19	121.60	320.75	304.75	359.56	403.94	578.21	740.57	664.33
C18:2 isóm.	0.17						1.56		7.53	8.09	6.49
C18:3	0.79			10.04	28.05	23.44	27.55	34.06	46.78	69.96	55.16
C18:4	0.06										
C20	0.24		0.56							6.82	5.92
C20:1	0.92										
C20:1 (9)			1.39					7.63	5.27		
C20:1 (11)			2.45	7.36	19.80	16.42	18.81	25.84	42.46	69.80	51.52
C20:2	0.39				11.66	10.12	12.46	16.18	24.59		27.89
∑FFA	99.92	707.8	748.03	1053	1958.2	1934.5	2478	3084	4186.97	5560.89	5569.9
∑SFA	32.41	301.9	497.5	521.2	670.3	791.6	984.5	1249	1386	1970.18	2104
∑MUFA	57.43	325.3	196.4	400	927.4	804.6	1092	1381	2212	2772.10	2712
∑PUFA	10.08	80.6	45.19	131.6	540.5	413.9	401.1	454.2	657.1	818.61	753.87
SFA / MUFA	0.564	0.928	2.533	1.303	0.723	0.984	0.901	0.905	0.626	0.711	0.776
SFA / PUFA	3.215	3.746	11.01	3.959	1.24	1.913	2.454	2.75	2.109	2.41	2.79

During the development of the fermentation which occurs at least during the first 6 days of the drying/smoking process (Sample 3), intrinsic and microbial lipolysis phenomena proceeded continuously up to day 180 (Sample 10), and was traduced on a 4 fold increase of the FFA level (1053 mg vs 4187 mg). Apart the initial phase where SFA content was still superior to MUFA (SFA/MUFA=1.3), the unsaturation level of the FFA profile gradually increased up reaching a SFA/MUFA relationship of 0.63 (55 days of ripening-S8). This is in agreement with the results reported by Chasco *et al*., (1993) for identical dry fermented sausages.

The conditions under which the processing stages take place are thought to be extremely important to the definition of the eating quality of the final product and to its stability over the storage period. Despite the formation of straight chain aldehydes butanal, pentanal and hexanal by a mechanism other than lipid peroxidation should not be ruled out, a significant correlation between their concentration, namely that of hexanal, and the rancid alteration of lipids has been observed (Shahidi *et al*., 1986).

From an extended study made on Paínho de Portalegre (results submitted for publication elsewhere) it could be observed that hexanal was never identified in all evaluated ripening periods (Table 3).

Table 3- Relative percentages % r.p. of volatile aldehydes detected along the drying/ripening stages.

	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10
2-Metylpropanal	-	3.73	0.0	0.0	1.63	1.36	0.0	0.0	0.0	0.0
Butanal	-	0.0	0.0	0.0	0.0	0.0	0.39	0.0	0.69	0.48
2-Metylbutanal	-	0.0	0.0	0.0	0.0	0.20	0.15	0.19	0.62	1.65
Pentanal	-	3.45	0.0	0.3	0.39	0.0	0.15	0.0	0.82	0.0
2-Metylpentanal	-	0.0	0.94	0.0	0.0	0.27	0.17	0.0	0.0	0.0
Hexanal	-	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Pentanal and 2-metylpentanal predominated at the early phases of processing (spiced raw materials stored at a chilling room - 3.45%r.p.) and during fermentation (first 6 days on drying/smoking house -0.94%r.p.), but its concentration decreased during on going stages to less than 0.5%. The presence of butanal was only noted from day 40 of ripening in minor concentrations (0.39%, 0.69% and 0.48% at day 40, 120 and 180 of storage ripening period, respectively). The detection of 2-metylpropanal in S2, with a value of 3.7%r.p., is probably a consequence of the utilization of linoleic acid by microbial agents (Grosch, 1987) and could explain the drop observed for this fatty acid in the free form at this processing stage (80.6mg to 45.2mg).

Conclusions

Painho de Portalegre is characterized by a strong lipolysis during the processing and storage ripening period, over 180 days, traduced by an expressive liberation of fatty acids, which is more pronounced in the period of greater microbial development (first 15 days of drying/smoking stage). Excepting the earlier processing period in which saturated fatty acids predominated, MUFA is the most important class of compounds. The reduced production of straight chain aldehydes, namely hexanal, can be an indicator of good lipid stability, associated to the natural ingredients used on spicing and the intense/long smoking phase.

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PREDICTING THE RISK OF HIDE CROSS-CONTAMINATION IN THE CATTLE LAIRAGE DURING HOLDING PRIOR TO SLAUGHTER

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Background

Foodborne pathogens carried asymptomatically in the intestines of cattle can be found on the hides of these animals [1], and pose a significant risk of contamination of the subsequent carcass during the slaughter and dressing procedure[2]. Similarly, spoilage organisms present on the hide can be transferred to the carcass during harvest [3]. Although only a small proportion of animals arriving at the abattoir may be contaminated with significant pathogens, extensive cross-contamination can occur during the holding period [4]. Understanding of the factors affecting the rate of transfer of micro-organisms between animals could assist in developing a risk-based strategy to minimise the spread of foodborne pathogens during the pre-slaughter phase, thus minimising the risk of contamination of carcasses processed in the abattoir.

Objectives

This study aims to evaluate some factors affecting cross-contamination during the holding period prior to slaughter in the commercial cattle abattoir.

Materials and methods

91 groups of cattle were observed continuously throughout the holding period at a large commercial beef processing plant in the Southwest of England. One focal animal in each group was selected and each incident of contact between that animal and either the vertical structures of the holding pen or another animal within the group was recorded, in five-minute blocks throughout the holding period, a minimum of 30 minutes. Group size ranged from 1 to 18 animals, held in pens of 24 m^2 to 30 m^2, giving space allowances ranging from 1.88 m^2 to 26 m^2 per animal. The result sets were grouped into low ($\leq$ 2.5 m^2 per animal), medium (2.5 $\leq$ 5 m^2 per animal) and high (>5 m^2 per animal) stocking density and the number of contacts over time analysed by ANOVA using MINITAB Statistical Software.

Results and discussion

Over the 30-minute holding period, at all stocking densities, total number of contacts affecting the focal animal in each 5-minute block declined. At all stocking densities, the numbers of contacts with the vertical structures of the pen did not significantly decline over time, and there was no significant difference in the numbers of wall contacts occurring per time block between each stocking density. At medium and high stocking densities there was a significant difference between animal activity in the first ten minutes of holding and in the subsequent period, by comparing average contacts per animal per minute (ACAM) (Table 1). There was also a significant difference in ACAM over all time periods between stocking densities, animals at low stocking density experiencing fewer animal-to-animal contact incidents than animals at medium stocking densities, which in turn experience fewer animal-to-animal contact incidents than animals at high stocking densities. The relationship between ACAM over a 30-minute period ($ACAM_{30}$) and space allowance was not linear, whilst plotting $ACAM_{30}$ against $\log_{10}$ space allowance showed two distinct lines of best fit, one associated with medium to high stocking density, the other associated with low stocking density. Linear regression analysis of the line of best fit associated with low stocking density identified that to achieve no animal-to-animal contacts, each animal would require a space allowance of 17.22 m^2, whereas linear regression analysis of the line of best fit associated with medium to high stocking densities suggested that a space allowance of 4.22 m^2 would lead to no animal-to-animal contacts.

Conclusions

It is likely that each physical contact of animals with lairage's structures and other animals results in some extent of transfer of microbial contamination, if one of hides/contact surfaces is contaminated. During the holding period at the lairage, contact between a beef animal and the structure of the holding pen is inevitable. Furthermore, micro-organisms remaining on the pen walls when one group of animals is removed are likely to be transferred to animals' hides in subsequent groups of cattle. Contact between animals does increase with restricted space allowance, but to eliminate the risk of animal-to-animal transfer of micro-organisms, each animal would require a substantial amount of space. Animal interactions at low stocking densities differs from that at medium to high stocking densities, those at lower densities maintaining a level of interaction over the entire lairage holding period, whilst animals at medium to high stocking densities appear to settle down after the initial ten-minute period, reducing interactions. Use of ACAM factors could assist in predicting the risk of hide cross-contamination during the holding period, by estimating the numbers of contacts that would occur in a group of animals at a particular stocking density, over a set period of time. As the hide contamination is a key factor for carcass contamination, this would be the first step in risk-assessment-based identification and development of best strategies to improve meat safety through controls of hide contamination.

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Table 1 – Average contacts per animal per minute at different stocking densities

Average Contacts per Animal per Minute (ACAM)	Contact Type	Space Allowance per animal		
		≤ 2.5 m^2	$2.5 \leq 5$ m^2	>5 m^2
First 10 minutes ($ACAM_{10}$)	animal-animal	12.55	6.9	1.81
	animal-wall	0.99	1.1	1.11
Subsequent Period	animal-animal	8.17	4.66	2.13
	animal-wall	0.60	0.44	0.56
Over entire 30 minutes ($ACAM_{30}$)	animal-animal	9.63	5.33	1.71
	animal-wall	0.73	0.66	0.72

IDENTIFICATION OF PEDIOCIN PA-1 PRODUCING *PEDIOCOCCUS PENTOSACEUS* TISTR 536 ISOLATED FROM NHAM (THAI FERMENTED MEAT)

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Background

Nham, traditional Thai fermented pork, is normally consumed without cooking and considered as a ready-to-eat food after 3-4 days of spontaneous fermentation. The reports on occurrence of the most common contaminant strain of *Salmonella anatum* in the product [1, 2, 3] are therefore a serious public health concern. Since the advantage of using lactic starter cultures was reported to have a positive effect on the microbiological quality and safety of various fermented products [4, 5], thus, the use of lactic starter cultures to control *S. anatum* in Nham was studied [1, 6]. The selection of the most potent bacteriocin-producing lactic acid bacteria (LAB) from the spontaneous fermentation of this thai fermented meat product had been studied [7, 8]. The strain of TISTR 536, which was first isolated from Nham [9] and kept as LAB culture collection at Thailand Institute of Scientific and Technological Research (TISTR), was later studied for bacteriocins production and reported as a bacteriocin-producing strain [8]. The strain exerted the best antimicrobial spectra of their produces on various indicators, which included the opportunistic food pathogens of *Listeria monocytogenes* and *Enterococcus faecalis* [8]. This strain exhibited no effect on *Staphylococcus carnosus,* which may enhance adverse effects on colour and aroma of Nham.

Objectives

According to the report on antagonistic substances produced from LAB strain TISTR 536 [8], thus, this report was conducted to identify the strain of TISTR 536 by using 16S rDNA sequence analyses and commercial kit carbohydrate fermentation patterns of API 50 CHL. In addition, identification of bacteriocins produced from this strain was also reported in the study.

Materials and methods

Bacterial strains and culture media : Bacteriocin-producing strain of TISTR 536 obtained from Bangkok MIRCENS in lyophilized form was used in this study. Before experimental use, the culture was cultivated twice in de Man Rogasa Sharp (MRS) broth [10] and incubated at 30^{o} C for 20-24 hours.
Escherichia coli JM 109 was used as host cell for PCR cloning and sequencing analysis. The strain was propagated in LB medium containing 50 µg/ml of ampicillin at 37^{o} C [11].

Identification of TISTR 536 : Carbohydrate fermentation patterns for the isolated strain of TISTR 536 were determined by using API CHL 50 test (bioMorieux Vitek, Inc., Hazelwood, Mo.) and 16S rRNA sequence analyses were performed by using the following methods :
Partial phenotypic characterization of the strain was performed by firstly preparing overnight cultured of TISTR 536 in MRS broth. 2 ml of the overnight cultured was harvested by centrifugation. The cells were then resuspended in 80 µl of TE buffer (50 mM Tris, 50 mM EDTA, pH 8). Lysis was initiated by the addition of 5 mg/ml lysozyme. After incubation at 37^{o} C for 30 min, the mixture was further provided with MagExtracter-Genome (TOYOBO) as specified by manufacturer. 16S rDNA gene was applied from genomic DNA using primer 1101A (5'- AACGAGCGMRACCC–3') and 1407B 5'-GACGGGCGGTGTGTRC –3') based on primers described by Kawamura, *et al*. Reaction [50 µl contained 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1.5 mM $MgCl_2$ and 0.1% Triton X-100, TOYOBO Co. Ltd.), 200 µmol of each deoxynucleotide triphosphate (TOYOBO Co. Ltd.), 20 pmol of primer and 1 U of Taq polymerase (TOYOBO Co. Ltd.)] was set up in a tube containing 200 ng of template DNA and genomic DNA amplification was then performed using Astec program temp control system PC-800. The program was

3 min at 94° C for 1 cycle followed by 30 cycles of 94° C at 30 sec, 55° C at 30 sec and 72° C at 1 min. The additional step for extending incomplete products was performed at 72° C for 5 min. PCR product was TA cloned and transformed into E. *coli* JM109. Selected white colonies were picked and transferred into 20 µl of PCR solution for confirming the insert using M13 forward and reverse primers (5'-GAGTGGGAACTAGAATAAGCGCGTA–3' and 5'–TTTCACACAGGAAGCTAT GAC –3' respectively). Plasmid DNA of selected transformants was isolated using MagExtracter-Plasmid (TOYOBO). The sequence was determined by using Thermo Sequenase fluorescent-labeled primer cycle sequencing kit (Pharmacia Biotech) with an automatic sequencer (ALF express, Pharmacia Biotech). The results from the sequencing analysis were analyzed using catalogued sequences in GenBank with the BLAST tool at National Center for Biotechnology Information (NCBI).

Determination of the concentration of antimicrobial produced from TISTR 536 : The study was conducted by inoculating 1 % an overnight culture of the selected potent LAB strains and culturing for 24 hours at 30° C. The culture of TISTR 536 were then centrifuged at 2,700 x g for 10 minutes. The supernatant from the culture was adjusted to pH 7.0 with 5.0 N NaOH and then filter-sterilized with 0.20 µm pore-size polysulfone (Cica, Tokyo). The cell-free supernatant was determined for antagonistic activity by using spot on lown method [12, 13] against the indicator strains (Table 1). The titer expressed in activity units/ml (AU/ml) was defined as the highest dilution factor of bacteriocin preparation that still caused a clearly visible zone of inhibition in the indicator lawn.

Bacteriocins purification : The cell-free supernatant of 2 liters culture incubated at 30° C of TISTR 536 was purified by a four step procedures as described by Ennahar *et al.* (1999) [12]. The final sample containing the purified bacteriocins was dried by Speed-Vac rotary evaporator (Savant Instruments) and stored at –20°C.

Mass spectrometric : The molecular masses of purified bacteriocins were determined using a Accu TOF spectrometer, model JMS-T100LC (Agilent Technologies, Germany).

PCR analysis and DNA sequencing of bacteriocin from TISTR 536 : The total DNA of TISTR 536 was isolated by using the method described by Anderson and McKay [14]. Pediocin PA-1 primer designed and synthesized (Hokkaido System Science Co. Ltd., Hokkaido, Japan) for PCR amplification are Pedi-1F (5' –GAGTGGGAACTAGAATAAGCGCGT A –3') and Pedi-1R (5' –TTACTCTTATTCATAAAATCACCCC –3'). DNA analyses and sequence alignments were carried out using the BLAST program [15].

Results and Discussion

Identification of TISTR 536 : Microscopic results of TISTR 536 revealed that this strain was gram-positve, coccus-shape bacteria and existed in pairs and tetrads. Based on the microscopic results and the rapid API carbohydrate fermentation patterns (Table 2) implied that the microorganism was 92.4 % identical to *Pediococcus pentosaceus*. By the complete DNA sequence analysis, the results showed the 100% of identity related to *P. pentosaceus*. Thus, this was confirmed that LAB strain TISTR 536 is classified as *P. pentosaceus*.

Determination of the concentration of antimicrobial produced from TISTR 536 : The confirmation of antagonistic produced from TISTR 536 was reexamined with 20 indicator strains using MRS cell-free cultured supernatant spot on lawn (Table 3). The results revealed that the LAB strain TISTR 536 could produce bactericidal substances to inhibit various bacterial indicators and exhibited most effect on *Kocuria varians*, *Lactobacillus plantarum* (ATCC 14917T), *L. sakei* subsp. *sakei*, *Listeria innocua* and *Lis. monocytogenes.*

Table 1 : Bacterial strains used as indicators in this study

Indicator	Source [a]	Culture condition [b]
Gram positive		
Pediococcus pentosaceus	JCM 5885 and 5890 T	MRS 30° C anaerobic
Lactobacillus plantarum	ATCC 8014	MRS 30° C anaerobic
L. plantarum	ATCC 14917 T	MRS 30° C anaerobic
L. sakei subsp. *sakei*	JCM 1157 T	MRS 30° C anaerobic
Lactococcus lactis subsp. *lactis*	ATCC 19435 T	MRS 30° C anaerobic
Lc. lactis subsp. *lactis*	NCDO 497	MRS 30° C anaerobic
Lc. lactis subsp. *lactis*	JCM 7638	MRS 30° C anaerobic
Lc. lactis subsp. *cremoris*	TUA 1344L	MRS 30° C anaerobic
Leuconostoc mesenteroides subsp. *mesenteroides*	JCM 6124 T	MRS 30° C anaerobic
Micrococcus luteus	IFO 12708	TSB-YE 37° C aerobic
Listeria innocua	ATCC 33090 T	TSB-YE 37° C aerobic
Lis. monocytogenes	ATCC 19117	TSB-YE 37° C aerobic
Enterococcus faecalis	JCM 5803 T	TSB-YE 37° C aerobic
Kokuria varians	LTH 1545	TSB-YE 37° C aerobic
Staphylococcus carnosus	LTH 2102	TSB-YE 37° C aerobic
S. aureus subsp. *aureus*	ATCC 12600 T	TSB-YE 37° C aerobic
Bacillus circulans	JCM 2504 T	TSB-YE 30° C aerobic
B. coagulans	JCM 2257 T	TSB-YE 37° C aerobic
B subtilis	JCM 1465 T	TSB-YE 30° C aerobic
Gram negative		
Escherichia coli	JCM 109	TSB 37° C aerobic
Salmonella anatum	WHO-BKK	TSB 37° C aerobic

[a] ATCC, American Type Culture Collection, Rockville, Md; JCM, Japanese Culture of Microorganisms, Japan; JM, commercial strain from Toyobo, Osaka, Japan; LTH, Lebensmitteltechnologie Hohenheim University, Stuttgart, Germany; TUA, Tokyu University of Agriculture, Japan; IFO, Institute for Fermentation, Osaka, Japan; WHO-BKK, World Health Organization, Salmonella-Shigella Center, Bangkok, Thailand.
[b] MRS medium (Oxoid); TSBYE, Trypticase soy broth (Difco) + 0.6 % Yeast extract (Difco)

Table 2 : API CHI 50 carbohydrate fermentation pattern of TISTR 536

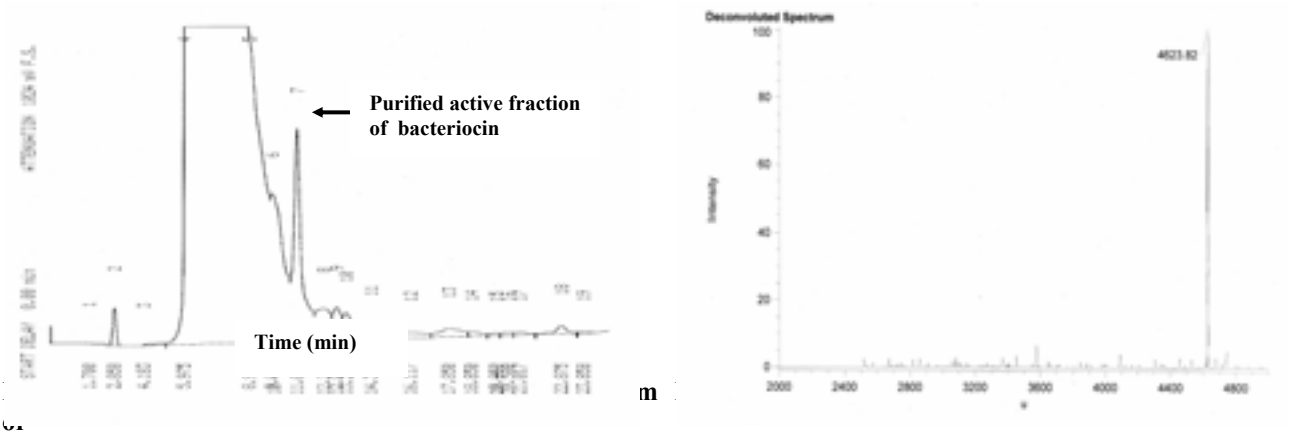
Carbohydrate	Result	Carbohydrate	Results	Carbohydrate	Results
Glycerol	-	Mannitol	-	Melezitose	-
Erythritol	-	Sorbitol	-	D-Raffinose	-
D-Arabinose	-	α Methyl-D-mannoside	-	Amidon	-
L-Arabinose	-	α Methyl-D-glucoside	-	Glycogene	-
Ribose	+	N Acetyl glucosamine	+	Xylitol	-
D-Xylose	-	Amygdaline	+	β Gentiobiose	+
L-Xylose	-	Arbutine	+	D-Turanose	-
Adonitol	-	Esculine	+	D-Lyxose	-
β Methyl-xyloside	-	Salocine	+	D-Tagatose	+
Galactose	+	Cellobiose	+	D-Fucose	-
D-Glucose	+	Maltose	+	L-Fucose	-
D-Fructose	+	Lactose	-	D-Arabitol	-
D-Mannose	+	Melibiose	-	L-Arabitol	-
L-Sorbose	-	Saccharose	-	Gluconate	-
Rhamnose	-	Trehalose	+	2 ceto-gluconate	-
Dulcitol	-	Inulin	-	5 ceto-gluconate	-
Inositol	-				

+ = positive result of Carbohydrate fermentation after incubating at 35° C for 48 hours ,
- = no fermentation occurred after incubating at 35° C for 48 hours

Table 3 : Inhibitory spectrum results of antagonistic substances produced by TISTR 536 using MRS cell-free cultured supernatant spot on lawn (AU/ml)

Indicator strains (Source)	Cell-free cultured spot on lawn (AU/ml)
Bacillus circulans (JCM 2504^T)	0
B. coagulans (JCM 2257^T)	0
B. subtilis (JCM 1465^T)	0
Enterococcus faecalis (JCM 5803 ^{T})	800
Escherichia coli (JM 109)	0
Kocuria varians (LTH 1545)	6,400
Lactobacillus plantarum (ATCC 8014)	0
L. plantarum (ATCC 14917^T)	6,400
L. sakei subsp. *sakei* (JCM 1157 ^{T})	6,400
Lactococcus lactis subsp. *lactis* (ATCC 19435^T)	0
Lc. lactis subsp. *cremoris* (TUA 1344L)	1,600
Leuconostoc mesenteroides (JCM 6124^T)	1,600
Listeria innocua (ATCC 33090 ^{T})	6,400
Lis monocytogenes (ATCC 19117)	6,400
Micrococcus luteus (IFO 12708)	0
Pediococcus pentosaceus (JCM 5885)	400
P. pentosaceus (JCM 5890 ^{T})	200
Salmonella anatum (WHO-BKK)	0
Staphylococcus aureus (ATCC 12600 ^{T})	0
S. carnosus (LTH 2102)	0

Bacteriocin purification and Molecular mass determination : The bacteriocins from TISTR 536 were purified after subjecting to C_2/C_{18} reverse-phase (PepRPC HR 5/5, Amersham Pharmacia Biotech) chromatography. The active fraction of purified bacteriocin (Fig. 1) were then analyzed by ion spray mass spectrometry. The results (Fig. 2) implied that the molecular mass of this bacteriocin was related to pediocin PA-1 (4,623.82 Da) produced from *P. acidilactici* PAC1.0 [16] and *L. plantarum* WHE 92 [17].



TISTR 536 after subjected to C_2/C_{18} reverse-phase HPLC

bacteriocin from TISTR 536

PCR analysis and DNA sequencing of bacteriocin from TISTR 536 : In order to prove that the bacteriocin produced by strain TISTR 536 was pediocin PA-1, PCR analysis using the known sequences of the pediocin structural gene was performed. The expected 300 bp fragment containing the structural gene of pediocin PA-1 of TISTR 536 was amplified and then sequenced. The results (Fig. 3) indicated that the sequences were 100% identical to that of pediocin PA-1.

```
1    GCGTTGATAGGCCAGGTTTCAAAAATTGACCAAGATCGTTAACCAGTTTGGTGCGAAAAT    60

61   ATCTAACTAATACTTGATATTTAAATTGAGTGGGAACTAGAATAAGCGCGTATTAAGGAT   120

121  AATTTAAGAAGAAGGAGATTTTTGTGATGAAAAAAATTGAAAAATTAACTGAAAAAGAAA   180
                                   K   K   I   E   K   L   T   E   K   E   M

181  TGGCCAATATCATTGGTGGTAAATACTACGGTAATGGGGTTACTTGTGGCAAACATTCCT   240
       A   N   I   I   G   G   K   Y   Y   G   N   G   V   T   C   G   K   H   S   C

241  GCTCTGTTGACTGGGGTAAGGCTACCACTTGCATAATCAATAATGGAGCTATGGCATGGG   300
       S   V   D   W   G   K   A   T   T   C   I   I   N   N   G   A   M   A   W   A

301  CTACTGGTGGACATCAAGGTAATCATAAATGCTAGCATTATGCTGAGCTGGCATCAATAA   360
       T   G   G   H   Q   G   N   H   K   C   *

361  AGGGGTGATTTTATGAATAAGACTAAGTCGGAACATATTAAACAACAAGCTTTGGACTTA   420

421  TTTACTAGGCTACAGTTTTTACTACAGAAGC                                451
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BACTERIOCIN PEDIOCIN PA-1 PRECURSOR

```
Identities = 61/61 (100%), Positives = 61/61 (100%)
Score =  138 bits (347), Expect = 7e-32

Query :   KKIEKLTEKEMANIIGGKYYGNGVTCGKHSCSVDWGKATTCIINNGAMAWATGGHQGNHK
      :   KKIEKLTEKEMANIIGGKYYGNGVTCGKHSCSVDWGKATTCIINNGAMAWATGGHQGNHK
Subject :KKIEKLTEKEMANIIGGKYYGNGVTCGKHSCSVDWGKATTCIINNGAMAWATGGHQGNHK
```

Figure 3 : Nucleotide sequence and deduced amino acid sequence of the pediocin PA-1 gene isolated from *P. pentosaceus* TISTR 536. Primers are in bold and underline. Stop codon is shown by asterisk.

Conclusions :

The study concluded that the strain TISTR 536 is *P. pentosaceus* and this strain can produce pediocin PA-1. *P. pentosaceus* TISTR 536 is the first pediocin PA-1 producer strain isolated from Nham, a type of thai traditional fermented meat products.

Acknowledgements :

We would like to acknowledge the Japan Society for the Promotion of Science (JSPS) and the National Research Council of Thailand (NRCT) in funding this research under RONPAKU (Ph.D. dissertation) program.

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MICROBIOLOGICAL SAFETY IN SUCCEDANEUM ITALIAN SALAMI

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Background

The Italian salami is characterized by being a fermented sausage that takes thirty days for its production, where in the first seven days the fermentation occurs and in the others twenty-three days there is the formation of its characteristic flavor (Fernández *et al* 2001 and Demeyer *et al,* 1996). The substitution of starter cultures in Italian salami production by the addition of encapsulated organic acids followed by cooking will assure a microbiological safety product and reduce the time of its production (Terra, 1998 and Lizaso *et al* 1999).

Objectives

The objective of this work was to produce differentiated Italian salami by substituting the starter cultures by the addition of encapsulated organic acids followed by cooking. This fact will make possible to produce similar Italian salami in a shorter time and without the need of ripening house.

Materials and methods

To the elaboration of a succedaneum Italian salami, three treatments were used as follow: Treatment 1 - control, Treatment 2 - received 500g of MEATSHURE encapsulated citric acid and treatment 3 – received 400g of MEATSHURE encapsulated lactic acid. The pork meat was cut in a 16 mm disk and the bovine meat was cut in a 3 mm disk. After grinding salt, cure meat mixture and others ingredients were added to the basic mass (Terra, 1998). Starting from the basic mass, the three treatments were separated, where T1 is the basic mass (control), T2 and T3 had the addition of encapsulated organic acids as above. The treatments were equally stuffed in fibrous case (40 mm), previously put in a 2% lactic acid solution, identified and cooked in the incubator. The encapsulated citric acid (MEATSHURE TM 341) is composed by 50% of citric acid and 48 - 52% of vegetable oil partially hydrogenated. The encapsulated lactic acid (MEATSHURE TM 509) is composed by 30% of lactic acid, 48 - 52% of calcium lactate and 48 - 52% of partially hydrogenated of cotton and soybean oils. The proportion of encapsulated organic acids added to the treatment of the succedaneum Italian salami was 1% of citric acid and 0,82% of lactic acid. Total mesophilic aerobic microorganisms, coagulase positive *Staphylococcus* and *Escherichia coli* determinations were done.

Results and discussion

The succedaneum Italian salami was done in 24 hours. The experimental results demonstrated the reduction of meat initial pH (6,1) to 5,3 in the treatment with encapsulated lactic acid and 5,1 in the treatment with encapsulated citric acid. The initial microbiological counts before the acids addition and the cooking was 10^6 UFC·g^{-1} for the mesophilic aerobic bacteria, 10^3 UFC·g^{-1} for *E. coli* and coagulase positive *Staphylococcus*. The Italian salami treated with encapsulated organic acids followed by cooking presented a significant microbiological reduction. The salamis treated with lactic acid showed 10^3 UFC·g^{-1} for mesophilic aerobic microorganisms, 10^2 UFC·g^{-1} for *Staphylococcus* and 10^1 UFC·g^{-1} for *E. coli* (Figure 1). In the salami treated with citric acid presented less than 10^1 UFC·g^{-1} counts for all tested microorganisms (Figure 2). In none of the salamis treated with encapsulated acids followed by cooking was observed the presence of coagulase positive *Staphylococcus*. The addition of encapsulated organic acids in the salami partially substituted the action of starter cultures, acidifying the mass and consequently making a safe product for the costumer. The reduction of microbial contamination followed the pH reduction in the treated samples presented a larger microbiological safety for the succedaneum Italian salami.

Conclusions

The production of acidified and cooked Italian salami demonstrated to be an option for microbiological safe products with low cost and reduced time of production.

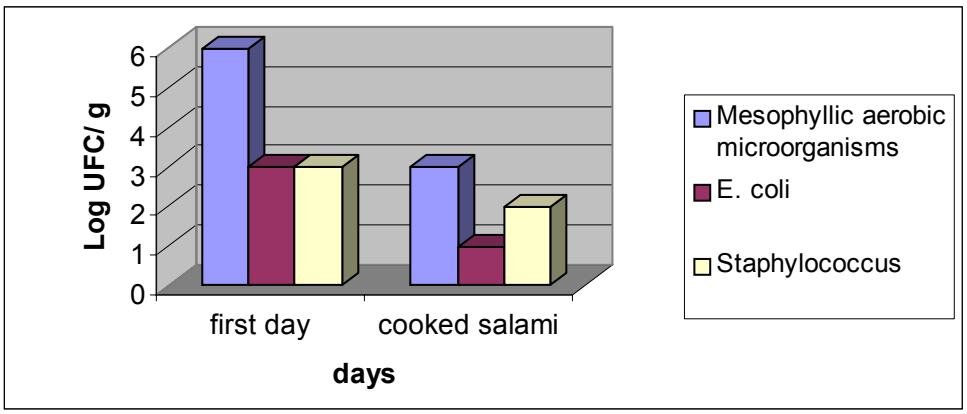

Figure 1 – Mesophilic aerobic microorganisms, *E.* coli, and coagulase positive *Staphylococcus* of a cooked salami treated with encapsulated lactic acid.

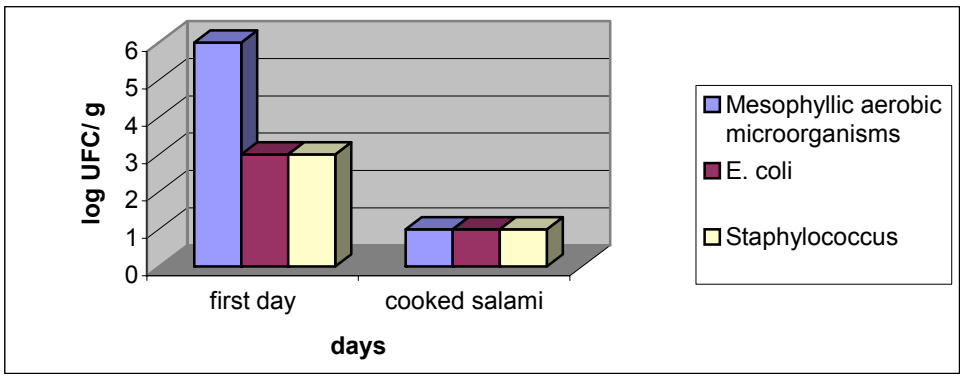

Figure 2 - Mesophilic aerobic microorganisms, *E.* coli, and coagulase positive *Staphylococcus* of a cooked salami treated with encapsulated citric acid.

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ENRICHMENT OF THE ITALIAN SALAMI WITH INSOLUBLE WHEAT FIBER

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Background

Since the old times, the Italian salami is known as a sausage of thick mass, fermented and ripened (Lizaso et al, 1999). The fat content of the salamis results in a consumption obstacle for this product by people who show cardiovascular and nutritional problems. The salami's fat is partly responsible for the sensorial characteristics of the product, when the tissue enzymes and bacterial lipolysis takes place. The drastic reduction of salami's fat would implicate in sensorial alterations, which are capable to turn the product unacceptable by the consumer, such as the aroma and flavor alterations (Demeyer et al, 1996). The fiber addition in the salami production without reducing the fat content could be a way of improving the nutritional value without altering the sensorial characteristics of this meat product.

Objectives

The objective of this work was to evaluate the effect of the addition of insoluble wheat fiber to the Italian salami, on its acceptability by a non-trained panel and the pH, moisture, fat content and water activity changes.

Materials and methods

For this experiment the Italian salami was elaborated using three treatments as following: Treatment 1: control, without the addition of starter culture nor the insoluble wheat fiber; Treatment 2: only the addition of starter culture and the Treatment 3: the addition of the starter culture as well as the insoluble wheat fiber. All the treatments were obtained by grinding the pork and bovine meats and adding salt, cure salts, seasonings and antioxidant. The stuffing was done using a natural casing (Terra, 1998). The starter culture used was a combination of *Lactobacillus plantarum* and *Staphylococcus xylosus*. Each treatment had 18 samples. Moisture, fat content, pH and water activity were determined (Terra & Brum, 1988). The sensorial analysis was accomplished by 15 non-trained judges. The analyzed sensorial parameters were appearance, color, odor, flavor and texture.

Results and discussion

The insoluble wheat fiber addition in the Italian salami reduced the moisture in the treatment 3 (Table 1), reducing the water activity (a_w) of the product. The reduction of a_w (Table 1) caused by pH drop reduces the water availability for biochemical and physical-chemical reactions, which are necessary for the growth and the multiplication of microorganisms and the possible toxins formation (Jay, 1994). The reduction of the pH values is responsible for the water liberation from the product, for the formation of sensorial characteristics of the salami and for the aid the homofermentative acid lactic bacteria to overcome the contaminant flora through the competitive antagonism. The fat content was important for endogenous and bacterial lipases, because they are the main responsible enzymes for the liberation of free fatty acids during ripening, with preferential release of poly-unsaturated fatty acids (Demeyer *et al*, 1996). The importance of lipolysis for lipid oxidation and thus, flavour promoting effect is often assumed. All the treatments were approved with grades superior than 5.0 (Table 2). The starter culture in the treatments T2 and T3 contributed for the acceptability of the product, and the insoluble fiber of wheat did not significantly differ ($p<0.05$) in the acidification and flavoring.

Table 1. The pH, moisture, fat and water activity values of the Italian salami treated with insoluble wheat fiber in the presence or absence of *Lactobacillus plantarum* and *Staphylococcus xylosus*

	Determinations			
Treatments	**pH**	**M(%)**	**F(%)**	**Aw**
T1	4.8	32.1	32.7	0.9
T2	5.2	39.4	31.6	0.86
T3	5.1	37.9	32.1	0.82
Mean	5.03	36.46	32.10	0.88

T1= control
T2= Italian salami treated with *Lactobacillus plantarum* and *Staphylococcus* ***xylosus***
T3= Italian salami treated with *Lactobacillus plantarum* and *Staphylococcus xylosus* and insoluble wheat fiber
M= moisture **F**= fat **Aw**= water activity

Table 2. Sensorial characteristics of the Italian salami treated with insoluble wheat fiber in the presence or absence of *Lactobacillus plantarum* and *Staphylococcus xylosus*

	Sensorial properties				
Treatments	**Appearence***	**Color***	**Odor***	**Flavor***	**Texture***
T1	7.8	7,9	7.0	6.0	6.0
T2	8.0	8.5	8.2	8.5	8.1
T3	6.9	6.5	6.3	6.9	6.5
Mean	7.56	7.9	7.0	6.0	6.0

T1= control
T2= Italian salami treated with *Lactobacillus plantarum* and *Staphylococcus xylosus*
T3= Italian salami treated with *Lactobacillus plantarum* and *Staphylococcus xylosus* and insoluble wheat fiber
*The values correspond as: > 5.0 = acceptable; < 5.0= unacceptable

Conclusions

The insoluble wheat fiber addition can enrich a product without altering the sensorial characteristics. The insoluble wheat fiber added to a fermented sausage is a new meat product and it is a healthier option for the consumer.

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MICROBIOLOGICAL QUALITY OF FRESH MEAT PRODUCTS STORED UNDER MODIFIED ATMOSPHERES

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Background

The high quality and the safety of fresh meat products are important features to be conserved during the production, the distribution and the storage.
During the last years, several studies were carried out in order to reduce the spoilage bacteria and, consequently, to extend the shelf-life of the products. Djenane *et al*. (2002) showed small but significant inhibition of the growth of psychrotrophic microorganisms with the application of rosemary extract on the surface of beef steaks, although Sanchez-Escalant *et al*. (2001) demonstrated a lack of effect of the antioxidants on microbial growth. Zhagh *et al*. (1996) reported that the storage of beef in presence of lactic acid reduces the initial number of spoilage bacteria. Moreover, the modified atmosphere packaging (MAP) is well known as a method for extending the shelf-life of meat products (Luno *et al*., 2000; Djenane *et al*., 2002; Jajasingh *et al*., 2001).
In a recent study, Djenane *et al.* (2003) showed that either lactic acid bacteria and *B. termosphacta* are significantly inhibited in beef steaks packaged in CO_2 40% and the shelf-life of these products, previously treated with lactic acid and a mixture of natural antioxidants, could be extended. Even though, the use of modified atmosphere with high CO_2 and low O_2 levels could cause meat bleaching (Ledward, 1970; 1984).
Jayasingh *et al*. (2001) reported that the colour stability of ground beef, packaged with CO 0.5%, was strongly improved. However, the consumers require safe and natural products (Hugas *et al.*, 2002) without additives such as preservatives and antioxidants and without the use of toxic gasses.
In this perspective, the present study aimed to compare three different gas mixtures. The screened MAP were obtained with low CO_2 and high O_2 concentration (MAP1), high CO_2 and O_2 concentration (MAP2), or high CO_2 and low O_2 concentration (MAP3).

Objectives

The aim of this study was to investigate the effect of different modified atmosphere packaging on the shelf-life of fresh meat products in order to harmonise the safety with the sensorial quality of the products.

Materials and methods

Use of modified atmosphere packaging. A mix of triturated pork lean, fat (10%), NaCl (2.5%) and black pepper (0.15%) was used to fill casing. After filling, the products were divided into four batches. The first (MAP1) was packaged in modified atmosphere containing CO_2 20%, O_2 70% and N_2 10%; the second (MAP2) was packaged in modified atmosphere containing CO_2 40% and O_2 60%; the 3rd (MAP3) was packaged in modified atmosphere containing CO_2 40%, O_2 30% and N_2 30%; the last batch was used as a Control (C) and packaged without gas mixtures. Each batch was stored at +4 °C for 12 day.
pH measurement. Potentiometric measurement of pH was made at 0, 3, 6 and 12 days of storage by inserting a pin electrode of a pH-meter (Crison 2001) directly into each sample. The results were expressed as the mean of four determinations performed on different parts of the samples.
Colour determination. Colour was measured on sausages at 0, 3, 6, and 12 days of storage, using the Hunter L*, a*, b* system (CIE, 1978) with a reflectance spectrophotometer (Minolta CR300b).
Microbial analyses. Total and faecal coliforms were counted on VRBLA after incubation for 48 h at 37 °C and 44 °C respectively.
Total mesophylic bacteria were counted on Plate Count Agar (Oxoid) after incubation at 28°C for 48 h.

Brochothrix thermosphacta was enumerated on STA Agar base (Oxoid) with STA selective supplement (Oxoid) after incubation at 37°C for 48 h.
Pseudomonas spp. were counted on Pseudomonas Agar (Oxoid) with SR102E supplement (Oxoid) after incubation at 22°C for 24 h.
Water holding capacity, cooking loss and shear force analyses. Water holding capacity (WHC) was measured with the procedure described by Monetti (1997), and values were expressed as meat expulsed water. For cooking loss determination, sausages were weighed and cooked at 177 °C in a convection oven to an internal temperature of 74 °C. The temperature was measured with thermocouple probe inserted into the center of the sample. After cooling at room temperature, sausages were weighed, and cooking loss was determined as percentage reduction of weight.
For Warner-Bratzler shear force determination cooked sausages were cooled, wrapped in freezer paper and held overnight at 4 °C before WB shear values were obtained on four 1.27 cm-diameter cores. Each core was sheared three times at a crosshead speed of 230 mm/min. Average shear values for each sample were calculated on the basis of 12 measurements.

Results and discussion

The packaging without modified atmospheres caused an increase in undesirable microorganisms producing alterations. In fact, the samples from batch C showed an appreciable increase in psycrotrophic microorganisms, such as *Pseudomonas* spp., able to degrade the proteins producing off-flavours and free amines.
B. thermosphacta is described by several Authors (Grau, 1980; Nissen *et al*., 1996) as an important and undesirable microorganism in the fresh meat products. This species occurs constantly in the samples packaged without modified atmospheres and in the present work showed an increase in counts during the storage time in the Control samples. In this batch an increase of all the other microbial groups was also observed.
In this research we observed that CO_2 produces an appreciable action on microbial growth in batches stored in modified atmosphere, as previously demonstrated by other Authors (Djenane *et al*., 2003), but the effect and the entity of the antimicrobial activity are both due to the CO_2 concentration and to the presence of other gasses. In fact, the batches treated with a modified atmosphere of CO_2 20% (MAP1) showed microbial counts very similar to those of the Control batch (without modified atmosphere).
CO_2 40% determined the best results on the assayed fresh sausages but this CO_2 percentage showed different effects on microbial growth when combined with different O_2 concentrations: the mixture CO_2 40% and O_2 60% (MAP2) caused a sensitive inhibitory effect on microbial growth but no effect was revealed in the samples packaged with CO_2 40% and O_2 30% (MAP3). In fact this combination evidenced a lack of effect on microbial counts and the samples showed an increase of the altering microorganism, even though their levels were slightly lower when compared to those of MAP1 and of the Control batch. Concentration of CO_2 20% (MAP1) didn't seem to cause any inhibitory effect on the spoilage bacteria.
These results confirmed that microorganisms are CO_2-sensible in presence of a high O_2 concentration while they are not CO_2-sensible in a low O_2 concentration (Mano *et al*., 2000).
The gas composition of the MAP2 determined also a positive effect on the definition and maintenance of the red colour, the most important parameter for the consumer's choice. In the samples from MAP2 the high O_2 concentration permitted the presence of myoglobine in oxidative state, which is responsible for the red colour, and at the same time, the high CO_2 concentration assured the safety of the product. The above phenomena have not been recorded in the samples from the other batches.
In conclusion, the gas mixture of MAP2 determined a good inhibitory effect on the microorganisms, assuring the prevention of alterative factors, and positively affecting some rheological parameters.
In fact, greater WHC showed by those sausages determined less cooking loss, while the treatment didn't modify the juiciness. In addition, sausages from MAP2 showed a more reduced shear value.

Conclusions

By the results obtained from microbiological, physical and rheological analyses it was possible to understand the playing role of the different atmosphere compositions on the microbial evolution and on the quality of sausages during the storage. In particular the use of techniques that modify in a very significant way the microbial growth in fresh sausages allowed us to assure the preservation of the most important quality parameters and the extension of the shelf-life.

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RESULTS OF PHYSICOCHEMICAL AND SENSORIAL INVESTIGATION OF DIFFERENT TYPE OF FERMENTED SAUSAGES DURING FERMENTATION AND RIPENING PROCESS

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Background

The production of naturally fermented sausages in the Mediterranean countries and the southern part of Central Europe has a very long tradition. In the second half of the 20th century, sausage production has been gradually industrialized and a very large assortment of fermented sausages has been developed. Aiming at maintaining the traditional sensorial quality of dry sausages and to assure their safety, although produced in industrial scale, it is important to develop and use protective cultures, after selection of strains isolated from naturally fermented products. A prerequisite for this research is to identify the main differences in the traditional production of fermented sausages, in various countries, and to study the evolution of physicochemical and sensorial characteristics of the sausages, during natural fermentation and ripening.

Key words: fermented sausages, physicochemical features, sensorial characteristics

Objectives

The main task of the research was to investigate the physicochemical features (pH, a_w and NaCl content) and the sensory characteristics of certain naturally fermented sausages, as produced traditionally in different countries (Greece, Serbia and Montenegro, Bosnia and Herzegovina, Croatia, Hungary and Italy). At the beginning, sausage composition was defined and then, during the processes of fermentation and ripening, the physicochemical and sensorial characteristics were investigated.

Materials and methods

For the purpose of the investigations, typical traditional sausages were produced in local meat processing plants, without using starter cultures. Sausages composition is presented in the table 1. The specifications applied in the preparation stage (final size of meat and fat pieces and used casings) as well as the critical technological parameters (i.e. temperature, relative humidity and duration) in the subsequent stages of traditional production (i.e. draining of stuffed sausages, smoking, fermentation, ripening and drying), are presented in table 2.

Three batches of sausages were used for the experiments, carried out within a 3 month period. Samples were taken from each batch at 0, 2, 4, 7, 14 and 28 days after formulation. Every sampling day, 3 sausages were transported to the laboratories and subjected to physicochemical analysis (moisture, NaCl, nitrite and nitrate content, pH and a_w). Standard methods were used for the analysis.

At the end of each process, sausage samples were subjected to sensory analysis. A panel of 10 persons was created in each country. In a 10 degrees scale, the panelists had to grade the produced sausages for coherence, smell, acidity, tenderness, flavor, after taste and overall impression. The differences in the sensory scores were further evaluated using a statistical program.

Results and discussion

Sausage composition, dimensions as well as fermentation-ripening processes varied among the different countries. Serbian, Hungarian and Italian sausages are produced by pork meat, only. The Bosnian sausages are produced by beef meat only, while the Croatian and Greek producers use mixed pork and beef meat. Other ingredients are sugars, salt, fat and spices (it might be simply black pepper for Italian sausages, garlic and paprika for Serbian sausages or spice mixture). The size of sausages varied between 28 and 50 mm in

diameter. Smoking was applied for the sausages produced in Serbia, Bosnia and Croatia, partial smoking in Hungary and Greece, while in Italy no smoking was performed. Ripening of the sausages was carried out under controlled conditions of temperature and relative humidity. In general, temperature higher than 12°C and relative humidity 60-80%, were the conditions of ripening in all countries, except for Serbia where ripening temperature was at 5-12°C. Sausages were considered ready for consumption on the 28th day, which was also considered the final time point of experiments. It should be noted that for the Croatian sausages, ripening times were usually shorter, but for purposes of harmonization with the rest of the partners, experiments were carried out beyond the average time of release to the market.

In sausages examined in Greece the most intensive decrease of pH (table 3) was observed during the fermentation and ripening procedure (6.25 to 4.90). The lowest starting pH (5.47) was detected in sausages produced in Serbia and Montenegro, but its decrease during processing was very slow (final pH was 5.27). In sausages produced by beef meat (Bosnia and Herzegovina), decreasing of pH had the most correct flow (from 6.15 to 4.86). The Croatian, Bosnian and Italian sausages showed a pH decrease till 7th day, and a slow increase in the rest 21 days of production. In sausages investigated in Hungary pH changes had the similar flow, but at a little higher level than the sausages from Serbia and Montenegro. Generally, final pH values were between 4.86 and 5.66. The most acid sausages were produced in Bosnia and Herzegovina (4.86) and Greece (4.90) and the most basic in Italy (5.66).

Salt content (table 4), just after the preparation (day 0), was between 1.51% (Croatia) and 2.52% (Italy). Proportionally to starting content and degree of moisture losses, an increase of NaCl content was observed. At the final product, the highest concentration of salt was determined in Hungarian sausages (4.71%).

Significant differences were observed in a_w values (table 5). After 28 days, a_w values in sausages investigated in Bosnia and Herzegovina, Croatia and Italy were higher than 0.90, while the lowest a_w values in the final product were found in sausages prepared in Greece, Serbia and Montenegro.

Sensorial characteristics of sausages (Figure 1), processed in the traditional way, were evaluated very high (overall impression was above 70), except products examined in Croatia. Differences between investigated sausages in other countries and Croatia were probably due to the prolongation of the usual process. Sensorial characteristics of sausages, in all 6 countries, showed that they were of very high quality with characteristic smell, flavor and coherence.

Conclusions

There are differences among the produced fermented sausages in the 6 countries, as far as composition, size and fermentation-ripening process. Physicochemical changes that occurred during ripening, can be summarized as follows for all countries:

1. Decrease of pH (final value between 4.86 and 5.66; sausages produced in Bosnia, Herzegovina and Greece are the most acid, and in Italy the most basic);
2. Decrease of a_w (final values between 0.78 and 0.94; Greek sausages had significantly lower a_w compared with the ones from other countries);
3. Increase of NaCl content (final values between 2.29 in Croatian sausages and 4.71 in the Hungarian ones).
4. The sensory analysis of the final products showed an overall acceptability of the products, above 70% for all partners, except for Croatia, due to the extension of the regular ripening time.

Acknowledgment

This research has been carried out under the umbrella of the “SAFETYSAUSAGE” project, which is funded by the E.C. within the framework of the INCO-DEV program (Contract No ICA4-CT-2002-10037). The authors gratefully acknowledge the Hungarian Meat Research Institute, the Agricultural University of Athens and the University of Udine for their physicochemical and organoleptic results, BEKKA SA, “OVAKO” Meat Industry and HUNNIA Meat Processing and Service Ltd for the production of sausages and Dr. Rantsios for his overall advice and support.

Sausage composition

Table 1

Ingredients	Composition (%)					
	Greece	SM	BH	Croatia	Hungary	Italy
Pork meat	35	70	-	62	63.5	60
Beef meat	35	-	96[4]	10	-	-
Pork back fat	30	30	-	25	32.5	40
Salt	2.5	2.6	2.5	2.5	2.3	2.5
Sugars	1.5[1]	0.5[3]	0.3[5]	0.3	0.1[1]	1.5
Skim milk pow.	2.5	-	-	-	-	-
Spices	0.3[2]	-	-	3.0[6]	1,5[2]	-
Garlic	0.1	0.8	1.0	-	0.4	-
White wine	0.2	-	-	-	-	-
$NaNO_3$	0.02	-	-	-	-	+[7]
$NaNO_2$	0.02	0.016	0.015	0.013	0.01	0.02[7]
Na-ascorbate	0.06	-	-	-	-	-
Paprika	-	0.6	-	-	-	-
Black pepper	-	-	0.2	-	-	0.07

[1]commercial mixture of maltodextrins with salt, Na-ascorbate, KH_2-phosphate and essential oils;[2]black pepper, red pepper and cloves; [3]dextrose; [4]beef meat with max. 20% of viable fatty tissue from cattle not older than 5 years; [5]commercial mixture of sugars as at [1]without essential oils;[6]black pepper, red pepper and garlic; [7] blend of $NaNO_2$ and $NaNO_3$

Technological parameters in the production of traditional fermented sausages

Table 2

Parameter	Greece	SM	BH	Croatia	Hungary	Italy
Preparing						
-meat pieces (mm)	10	8	10	12	-	-
-final fat size (mm)	2	5	2-3	2	3-5	
-casing	synthetic	natural	synthetic	natural	natural	natural
-diameter (mm)	45	30-32	32	32-34	28	50
Draining						
-temperature (°C)	15-18	environ.	15-18	20	15-18	22
- rel. humidity (%)	-	-	-	95	-	85
-duration	2-3h	1 day	2-3h	12h	2-3h	2 days
Smoking	after 2nd day[1]	Classic[2]				
-temperature (°C)	24	-	15	20	20	
-rel. humidity (%)	-	-	90	85-90	75	
-duration	2-3h	4days	7days[4]	2 days	2h[6]	
Fermentation		Traditional	Traditional	Tradiotional		
- temperature (°C)	24-20	drying and	drying and	drying and	20	12[7]
- rel. humidity (%)	94-86	ripening	ripening	ripeniing	80	60-90
- duration	7 days				2 days	5 days
Ripening						
- temperature (°C)	15-16	5-12[3]	14-18	20-16	15	12
- rel. humidity (%)	80	85-60	90-75	90-75	75	65-85
- duration	21 days	21 days	21 days	26 days[5]	14 days	21 days

[1]smoking performed after 2nd day of fermentation; [2]smoking process in classic smoking chamber without thepossibility for air conditioning with open burning fire; [3]temperature depended of the outdoor temperature; [4]smoking regime was 4h of smoking and 4h pause during 24h; [5]usual duration of drying and ripening is 19 days; [6]after draining sausages were in fermentation chamber 8h at 17°C and 70% RH, prior of smoking; [7]temperature was decreased from 22°C to 12°C with a rate of 2°C per day

Changes of pH during fermentation and ripening of sausages (mean values of three batches)

Table 3

Country	Day					
	0	2	4	7	14	28
Greece	6.25	5.70	5.37	4.91	4.85	4.90
SM	5.47	5.34	5.26	5.15	5.06	5.27
BH	6.15	5.58	5.03	4.81	4.82	4.86
Croatia	6.15	6.00	5.84	5.21	5.23	5.38
Hungary	5.89	5.84	5.79	5.64	5.48	5.53
Italy	5.73	5.54	5.40	5.34	5.50	5.66

Changes of NaCl content (%) during fermentation and ripening of sausages (mean values of three batches)

Table 4

Country	Day					
	0	2	4	7	14	28
Greece	2.39	2.66	3.06	3.30	3.89	4.05
SM	2.42	2.69	2.78	3.09	3.15	3.73
BH	2.36	3.35	3.36	3.40	3.80	4.32
Croatia	1.51	1.54	1.69	2.05	2.22	2.29
Hungary	2.50	2.86	3.44	3.43	3.94	4.71
Italy	2.52	2.66	2.72	2.95	3.11	3.34

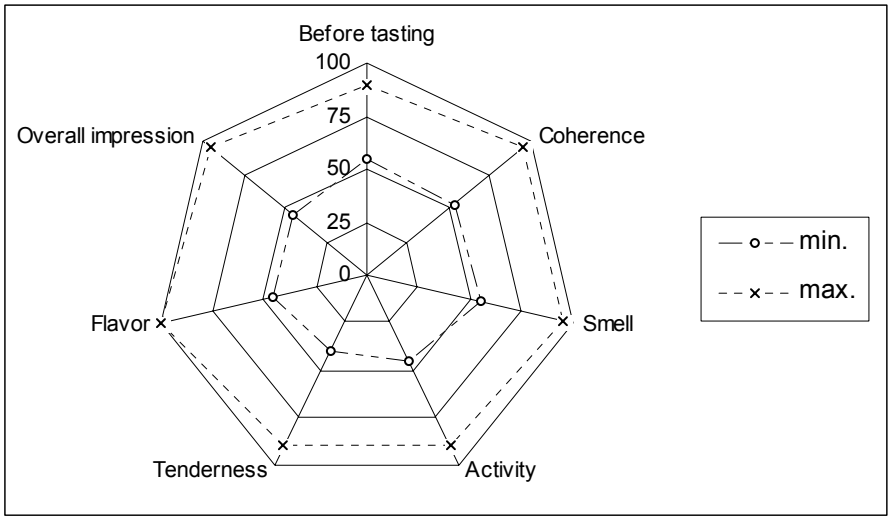
Changes in a_w value during fermentation and ripening of sausages (mean values of three batches)

Table 5

Country	Day					
	0	2	4	7	14	28
Greece	0.86	0.85	0.85	0.84	0.83	0.78
SM	0.92	0.92	0.90	0.90	0.87	0.85
BH	0.96	0.94	0.92	0.91	0.90	0.90
Croatia	0.97	0.96	0.96	0.96	0.95	0.94
Hungary	0.96	0.95	0.94	0.94	0.92	0.86
Italy	0.97	0.95	0.93	0.93	0.93	0.92

Sensorial analysis of final product

Figure 1



COMPARISON OF SENSORY AND ANALYTICAL PROPERTIES OF DRY SAUSAGE PRODUCED WITH DIFFERENT PROCESSING PROCEDURES

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Background

Traditional processing of typical German dry sausage followed by ripening in drying chambers takes a long time (up to 20 days), requires huge storeroom capacity, is energy intensive and often results in low production rates. A faster method of producing dry sausage would allow considerable energy savings while raising the production rate. This could have interesting applications in the meat industry.
The concept of adding a certain amount of dried fermented meat in production of dry sausage would not only shorten the ripening time, but also raise the question if there are differences between the final products in terms of organoleptic characteristics such as odor and taste.
In earlier investigations, a number of volatile organic compounds released from dry sausage were analyzed by gas chromatography/mass spectrometry [1, 2, 3, 4]. In dry sausage, identified volatile constituents are mainly derived from added spices (pepper, garlic etc.) and as a result of light and oxygen induced lipid oxidation as well as enzymatical degradation of carbohydrates, proteins and lipids.
Also, sensory properties determined by test panels were reviewed for correlation with more objective and reproducible analytical methods such as GC/MS and electronic nose [5].

Objectives

The objective of this study was to develop a novel method to shorten the ripening time of dry sausage by adding a certain amount of dried fermented meat. In comparison with a traditional German dry sausage product, the overall properties such as odor and taste were determined by sensory evaluation and correlation with GC/MS and electronic nose results have been investigated.

Materials and methods

Production of dried fermented meat: Preparation of meat: Refrigerated lean pork (estimated fat content of 10%) was coarsely cut into pieces. 2.8% nitrite curing salt, 1.2% mixture of spices, 0.3% dextrose and a 0.05% starter cultures compound (containing *L. sakei* + *Staph. carnosus*) (Bitec, Germany) were added. All ingredients were mixed in a blender and minced through an 8 mm plate. Fermentation: The meat was fermented in vacuum-packed bags at 24°C for at least 40 hours depending on the change of the pH value (below pH 5.0). Drying: The meat was air-dried at 50°C. The drying process was completed when the weight was reduced by 60%. The dried fermented meat was chopped again on high speed in the cutter, packed in bags and stored frozen at -18°C.
Production of dry sausage (batch 1, control): Traditional dry sausages were prepared according to the following weight based formula: 30% lean frozen pork (estimated fat content of 10%), 30% 3 mm minced beef (estimated fat content of 8%), 30% frozen pork back fat (estimated fat content of 90%) and 10% 3 mm minced lean pork. Additives and spices were added per kilogram: 28 g nitrite curing salt, 12 g mixture of spices and 0.5 g of a starter cultures compound (containing *L. sakei* + *Staph. carnosus*) (Bitec, Germany). The mass was stuffed into 65 mm diameter fiber-reinforced cellulose casings (Nalo Fibrous, Kalle, Germany).
Production of dry sausage with dried fermented meat (batch 2): Dry sausages were prepared according to the following weight based formula: 5% lean frozen pork, 30% 3 mm minced beef, 30% frozen pork back fat, 10% 3 mm minced pork and 10% dried fermented meat. Additives and spices were added per kilogram (except dried fermented meat): 28 g nitrite curing salt, 12 g mixture of spices, 1 g sodium diphosphate and 0.5 g of a starter cultures compound (containing *L. sakei* + *Staph. carnosus*) (Bitec, Germany). The lack of freezing capacity during chopping makes it necessary to add liquid N_2 periodically. The mass was stuffed into 60 mm diameter fiber-reinforced cellulose casings (Nalo Fibrous, Kalle, Germany).

Ripening: The sausages were placed in a drying chamber under the following conditions: 2 days at 24°C, 88 – 92% relative humidity (RH); 2 days at 20°C, 85 – 88% RH; 3 days at 18°C, 82 – 86% RH and finally the dry sausages were ripened another day (control 8 days) at 14°C, 75 – 85% RH until a weight loss of 25% and another 22 days (control 29 days) at 14°C, 75 – 85% RH until a weight loss of 35% was reached. The sausages were smoked after 2, 3 and 5 days using friction smoke for 30 min each time.
pH measurement: The course of pH while ripening was measured using a spear tip electrode (Schott, Germany). The electrode was calibrated with two buffer solutions of pH 4.000 and 7.000.
Weight loss: The sausages were weighed once a day (Universal pro 32/34 F, Sartorius, Germany) until a weight loss of 25% and 35% was reached.
Sensory evaluation: A sensory panel determined the odor and taste of the dry sausages after a weight loss of 25% and 35% was reached.
Gas chromatography/mass spectrometry: In a 500 ml gas tight glass jar, 50 g of homogenized sample was equilibrated for 30 min at 42°C in a water bath. Then, each sample was purged for 15 min with 200 ml 99,995% pure N_2/min using an automatic sampling device (GS 301, Desaga, Germany). The extracted volatiles were trapped onto 225 mg of Carbotrap 349 (Perkin-Elmer, Germany) in a stainless steel thermodesorption tube. Each sample was analyzed in triplicates. The Carbotrap tubes were thermally desorbed at 300°C in a thermal desorber (ATD 400, Perkin-Elmer, Germany). The compounds were separated in a gas chromatograph (GC 6890, Agilent, Germany) equipped with a DB-5 low polar column and identified using a mass spectrometer (MSD 5973, Agilent, Germany). The temperature was programmed at 40°C for 3 min, from 40°C to 230°C at 7°C/min and to 260°C at 12°C/min and then held at 260°C for 15 min. The mass spectra were recorded in electronic impact mode at 70 eV, from 30 to 250 m/z. Identification of compounds was based on mass spectra from a library database (NBS 75K, Agilent, Germany).
Electronic nose: In a 22 ml headspace vial sealed with a silicon/PTFE septum, 10 g of homogenized sample was equilibrated for 30 min at 50°C in a headspace autosampler (HSS 7694, Agilent, Germany). Each sample was analyzed four times. An aliquot of 3 ml headspace was transferred with the carrier gas to a chemical sensor system based on 4 different metal oxide semiconductor (MOS) gas sensors (VOCmeter-Hybrid, AppliedSensor, Germany). The evaluation by means of a principal component analysis (PCA) was performed using a commercial software package (Argus, AppliedSensor, Germany).

Results and discussion

In the following, different characteristics such as weight loss and pH value between the batches were investigated. A fundamental advantage of batch 2 is the shortening of ripening time. Figure 1 shows the weight loss during ripening. In comparison with the control, the batch with dried fermented meat reaches a weight loss of 25% and 35% after 8 and 30 days, respectively, whereas the control loses the corresponding weights not before 15 and 44 days, respectively. As can be seen, batch 2 already has a 15% weight loss at the beginning of the ripening process. This can be explained by the fact that moisture has been removed from the fermented meat during drying.
After adding the starter cultures to the meat mixture a course in the pH value over the ripening period was obtained (Figure 2). In comparison, the traditional product and the new technology started at different pH values but met after 2 days. The traditional product began at a higher pH level with a steeper decrease within the first day. The batch produced with dried fermented meat started at pH 5.7 and decreased more slowly the first day. This can be explained by the fact that the water activity of this batch was lower than the traditional one and for this reason the starter cultures needed a longer period to grow and to produce lactic acid.
The determination of the odor after a weight loss of 25% and 35% by a sensory panel resulted in no difference at all between the two batches. On the contrary, the taste differed clearly. Batch 1 showed a typical distinct fermented flavor which has been developed during the longer ripening time.
The headspace GC/MS analyses of batch 1 and 2 at the beginning of the ripening process, at 25% and 35% of weight loss are thought to result from a combination of added spices, oxidation processes and microbial activities. Figure 3 represents a typical headspace gas chromatogram of a dry sausage sample. The individual compounds identified by mass spectrometry are shown in Table 1. Between the batches there were hardly any qualitative but more quantitative differences regarding individual volatile compounds. Regarding the hexanal values, both batches show an increase over ripening time (Figure 4), where batch 2 starts at a comparatively high value which might be induced by adding dried fermented meat.
Figure 5 presents a PCA plot of batch 1 and 2 at different ripening stages resulting from electronic nose measurements. Here, all volatile compounds present in the headspace are measured as a sum parameter using

4 MOS sensors with different selectivities to give a specific aroma fingerprint for each individual sample. It can be seen that there is a clear difference between the samples at the beginning and at the end of the ripening time, but hardly any differences between the ripened samples or individual batches. This is in correlation with the odor results obtained by the sensory panel.

Conclusions

The aim of this study was to show that the use of dried fermented meat in the production of dry sausage can shorten the ripening time by about one week. However, this new processing procedure leads to a less intense dry sausage taste. Furthermore, the investigations have shown that analytical tools such as headspace GC/MS and electronic nose can be used to provide more objective and reproducible results, but with the limitation that only the human being can specifically describe taste and odor impressions.

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Data in the form of tables, charts and figures

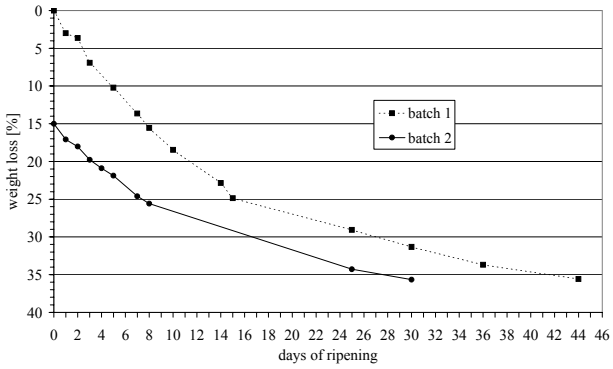

Fig. 1: Plot of weight until 35% loss.

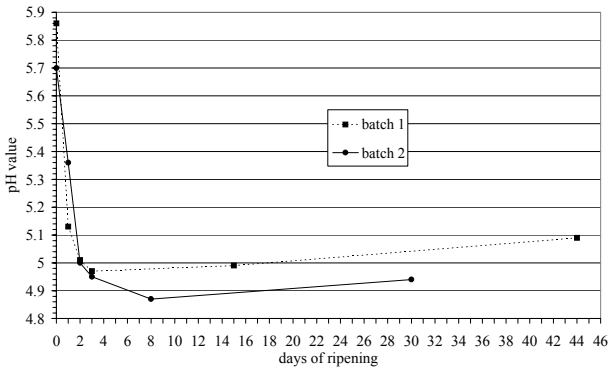

Fig. 2: Plot of pH change during ripening.

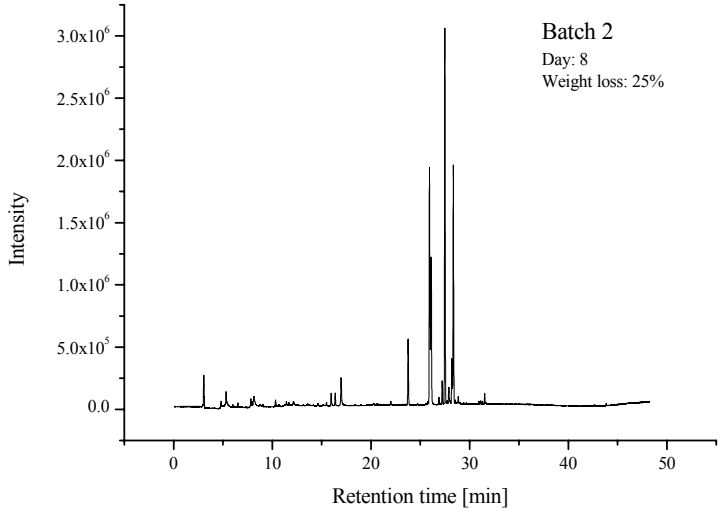

Fig. 3: Typical headspace chromatogram of a dry sausage sample. See table 1 for peak identifications.

Peak	Time	Compound	Origin of compound
1	5.33	Pentane	Lipid oxidation
2	6.52	Dimethyl-disulfide	Garlic/spice mixture
3	7.83	Hexane	Lipid oxidation
4	7.96	3-Methyl-pentane	Lipid oxidation
5	8.13	2-Butanone	Carbohydrate fermentation
6	10.34	3-Methyl-butanal	Amino acid catabolism
7	11.70	Heptane	Lipid oxidation
8	12.15	Pentanal	Lipid oxidation
9	15.97	3-Methyl-heptane	Lipid oxidation
10	16.38	Octane	Lipid oxidation
11	16.98	Hexanal	Lipid oxidation
12	22.02	Heptanal	Lipid oxidation
13	23.77	α-Pinene	Pepper/spice mixture
14	25.94	Pentamethyl-heptane	Lipid oxidation
15	26.11	β-Pinene	Pepper/spice mixture
16	27.23	α-Phellandrene	Pepper/spice mixture
17	27.49	3-Carene	Pepper/spice mixture
18	27.90	Tetramethyl-octane	Lipid oxidation
19	28.21	1-Methyl-4-(1-methylethenyl)-benzene	Pepper/spice mixture
20	28.36	Limonene	Pepper/spice mixture
21	28.86	Dimethyl-octane	Lipid oxidation
22	31.54	Nonanal	Lipid oxidation

Tab. 1: Volatile compounds identified in a dry sausage sample.

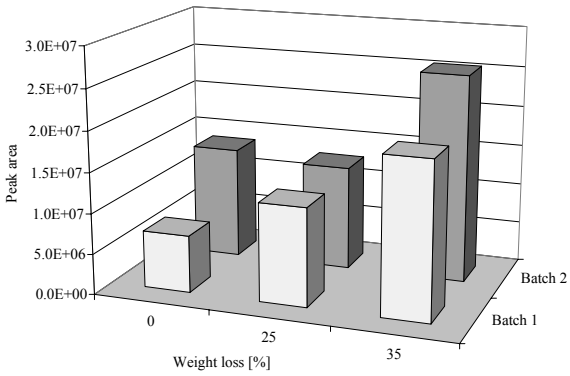

Fig. 4: Area of hexanal peaks at different ripening stages.

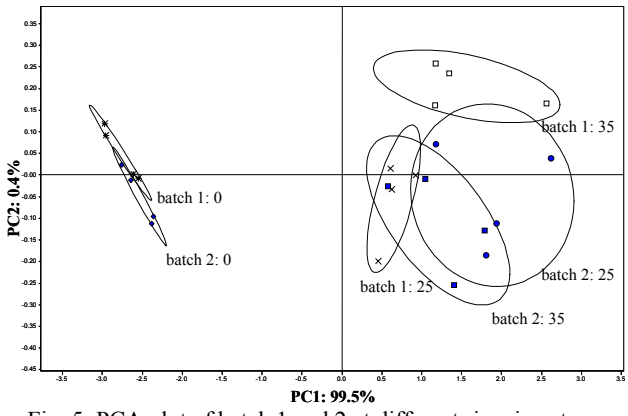

Fig. 5: PCA plot of batch 1 and 2 at different ripening stages.

MICROBIOLOGICAL CONTAMINATION OF LAMB CARCASSES: EFFECT OF ABATTOIR SIZE, SAMPLING SITE AND TIME OF STORAGE

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Background

The interest in meat hygiene has increased in recent years, mainly due to the current crisis suffered by the meat sector. These emergencies have alerted the European Union (EU) to perform an extensive normative for controlling hygienic-sanitary quality of meat "from the farm to the fork", therefore including slaughtering and dressing procedures in this slogan. The European regulatory authorities have set up the microbiological assessment of carcasses before chilling in the abattoirs (Directive EC/471/2001). However, carcass quality during chilled storage has only been recommended by the ICMSF, but not by EU requirements. On the other hand, in the products with undisputed prestige that have attained their own official label, such as Manchego Spanish Breed Lamb, it is necessary for their organoleptic quality also to be accompanied by optimum microbiological quality during the pre-sale period.

Objectives

The aim of this work was to evaluate the effects on microbiological development of carcass lamb chilled at 4ºC of the next factors: size of the abattoir (number of animals slaughtered per week), the sampling site (flank, neck and rump), and the time of storage (at 24 h and 6 days post-slaughter which is the maximum time permitted for sale in "Manchego Lamb Denomination of Origin").

Materials and methods

In June, forty lambs of the Manchega breed were slaughtered at 25 kg live weight using standard commercial procedures. The slaughter was carried out in two commercial abattoirs of different size (located in the same city): a small abattoir and a big abattoir, (less than 2000 and more than 8000 animals slaughtered per week respectively, including pigs, lambs and beef). In each abattoir 20 lambs were slaughtered and the carcasses remained at 4ºC for 6 days post-slaughter in a chilling room. None of the carcass exhibited 24-h pH values higher than 5,7.

In all carcasses, the sampling (according to EC Decision 471/2001) was carried out by swabbing areas of 100 cm^2 in three different sites of carcass (rump, flank and neck), and at 1 and 6 days post-slaughter. Samples were stored at 4ºC in sterile tubes containing 10 ml of peptone water until examination, after no more than 3 hours.

Each sample was homogenised for 60 seconds, and additional serial 10-fold dilutions of homogenates were made in peptone water and were inoculated in Petri dishes for enumerations of: Mesophiles, pouring on Plate Count Agar (Scharlau Chemie, Barcelona, Spain) at 32°C for 2 days; psychrotrophs, pouring on PCA at 7ºC for 10 days; enterobacteria, using pour plates of Violet Red Bile Dextrose agar (Scharlau Chemie, Barcelona, Spain) and incubated at 32°C for 48 h; and Yeasts and Moulds, were determined on Rosa Bengala agar (Scharlau Chemie, Barcelona, Spain) incubated at 25ºC for 3 days. All microbial counts were expressed as base –10 logarithms of colony forming units per cm^2 of surface area (log CFU cm^{-2}).

Effects of abattoir, time of storage, sampling site and their interactions, on each microbial group was determined using a general lineal model (GLM). Factors and interactions that did not achieve significance were excluded from the analysis to increase the degrees of freedom of the residuals. Differences between sites of carcass in each abattoir were examined using a analysis of variance (ANOVA). When the differences among sites were significant ($p< 0.05$), Tukey's test was carried out to check the differences between pairs of groups. The effect of time of storage in refrigeration for each site was analysed using ANOVA at a significance level of $P< 0.05$. The correlation between pairs of microbial groups were examined using a simple correlation. Data were analysed using the SAS (1998) statistical package.

Results and discussion

GLM shows that the number of microorganisms found on carcasses varied significantly between abattoirs, sampling sites and time of storage (Table 1). In general for all microbial groups we found significant interactions between these factors. Other works (Sumner et al., 2002) reported that there were little difference in the mean values of aerobic viable count of the carcasses produced in abattoirs and very small plants.

After 1 day post-mortem in both, small and big abattoir (Table 2 and 3, respectively), the highest microbial recovery were observed on the flank ($P < 0.05$). After 6 days post-slaughter in the small abattoir the differences among sites only remained for enterobacteria ($P < 0.05$), and the lowest recovery was in the rump. However after this time in the big abattoir there were significant differences among sites for all microorganisms groups analysed.

In general, all microbial groups increased with time in both abattoirs. But in the small abattoir significant differences were not found in mesophiles neither psychrotrophs in flank site with time.

The correlations between the microbial groups assayed achieved significance in the four groups. Thus, mesophiles showed a positive correlation with psychrotrophs ($R= 0.881$, $P < 0.01$), enterobacteria ($R= 0.641$, $P < 0.01$), and yeasts and moulds ($R= 0.711$, $P < 0.01$); psychrotrophs were positively correlated with enterobacteria ($R= 0.674$, $P < 0.01$), and yeasts and moulds ($R= 0.702$, $P < 0.01$); and enterobacteria with yeasts and moulds ($R= 0.626$, $P < 0.01$).

The mean of mesophiles per cm^2 in this work ranges from 1.37 to 4.91 log, whereas the means counts of psychrotrophs varied from 0.85 to 3.91 log. According to these results, it could be concluded that the slaughtering practices in both abattoirs were acceptable. Moreover, the counts after 6 days of chilled storage are in agreement with the current EU requirements for ovine carcasses immediately after slaughter. Both, mesophiles and psychrotrophs were recovered from 95% of samples analysed in both abattoirs after 1 day at counts inferior than 3.5 log CFU/cm^2. After 6 days only 17.8% and 15.6% of samples recovered for mesophiles and psychrotrophs, respectively, showed counts superior than this level.

Our results are in the range found by other authors in a similar period of time. Along this line, Sumner *et al.* (2002) found that 364 chilled lamb carcasses from 17 Australian abattoirs had a mean value of aerobic viable counts of 2.59 log CFU/cm^2. In three Swiss abattoirs the median counts for aerobic total counts ranged from 2.5 to 3.8 log CFU/cm^2 (Zweifel and Stephan, 2003). Other studies showed mean values of mesophiles on chilled carcasses which ranged from 3 to 5 log CFU/cm^2 (Prieto et al., 1991; Vanderlinde et al., 1999; Duffy et al., 2001).

Enterobacteria values were less than 1 log CFU/cm^2 in 90% of samples after 1 day of storage. This group was only presented in 50% of samples at this time. After 6 days the 75% of samples showed count inferior than 2 log CFU/cm^2 . The EU requirements establishes count above 2.5 log CFU/cm^2 as unacceptable for this microbial group in carcasses before chilling.

The results also showed a low occurrence of yeasts and moulds. In 75% of samples analysed overnight this microbial group was not recovered. Counts were inferior to 3 log CFU/cm^2 in 95% of the samples stored for 6 days. These results suggest that yeasts and moulds contamination is apparently not a problem.

Conclusions

In general, the microbial counts for the four microbial groups analysed were higher in the big abattoir. In both abattoirs the mean values in the flank were higher than in the rump and in the neck. After six days post-slaughter there was an increase in all microbial groups analysed, however these levels were similar to those observed in other European or non abattoirs. According to these results, it could be concluded that the slaughtering practices in both abattoirs were good and the counts fell within the recommendations of International Commission on Microbiological Specifications for Foods (ICMSF).

Table 1. Factors (abattoir, sampling site, storage time) and interactions affecting mean level (log CFU/cm^2) of mesophiles, psycrotrophs, *Enterobacteriaceae,* and yeasts and moulds.

Model[a]	Mesophiles	Psychrotrophs	Enterobacteria	Yeasts & moulds
R^2	58.70	47.34	66.69	54.33
S.E.E. [b]	0.69	0.75	0.59	0.64
P value	0.000	0.000	0.000	0.000
Constant	2.64 ± 0.05	2.21 ± 0.05	0.82 ± 0.04	0.72 ± 0.05
Abattoir	- 0.50 ± 0.05***	- 0.33 ± 0.05***	- 0.09 ± 0.04*	- 0.38 ± 0.04***
Time	- 0.38 ± 0.05***	- 0.44 ± 0.05***	- 0.54 ± 0.04 ***	- 0.42 ± 0.04 ***
Site[1]	- 0.14 ± 0.07***	- 0.23 ± 0.08 ***	- 0.53 ± 0.06 ***	- 0.02 ± 0.06 ***
Site[2]	0.65 ± 0.06***	0.66 ± 0.08***	0.74 ± 0.06***	0.42 ± 0.06***
Site[1] x Time	-	- 0.01 ± 0.08 *	0.34 ± 0.06 ***	-
Site[2] x Time	-	0.18 ± 0.08*	- 0.33 ± 0.06***	-
Site[1] x Abattoir	0.10 ± 0.04***	0.04 ± 0.08 *	- 0.05 ± 0.06 ***	- 0.08 ± 0.06 ***
Site[2] x Abattoir	- 0.34 ± 0.07***	- 0.20 ± 0.08*	- 0.21 ± 0.06***	- 0.23 ± 0.06***
Time x Abattoir	-	-	-	0.14 ± 0.04 **
Site[1] x Time x Abattoir	- 0.21 ± 0.07	- 0.20 ± 0.08 ***	- 0.02 ± 0.06 ***	-
Site[2] x Time x Abattoir	0.30 ± 0.07***	0.29 ± 0.08***	0.23 ± 0.06***	-

[a] Significance levels for each factors are indicated as follows: *P< 0.05,** P< 0.01, and *** P< 0.001. The model shows only interaction that were significant at least for one microbial group.
[b] S.E.E., standard error of the estimate.

Table 2. Effect of sampling site (rump, flank or neck) on the microbial recovery (log CFU/cm^2) after 1 or 6 days post-slaughter in carcasses from the small abattoir.

Microbial group	Time	Rump	Flank	Neck	ANOVA
Mesophiles	1 day	1.57 ± 0.58^{ab}	2.29 ± 0.77^{b}	1.37 ± 1.30^{a}	**
	6 days	2.62 ± 0.86	2.62 ± 0.55	2.35 ± 0.69	NS
	ANOVA	***	NS	**	
Psychrotrophs	1 day	0.99 ± 0.95^{a}	2.34 ± 0.82^{b}	0.85 ± 1.04^{a}	***
	6 days	2.36 ± 1.02	2.31 ± 0,79	2.32 ± 0.84	NS
	ANOVA	**	NS	***	
Enterobacteria	1 day	nd	0.68 ± 0.95^{b}	0.07 ± 0.32^{a}	***
	6 days	0.31 ± 0.52^{a}	1.85 ± 0.68^{b}	1.47 ± 0.74^{b}	***
	ANOVA	**	***	***	
Yeasts & moulds	1 day	nd	0.188 ± 0.51^{a}	nd	*
	6 days	0.46 ± 0.89	0.89 ± 0.85	0.49 ± 0.80	NS
	ANOVA	*	**	**	

Significance levels for each factors are indicated as follows: *P< 0.05, ** P< 0.01, and *** P< 0.001; NS: not significance; $^{a, b, c}$ in the same row are significantly different (P< 0.05); nd: no growth were detected.

Table 3. Effect of sampling site (rump, flank or neck) on the microbial recovery (log CFU/cm^2) after 1 or 6 days post-slaughter in carcasses from the big abattoir.

Microbial group	Time	Rump	Flank	Neck	ANOVA
Mesophiles	0 days	2.82 ± 0.42^{a}	3.38 ± 0.25^{b}	2.13 ± 0.48^{c}	***
	6 days	2.96 ± 0.38^{a}	4.91 ± 0.78^{b}	2.66 ± 0.42^{a}	***
	ANOVA	NS	***	**	
Psychrotrophs	0 days	2.04 ± 0.58^{a}	2.87 ± 0.57^{b}	1.46 ± 0.45^{c}	***
	6 days	2.49 ± 0.41^{a}	3.91 ± 0.85^{b}	2.44 ± 0.44^{a}	***
	ANOVA	*	***	***	
Enterobacteria	0 days	0.17 ± 0.29^{a}	0.68 ± 0.64^{b}	0.02 ± 0.07^{a}	***
	6 days	0.69 ± 0.41^{a}	3.05 ± 0.86^{b}	0.86 ± 0.67^{a}	***
	ANOVA	***	***	***	
Yeasts & moulds	0 days	0.53 ± 0.81^{a}	1.10 ± 0.65^{b}	nd	***
	6 days	1.81 ± 0.77^{a}	2.42 ± 0.66^{a}	0.76 ± 0.85^{b}	***
	ANOVA	***	***	***	

Significance levels for each factors are indicated as follows: *P< 0.05, ** P< 0.01, and *** P< 0.001; NS: not significance; $^{a, b, c}$ in the same row are significantly different (P< 0.05); nd: no growth were detected.

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A MECHANISTIC SIMULATION MODEL TO STUDY THE EFFECTIVENESS OF INTERVENTIONS AGAINST *E.coli* O157 IN A DUTCH CATTLE SLAUGHTERHOUSE

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Background

Reducing the contamination level of beef carcasses with enteric pathogens such as *E.coli* O157:H7 (VTEC), by means of implementing preventive measures in the slaughterhouse, is regarded to be important. Cost-effectiveness analysis is a valuable tool to evaluate the existing preventive measures. For a reliable cost-effectiveness analysis, beside calculation of the costs for each intervention, a good insight into the benefits is needed. According to the definition of Food Safety Objectives (FSOs), any reduction in the frequency and/or concentration level of a microbiological hazard in a food product can be treated as an appropriate benefit for a certain intervention (Havelaar et al. 2004). Microbial decontamination methods are the commonly used preventive measures against the bacterial hazards in slaughtering plants. In the majority of studies in this field, scientists have been mostly interested on reduction in the number of bacterial Colony Forming Units (CFUs) on the surface of the meat, using interventions after experimentally contamination. Due to the essences and objectives of these types of studies, the effects of interventions, in terms of reduction in frequency of the contaminated beef units, will not be reported. Moreover, only few studies have been done to investigate the effectiveness (in terms of reduction in prevalence) of the intervention methods in the slaughtering process in real practice.
To fill this gap one can consider two approaches: 1) putting the interventions into practice in a slaughterhouse and observing the effects or 2) using computer simulations and performing sensitivity analysis. The first approach is not feasible to apply, because it is costly and disruptive (van der Gaag, 2003). Computer simulation is an attractive alternative to the implementation of explorative control strategies.

Objectives

The aim of the study presented in this paper is to build an epidemiological framework to investigate the effectiveness of different intervention methods along the beef slaughter line, in terms of reducing the frequency of contaminated beef quarters with VTEC. The result of this study will be used as a part of inputs needed for the cost-effectiveness analysis, which will be the next step in our research. Decision makers in the beef slaughtering industry are the potential users of the results.

Materials and methods

The general model described in this paper was build on a Microsoft Excel spread sheet using @Risk add-in software. In our model 500 cattle enter a typical Dutch industrial slaughterhouse on a daily basis. Two main sources of VTEC in/on the body of cattle on the farm can be recognized: 1-In the gastrointestinal tract (GI) and 2- On the hide (Heuvelink, 2001). Animals entering to the slaughterhouse can be grouped in the four groups, based on their GI tract and hide status: 1- [GI^+ H^+] 2- [GI^+ H^-] 3- [GI^- H^+] 4- [GI^- H^-].

Slaughter process

The following stages have been included in the model: *de-hiding*, *evisceration*, *splitting*, *fat/tail removing*, *trimming* (as a decontamination method), *washing with cold water* (for cooling down purpose) and *chilling*. Because the main possible interventions on the slaughter line are applied before the de-boning and fabrication stages, the beef carcass quarters (hind-quarters and fore-quarters), which are produced after the chilling stage, have been considered as the end product in our model. For each of the mentioned stages, a main risky event for VTEC transmission was identified from the literature: Direct contact of carcass surface with the hide of anus area for the de-hiding stage, rupture of the GI tract for the evisceration stage, contamination of splitter saw for the splitting stage, contact with contaminated knife for the fat trimming stage, spreading the bacteria from the hind-quarter to the fore-quarter for the washing stage and carcass-to-carcass contact for the chilling stage.

The output of the model is a distribution of the number of VTEC contaminated quarters of the beef carcasses, produced in one day at the end of *chilling* stage. Therefore, the prevalence of contaminated quarters in this model refers to the number of contaminated quarters out of 2000 produced quarters.

Stochastic Process of the model

Monte Carlo simulation with 10,000 iterations was implemented. One iteration of the model represents one day production of the slaughterhouse. As, during each slaughtering stage, two possible outcomes (contaminated versus not-contaminated) can be recognized, the *Binomial process* was chosen as the stochastic process of the model (Vose, 2000). In this model, quarters contaminated with no bacteria (zero CFU) are defined as negative, irrespective to the detection level of the routine bacteriological tests. In opposite, quarters with even one CFU contamination on their surface are treated as positive.

Three different stochastic processes are distinguished that determine the contamination status of a quarter in a stage. These processes are modelled as from equations 1 to 6. Let N denote the total number of quarters entering stage, $S_{(j)}^{+}$ the number of positive quarters after modelling the stochastic process by either $(j=1)$ the main risk factor or $(j=2)$ the environment or $(j=3)$ decontamination processes. $S_{(j)}^{-}$ is thus the number of negative quarters after each stochastic process. Considering Pr and Pe as probabilities of changing the status of a quarter from negative to positive due to the risky event or/and environment , and denoting Pd for changing the status from positive to negative by decontaminations, three levels of the model are written as:

1- Contamination due to the Risky Event of the stage

$$S_{(1)}^{+} = \text{Binomial}\ (S_{(0)}^{-};\ Pr) + S_{(0)}^{+} \qquad (1)$$

$$S_{(1)}^{-} = N - S_{(1)}^{+} \qquad (2)$$

2- Contamination by the Environment of the stage

$$S_{(2)}^{+} = \text{Binomial}\ (S_{(1)}^{-};\ Pe) + S_{(1)}^{+} \qquad (3)$$

$$S_{(2)}^{-} = N - S_{(2)}^{+} \qquad (4)$$

3- Decontamination

$$S_{(3)}^{-} = \text{Binomial}\ (S_{(2)}^{+};\ Pd) + S_{(2)}^{-} \qquad (5)$$

$$S_{(3)}^{+} = N - S_{(3)}^{-} \qquad (6)$$

The yearly prevalence of VTEC infection of dairy cattle (0.0096) in the Netherlands, infected in the GI, was used in the model to determine the number of infected cattle entering into the slaughtering line (Nauta, 2001). The other mentioned probabilities have been estimated based on available data found in the literature and experts opinion, which the list of these references is available with the authors.

Interventions

Interventions can reduce transmission probabilities of VTEC in certain stages of the slaughtering process. They also can reduce the transmission probability from the environment to the carcass and can change the contamination status of the quarters itself. Interventions can be categorized in three groups: *a*- cleaning and hygienic interventions; *b*- decontamination methods (and combinations); *c*- other interventions (e.g. replacing a stage by another or stopping a risky event).

We mainly focused on the decontamination methods and tried to compare their effectiveness, when they are used individually or in combination with other interventions. A linear relation between the reduction of CFUs and the reduction factor for the changing status probability (from positive to negative) was assumed. Using the data reported by Phebus et al (1997) for the level of reduction in number of CFUs in experimental studies, and mentioned linear relation, the level of reduction on changing the status probability for the five important decontamination methods were determined (the estimated reduction factors are given as: 0.0 for no intervention; 0.12 for hot water wash (H); 0.43 for lactic acid rinsing; 0.5 for trim (T); 0.51 for steam-vacuum (V); 0.57 for steam-pasteurization (S) and 0.99 for irradiation). Irradiation has been known as the most effective decontamination method by reducing the numbers of CFUs of VTEC by 10^6 CFU/cm^2 (Molins, 2001). Therefore irradiation was chosen for the upper bound of reduction factor. The default situation, where no intervention is applied, represents the lower bound. In total 18 interventions (including combinations) were examined in our model.

Results and discussion

Table 1 shows the baseline output of the model as well as the effects of implementing the interventions on the output. In the baseline situation, on average 18 contaminated beef quarters are produced in a working day. The distribution of the number of contaminated quarters in the baseline situation shows that in 95 % of the working days, the number of contaminated quarters is less than 33 quarters. Besides that, in 5% of the working days less than 6 contaminated quarters are produced. As it is expected, irradiation shows the highest effectiveness. In opposite hot water wash has the lowest effectiveness to reduce the frequency of contamination.

To make a comparison with the baseline situation, the distributions of two interventions, one from the middle of the table 1 (TW) and one from the top (VWLS) are illustrated in figure 1A. Applying TW and VWLS, the 95th percentiles values reduce to 18 and 6 quarters. In general a combination of interventions gives a better effect.

A comparison between our results and the study of Phebus et al (1997) shows an almost similar ranking order (table 1). However some differences exist. In the mentioned research, hot water washing of the carcasses after trimming (TW), had the first place of their list and applying only hot water wash was the least effective method to reduce the number of CFus/cm^2 of the meat surface. The most important difference is that, the combined intervention TWLS showed the most promising results in our study whereas, it's in the fifth place in their list. A possible reason for this is that in the laboratory trimming, freshly sanitized instruments are used and great control to prevent cross contamination is applied.

In a higher prevalence scenario (0.05), illustrated in figure 1B, the mean number of contaminated quarters will increase up to 95 quarters per day (125 quarters for 95 percentile). The TW and VWLS can reduce the mean number to 45 and 18 quarters, with 95 percentile as 68 and 20 respectively. This implies that the prevalence of infections at the farm is important and application of interventions at the slaughterhouse in such a situation would play a crucial role to keep the frequency of contaminated product in the acceptable level.

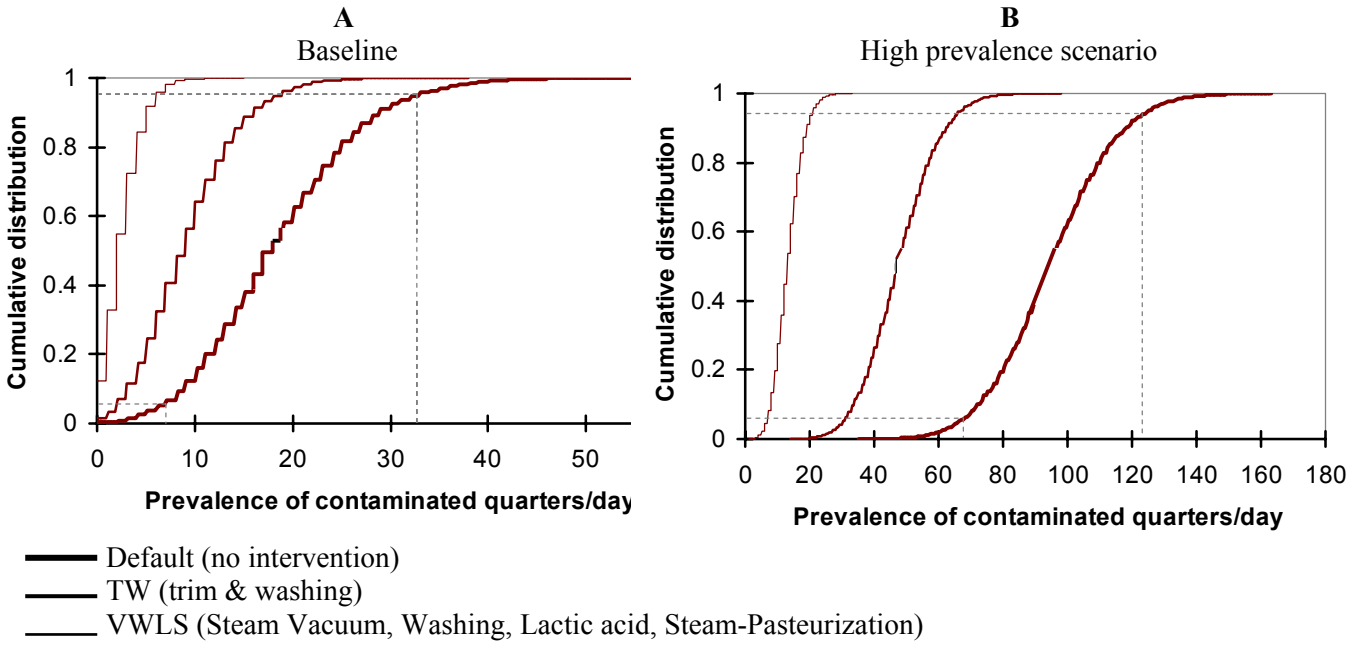

Figure 1 Distribution function for the number of contaminated beef quarters per day in with and without intervention situations. Graph A: baseline results, using yearly prevalence of GI infection for incoming animals to the slaughterhouse. Graph B: higher prevalence scenario (0.05).

Table 1 Baseline output of the model and the effects of interventions.

Interventions	Stage	Mean	5th & 95th percentiles	Rank order in this study	Rank order in Phebus's study
Irradiation	washing	00.22	0 – 1	1	na*
VWLS	de-hide/evisc/wash/split	02.58	0 – 6	2	2
TWLS	de-hide/evisc/wash/split	02.57	0 – 6	2	5
VWS	evisc/split/wash	04.16	1 – 9	3	6
TWS	de-hide/evisc/wash	05.00	1 – 11	4	3
TWS	de-hide/evisc/split	05.14	1 – 11	4	3
WS	evisc/wash	07.97	2 – 15	5	4
WS	evisc/split	08.21	2 – 16	6	4
VW	evisc/wash	09.13	2 – 17	7	7
Steam Pasteurization (S)	splitting	09.17	2 – 17	7	7
TW	de-hid/evisc	09.20	2 – 18	7	1
Trim (T)	de-hiding	10.38	3 – 20	8	9
Steam Vacuum (V)	evisceration	10.20	3 – 20	8	8
Trim (T)	splitting	10.46	3 – 20	8	9
Steam Vacuum (V)	splitting	10.40	3 – 20	9	8
Acid Lactic (L)	washing	11.36	3 – 21	10	na
Move chest opening**	de-hiding	15.34	5 – 28	11	na
Hot water wash (W)	washing	16.33	6 – 29	12	na
Hot water wash (W)	splitting	16.67	9 – 29	12	10
Baseline (without)		**18.42**	**6 – 33**	-	-

*na: These interventions either have not been considered in study of Phebus (1997) or have been applied in other sequence.
** Brisket region is opened in the de-hiding stage in our visited slaughterhouse.

Conclusions

Firstly, the results of this study show that, the use of computer simulation to evaluate the effectiveness of interventions along the slaughter line is a promising approach.
Secondly, the combinations of decontamination methods have more positive effect on reduction of frequency of contaminated quarters than individual interventions.
Thirdly, in a high prevalence scenario, application of interventions in the slaughterhouse plays a crucial role to keep the frequency of contaminated product in the acceptable level.
The forth and the last conclusion of this study is that, changing the place of the individual interventions in the slaughtering line has not a significant effect on the frequency of contamination. However the effectiveness of interventions is slightly increasing toward the end of the slaughter line.

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THE EFFECTS OF NITRITE AND TRADITIONAL COOKING PROCESS ON THE SURVIVAL OF *CLOSTRIDIUM SPOROGENES* AND AUTOXIDATION IN KAVURMA, A TRADITIONAL TURKISH FRIED MEAT PRODUCT

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Background

Kavurma is a traditionally deep fried (coarsely ground) meat product and produced to extend the shelf life of the product for 6-9 months. In the past, this technology was used only to conserve meat, but currently it is preferred as a processed meat variety; although, some people living in many places in Turkey and the Middle-East are still processing kavurma for preservation purposes. In traditional processing, the meat from beef or mutton is ground (approximately 4x5x6 cm) and mixed with 2.0 to 5.0 % salt, and then fried or cooked in animal fat using a double sided steam cauldron. After the cooking process, it is kept in almost anaerobic condition which is provided by solidified animal fat in the container. Recently, it has also become available in vacuum-packaged forms produced in modern meat processing plants and sold in department stores and markets (Vural and Oztan, 1989; Anon., 1996; Gungor, 2000; Yetim *et al*., 2003). The popularity of ready-to-eat foods is increasing in the world because of changing life styles, and kavurma is also considered as a ready-to-eat meat product since it is generally consumed without further processing or cooking. The technological and hygienic insufficiencies during the production of kavurma may cause quality deteriorations in the product as well as a health risk associated with microorganisms.

Deteriorations occurring in kavurma are generally due to microorganisms. Although, it is heat treated, it may contain a small microbial load. Microorganisms surviving during the heat treatment of kavurma may proliferate if the product is stored under inappropriate conditions. When the microorganisms present in kavurma are pathogenic or toxic, there is a potential health risk to the consumers. For example, clostridia are considered important bacterial group in the heat treated meat products, because of their facultative anaerobic and spore forming nature; *C. botulinum* produces a deadly toxin which causes botulism.

Research on kavurma is very limited in the literature. A study on kavurma sold on local markets indicated that some thermophiles, subtilis-mesenticus bacteria and micrococci were commonly present in kavurma samples (Inal, 1992). Additionally, Sarigol (1978) determined the microbial load in kavurma stored under different conditions and reported that lipolytic and proteolytic microorganisms, yeast, mold and staphylococci were present in home type and industrial type of kavurma. Vural and Oztan (1989) investigated the effects of α - tocopherol and potassium sorbate on the enhancement of shelf life of kavurma, and determined that control and antioxidant added samples initially contained less than 10 bacteria/g while samples with antimicrobial agent had no microorganisms. They observed that total viable microorganism of the control sample continuously increased during 6-month of storage. Another study conducted for the determination of microbiological, physical and chemical qualities of kavurma sold in Bursa (Turkey) market revealed that a potential health risk might be evident resulting from the consumption of kavurma when the procuct is not hygienically produced or it is stored under improper conditions (Tiryakioglu and Yucel, 1995). On the other hand, Cetin (2000) reported some pathogenic microorganisms, such as *Salmonella*, *Morganella*, *Yersinia* or *Klebsiella* in the kavurma samples obtained from local stores in Erzurum. He pointed out that the kavurma samples containing these pathogens may pose a probable public health risk. Yetim et al. (2003) investigated the formation of nitrosamines in kavurma prepared with nitrite addition and concluded that there was no detectable amount of nitrosamine compounds in the kavurma samples.

Objectives

The objective of this study was to determine the influence of nitrite addition and the traditional cooking process of kavurma on the survival and proliferation characteristics of *C. sporogenes,* representing the *C. botulinum*, and their effects on rancidity which was evaluated by TBA, FFA values and peroxide number.

Materials and methods.

Traditional Kavurma was manufactured as described by Gokalp et al. (1994). In this study, 4 types of kavurma were prepared as illustrated in Table 1). 1) Control, 2) 100 ppm nitrite, 3) *C. sporogenes* and 4) Nitrite + *C. sporogenes* containing samples. A steam jacketed cauldron was used for kavurma processing and the internal temperature of the meat-fat mixture was approximately 98°C during cooking that took approximately 1.5 hours. Kavurma samples were filled into sterile fibrose casings and sliced and vacuum packaged under aseptic conditions before storage. All the samples were kept for 6 months under refrigerated (4±1°C) conditions.

Table 1. Kavurma samples prepared in experiment,

Sample	Kavurma Types
1	Control (contains only meat and fat)
2	Nitrite added sample (100 ppm)*
3	*C. sporogenes* inoculated sample (10^6 cfu/g)**
4	Nitrite + *C. sporogenes* (100 ppm + 10^6 cfu/g)

* : $NaNO_2$ was dissolved in distilled water and added into beef stew before cooking,
**: *C. sporogenes,* strain no: 413; the bacteria was obtained from Pendik Veterinary Control and Research Institute (Istanbul, Turkey) and proliferated in Cooked meat medium (Difco) before inoculation.

For the analysis, 25 g of Kavurma samples were homogenized using a Waring blender at 6000 rpm in 225 of ml sterile serum physiological salt solution for microbiological analysis. *C. sporogenes* count in the samples was determined by the pour plate technique using Heart infusion agar (Oxoid). Plated samples with anaerobic kit (Oxoid) in anaerobic jars (Anaerocult, Oxoid) were incubated at 37 ^oC for 48 hr. Typical *C. sporogenes* colonies were counted after the incubation period (Anon, 1994). The TBA, Peroxide and FFA values of samples were determined using the methods described by Gokalp *et al*., (1993).
The collected data was subjected to ANOVA, and means were separated using the Duncan's Multiple Range Test.

Results and discussion

C. sporogenes counts
The number of *C. sporogenes* in the control and nitrite added samples were less than <1 log CFU/g. It was 3.08 log CFU/g for *C. sporogenes* inoculated and 2.99 log CFU/g for nitrite + *C. sporogenes* containing samples. Analysis of variance results revealed that nitrite addition and *C. sporogenes* inoculation had a significant effect ($p<0.05$) on the count of *C. sporogenes*. For example, if only *C. sporogenes* inoculated samples were considered, it was observed that clostridium count was slightly lower in the samples prepared with the addition of nitrite. Duncan test indicated that there was a statistically significant difference in terms of the number of *C. sporogenes* between those two samples (Table 2). Before the processing, 6 log CFU/g microorganisms were inoculated into the kavurma sample during production, and that number was decreased about 3 log CFU/g unit with the cooking processes. The results indicated that *C. sporogenes* were not completely eliminated during the heat treatment of kavurma production and survived the storage period. It was observed that the storage time significantly affected the number of *C. sporogenes* in kavurma (Table 3). *C. sporogenes* inoculated samples initially contained 3.27 log CFU/g organism which was reduced to 2.89 log CFU/g after six months of the storage. For the nitrite + *C. sporogenes* sample; the initial count was 3.21 log CFU/g, and the count was 2.73 log CFU/g after six months.

Table 2. Duncan results (averaged over storage time) for the effect of nitrite addition on *C. sporogenes* count and oxidation parameters of the kavurma samples

Sample Type	*C. sporogenes**	TBA	Peroxide Number	FFA Value
1	$<1^c$	1.96^a	6.07^a	0.879^b
2	$<1^c$	0.43^b	4.04^c	1.036^a
3	3.08^a	1.44^a	5.38^b	0.817^b
4	2.99^b	0.25^b	3.84^d	0.981^a

1: Control, 2: Nitrite added 3: *C. sporogenes* inoculated, 4: Nitrite + *C. sporogenes* containing. *log CFU /g sample, TBA: mg malonaldehyde/kg fat, Peroxide number: meq O_2/kg fat, FFA: mg KOH/g fat, $^{a-d}$: Different letters in a column are significantly different ($p<0.05$).

Table 3. Duncan results for the effect of storage time on *C. sporogenes* count and oxidation parameters of kavurma

Storage Time (month)	*C. sporogenes**	TBA	Peroxide Number	FFA Value
0	1.97 [a]	0.80[b]	4.04[c]	0.79[b]
1.5	1.93 [a]	0.81[b]	4.69[b]	0.86[b]
3	1.82 [b]	1.03[b]	5.14[a]	0.99[a]
6	1.76 [c]	1.43[a]	5.46[a]	1.07[a]

*log CFU /g sample, TBA: mg malonaldehyde/kg fat, Peroxide number: meq O_2/kg fat, FFA: mg KOH/g fat,
[a-c] : Different letters in a column are significantly different ($p<0.05$).

It was reported in the literature (Hamilton, 1990) that the spores of *C. botulinum* did not germinate in the medium with low water activity (<0.95). Similarly, the water activity of kavurma produced in this study was less than 0.95 (the data was not reported) which may have caused no increase in the number of viable *C. sporogenes* during storage. Storage temperature of kavurma samples was 4 °C which could be another factor for the reduction or not germination of *C. sporogenes* during storage. It could also be noted that the *C. sporogenes* count was lower for the samples prepared with nitrite addition as indicated in the literature by many researchers that nitrite has an antimicrobial effect against clostridia. It was stated that the antimicrobial effect of nitrite is due to prevention of growth of bacteria rather than directly killing them (Ockerman, 1983, Cassens, 1995).

TBA value

TBA value of the samples changed in the range of 0.125 – 2.56 mg malonaldehyde/kg sample. Kaya and Gokalp (1995) stated that TBA value would be a proper indicator for determination of rancidity in kavurma. ANOVA results indicated that the addition of nitrite and nitirite + *C. sporogenes* into samples had a significant effect ($p < 0.01$) on TBA values (Table 2). It was determined by Duncan test that nitrite added kavurma (samples 2 and 4) had significantly lower TBA values compared to non-nitrite samples (1 and 3). This occurrence was probably caused by antioxidant effect of nitrite. It is known that nitrite inhibits the formation of oxidative rancidity in meat products. Oxidized Fe^{+++} present in meat catalyzes the oxidation of fat but if the meat is cured with nitrite, reaction of NO with myoglobin reduces the heme molecule to Fe^{++} state which has no effect on the oxidation of fat. It was determined that TBA value significantly increased with time during storage (Table 3). The TBA value of the samples after 6 months of storage was higher than those of the samples at any other storage time. Vural and Oztan (1989) reported that initial TBA values of the control and with 30 ppm tocopherol added kavurma samples were 1.65 and 2.10, respectively. They determined that TBA values increased during storage, and after 6 months TBA value of control group increased to 2.32 while that of antioxidant added sample reached to 1.80 mg malonaldehyde/kg fat.

Peroxide number

The peroxide number of kavurma samples ranged between 3.21and 7.10 (meq O_2/kg). The statistical analysis revealed that the addition of nitrite had a significant effect ($P<0.01$) on the peroxide number of kavurma (Table 2). It was found that sample 2 and 4 (nitrite containing samples) had significantly lower peroxide values than that of non-nitrite containing samples, 1 and 3. The change in peroxide number of sample with storage time was statistically significant (Table 3). The peroxide number of all samples increased during the storage, however the peroxide value of the samples stored 3 months was not statistically significant from that of the samples stored for 6 months. Kavurma Standard (TS 978) states that peroxide level of kavurma shall be less than 20 meq O_2/kg, and all of the current samples contanied peroxide levels under that limit. Sarigol reported that home or industrial type kavurma samples had the peroxide value of 5 ve 9 meq O_2/kg, respectively, and the number increased depending on the storage time. Tiryakioglu and Yucel (1995) investigated the peroxide value of kavurma marketed in Bursa and measured an average of 61.44 (meq O_2/kg) peroxide. Kavurma is not consumed right after the manufacture; therefore it should keep its quality during the storage. The fat used in kavurma production is an important factor for the deterioration of quality of the product since it is prone to rancidity formation due to the oxidation (Vural and Oztan, 1989). For this reason, the use of certain amounts of nitrite could be recommended in kavaurma production to retard oxidation of the fat present in kavurma.

FFA Value

It was determined that the addition of nitrite had a significant effect on the FFA (Free Fatty Acid) value of the samples. The addition of nitrite caused a significant increase in the FFA values samples (Table 2). The FFA value of the control sample was 0.879 (mg KOH/g fat) while that of sample 2 (containing 100 ppm nitrite) was 1.036. Additionally, storage time significantly affected the FFA value of samples with FFA in kavurma samples increasing during storage. As seen in Table 3, average initial FFA value of samples was 0.79, and it increased to 1.07 after 6 months of storage.

Conclusions

The results of this study revealed that *C .sporogenes* count decreased during cooking of kavurma but they were not completely eliminated and survived during storage. The number of *C. sporogenes* slightly decreased during storage which could be attributed to proper storage conditions. *C. sporogenes* was not fully inactivated during kavurma processing in the samples inoculated with the bacteria, and it was concluded that they might reproduce and might pose a potential health risk, if the product is stored under inappropriate conditions. Nitrite addition reduced the TBA value and peroxide number of kavurma. Since higher TBA value and peroxide number is related to the oxidation of lipids, nitrite addition limited the rancidity formation in kavurma by lessening the autoxidation. In conclusion, considering the facts that nitrite has a slight antimicrobial effect and inhibits the autoxidation of fat in product while enhancing the other quality characteristics of meat product, a certain amount of nitrite could be added to traditional kavurma processing.

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50th International Congress of Meat Science and Technology

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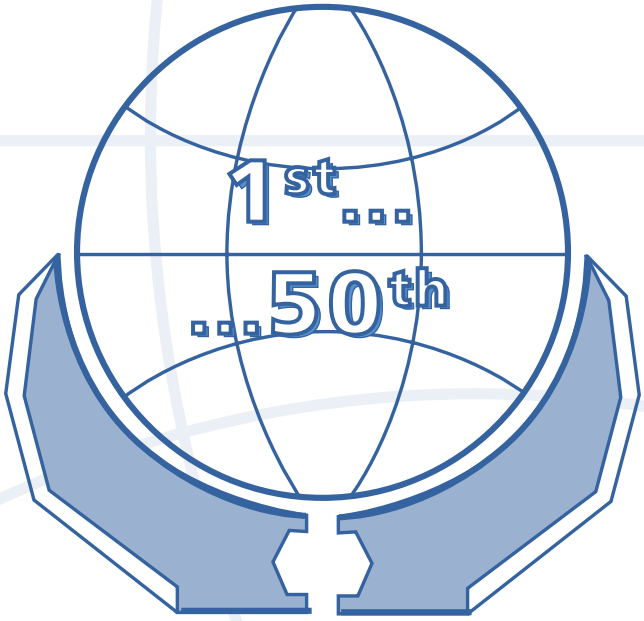

RECENT CONCERNS ABOUT STUNNING AND SLAUGHTER

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ABSTRACT

This review summarises information that is relevant to concerns that have recently been expressed about stunning and slaughter. It is known that captive bolt stunning can result in brain material passing to the lungs via the jugular veins. If future studies show that BSE prions pass beyond the lungs to the edible carcass, there will be a move away from captive bolt stunning in large cattle towards electrical stunning. Greater use of electrical stunning in large cattle will increase the importance of blood splash in the beef industry. The theoretical causes of blood splash are reviewed to improve our understanding of this problem. In some situations it can be due to excessive venous pressure causing rupture of a capillary bed some distance from the source of the pressure rise, but it is not known whether this applies to electrical stunning. Gas stunning is replacing electrical stunning for poultry because it can reduce blood spots, which is a similar condition to blood splash. Several gas stunning methods are now being used, but it is not clear which of these is the most humane. Anoxic stunning leads to carcass convulsions and this causes more carcass damage. In fish, recent developments in electrical stunning are showing promise in overcoming problems with carcass damage. It is recommended that rock lobsters should be chilled or frozen before butchery, to ensure a humane death.

Keywords: electrical stunning – gas stunning – captive bolt stunning – BSE – petechial haemorrhage – meat quality – shechita - restraint

INTRODUCTION

There have been two excellent reviews recently on concussion stunning and electrical stunning (Shaw 2002; Schütt-Abraham 1999). The focus in this paper is on recent information not covered in those reviews, and on aspects of stunning and slaughter that are likely to become more important in the next five to ten years. The topics that are discussed include

- BSE and stunning
- Electrical stunning in red meat species
- Electrical stunning in poultry
- Harvesting foetuses
- Fish and crustaceans
- Electrical stunning monitors
- Blood splash and bleeding efficiency
- Religious slaughter
- Gas stunning

In recent years, a large number of abattoirs have changed from working a single shift to two (night and day) shifts. This has allowed better use of infrastructure, and closure of excess slaughter capacity. The effects on the plants that have stayed open include, the need for increased chiller capacity, more precise scheduling of the arrival of stock or greater capacity in the livestock holding area or lairage, and closer attention to rectifying line breakdowns including failure of stunning equipment. Reliability of stunning equipment has become a higher priority, and there is greater willingness to invest in reliable and if necessary sophisticated equipment, especially if there are carcass or meat quality advantages.

BSE and Stunning

There is growing concern that captive bolt guns might be encouraging the distribution of BSE prions from the brain to the edible carcass. In addition, there have been worries about abattoir staff safety when handling CNS material, and particularly from the aerosol of cerebrospinal fluid and spinal cord macerate created during carcass splitting. The history and theory behind these threats is as follows. BSE was first discovered in the UK in November 1986, and by July 1991 about 60,000 cases had been confirmed in Britain. At that time it was well recognised in the medical profession that brain emboli can pass from the brain to the lungs in humans who experience penetrating head injuries (Ogilvy *et al* 1988; Kunz *et al* 1990). For example, brain material had been identified in the pulmonary blood vessels following gunshot wounds in the head (Hatfield & Challa 1980; Miyaishi *et al* 1994), and it had even been known for a bullet to migrate with brain matter to the pulmonary artery (Nehme 1980). The explanation is that when the brain is disrupted, homogenised material can be forced under pressure into the sinuses that drain the brain. Provided the heart is still beating, the particles of brain matter are carried to the heart and then lodge in the capillary bed of the lungs. The lung capillary network has a relatively fine diameter, and so it acts as quite an efficient filter for particulate brain matter that is irrigated out of the brain wound and into the pulmonary circulation (Conhaim *et al* 1998). It would not, however, filter out soluble proteins released from the brain.

In 1991, an attempt was made to introduce a pneumatically powered captive bolt stunner into Europe (Hantover ®). This gun was manufactured in the USA, and it vented some of the spent pneumatic pressure through its bolt into the cranium. This disrupted the brain and produced a relaxed carcass through a pneumatic pithing effect. The gun was disallowed in the UK because it was prone to forcing brain material out of the brain cavity through the bolt hole in the skull and into the operator's face. This was recognised as a hazard for the operator. At about the same time, a hot chining method was developed in the UK which avoided the inhalation of CSF aerosol by abattoir staff (Gregory & Murray 1992). The spinal column was removed intact from the eviscerated carcass using knife work and a pig breastbone opening saw. However, this method was not adopted by the UK meat industry at that time.

The next development came from the USA in 1996, when Garland *et al* (1996) reported that brain emboli could be recovered from 3% of the lungs of cattle stunned with the Hantover stunner. This finding was the death knell for that gun, which is now no longer allowed in the States. Schmidt *et al* (1999a) examined the hearts of cattle stunned with three types of captive bolt gun, for the presence of blood clots. They assumed that clots within the chambers of the heart were a sign of the presence of brain material which acted as nuclei for clot formation. This assumption was never evaluated and is now not widely accepted, and so this piece of work led to some confusion. Shaw & Gregory (2000) subsequently found that modest-sized blood clots occur in the chambers of the heart in over 8% of cartridge-powered captive bolt shot cattle, and in only one of the recovered clots was there material that could be confirmed histologically as originating in the brain. Schmidt *et al* (1999b) developed an assay for detecting filamentous protein of brain origin in beef, but it was not tested sufficiently to demonstrate whether the meat from captive bolt shot cattle presented a risk. Love *et al* (2000), using an ELISA for syntaxin 1B, subsequently found that brain material passes from the head in the blood carried by the jugular vein in captive bolt shot cattle, and the same group confirmed this phenomenon for sheep (Anil *et al* 2002). Daly *et al* (2002) confirmed earlier work by Mackey & Derrick (1979) that marker bacteria that gain access to the brain from captive bolt stunning equipment can end up in muscle. Daly emphasised that if bacteria can be distributed by this route, then so could prion proteins that leak from the brain. The magnitude of the threat of brain material passing through the lungs to the edible carcass in large enough quantities to pose a health risk for consumers has not been established, and it will be difficult to provide any precise answers without having a good estimate of the infective dose required to produce clinical Creutzfeldt-Jakob disease. If the outcome is that captive bolt stunning has to be discontinued, there would be a move towards electrical stunning. Non-penetrating bolt guns cause damage to the brain in some animals, and so they may have a comparable effect to the captive bolt. In which case they would be considered an inappropriate alternative.

When a captive bolt is used, brain tissue and cerebrospinal fluid can leak from the bolt hole in the head for at least 55 min in a suspended carcass (Prendergast *et al* 2003). This leakage contributes to contamination of the surrounding area of the hide as well as the bleeding area plus equipment in the abattoir, and it is thought that it may spread to carcass surfaces especially during the physical movement associated with hide pulling and head removal.

Electrical stunning in red meat species

The potential move away from the captive bolt method towards electrical stunning, raises two questions. How should electrical stunning be applied in large cattle, and does it have any drawbacks ? When using the Jarvis Electrical Stunner that was developed in New Zealand, the recommended minimum current to achieve a satisfactory stun is not less than 1.2 amp, and the current necessary to fibrillate the heart is greater than 1.5 amp (when using nose to brisket electrodes) (Wotton *et al* 2000). One of the shortcomings of electrical stunning in cattle is that it can cause blood splash (ecchymoses or petechial haemorrhage). Anecdotal experience at one plant in New Zealand indicates that blood splash is worse in cattle that carry hormone growth promoter implants, but this has not been authenticated with a controlled study. In earlier work with electrical stunning in cattle there were problems with broken sacrums, but this has now been controlled by using a brisket instead of rump electrode when fibrillating the heart.

About ten years ago, a survey in the UK showed that about 16% of pigs that were electrically stunned manually, were subjected to a repeat application of the current (Anil and McKinstry 1993). Some animals received a second application because the first failed to produce a satisfactory stun. Other pigs received a second current to control carcass kicking and facilitate shackling and hoisting. Some pigs were given a second stun because of an inadvertent delay in sticking. McKinstry & Anil (2004) showed that the effectiveness of a second stun is comparable to that of the first stun, in terms of the duration of epileptiform activity in the EEG, and the time to return of physical brainstem reflexes. The implication is that repeat application of a stunning current is an acceptable procedure when it has to be used, but that situations giving rise to its use should be controlled as far as possible.

Analysis of EEGs using Fast Fourier Transformation has allowed a finer interpretation of EEG traces in electrically stunned animals. This has modified some of our thinking. For example, Velarde *et al* (2002)

concluded that in sheep, normal rhythmic breathing activity restarts before epileptiform activity in the EEG has subsided following electrical stunning. Previously, it had been thought that breathing at this stage was abnormal and an involuntary consequence of the physical convulsions. Nevertheless, the resumption of breathing is still regarded as a useful indicator of the imminent return of consciousness.

PSE meat continues to be a problem for pigmeat processors. Besides causing drip, PSE meat is more prone to falling apart when it is sliced thinly. PSE can be limited to zones within a ham and this can make it more difficult to identify suspect carcasses (Franck *et al* 2003). Channon *et al* (2003) confirmed that over-application of electrical stunning increases the risk of PSE meat. This can happen in two ways. Either the current is applied for too long (e.g. 19 *vs* 4 s), or the current is too high (2.0 *vs* 1.3 A). Bertram *et al* (2002) came to the logical conclusion, from using ^{31}P NMR spectroscopy, that the early utilisation of phosphocreatine is an important component determining the link between the physical contractions at the time of stunning and slaughter and post-mortem pH decline.

Electrical stunning in poultry

Blood spots continue to be a problem in the poultry processing sector. However, two developments have helped to reduce their importance; high frequency electrical stunning and gas stunning. In many countries, high frequency electrical stunning has replaced 50 or 60 Hz frequency waterbath stunners. It is well recognised, from work on laboratory animals, that high frequencies (450 Hz and higher) produce less initial spiking and a more even muscle contraction when the current is applied (Rosenblueth and Cannon 1940). During sustained application of the current, muscle tension is lower and the physical contractions are more prone to subsiding, when using high frequencies (Rosenblueth and Luco 1937; 1939). High frequency electrical stunning in poultry usually results in a shorter-lasting stun, and following the current there can be more physical activity (Hillebrand *et al* 1996; Mouchonière *et al* 1999). The greater physical activity is presumably fascicular rather than fibrillary in origin, as it involves whole body responses, but it could also be linked to a lower prevalence of cardiac arrest and hence better oxygenation of nervous as well as muscle tissues. The main commercial advantage with high frequencies is fewer blood spots and other haemorrhages in the carcass, and this could be linked to the reduced muscle tension during stunning. High frequency stunning can also lead to less blood retention in the viscera, and this has benefits in terms of yield of trimmed livers for foie gras production (Turcsán *et al* 2003).

When unrestrained poultry are electrically stunned across the head, they develop severe wing flapping, and because of this, whole-body stunning in a waterbath is preferred. However, a system recently developed at Silsoe Research Institute employs head-only stunning immediately followed by a head to vent current, and this does not provoke any wing flapping. Savenije *et al* (2002a) showed that convulsions involving wing flapping are associated with an impressive acceleration in breast muscle ATP and glycogen depletion, pH decline and lactate accumulation, and the meat has a poorer water holding capacity. Electrical stunning promotes greater depletion of muscle glycogen in Type IIB myofibres (fast twitch glycolytic) in comparison with type IIA fibres (fast twitch oxidative glycolytic) (Iwamoto *et al* 2002).

Electrical waterbath stunners still operate at a constant voltage, even though constant current stunners, that supply current separately to each bird in a waterbath, have been designed, tested and developed (Kettlewell *et al* 1995).

Layer hens are more prone to a cardiac arrest at stunning than broilers, but in practice they are often poorly stunned because of loose-fitting shackles (Schütt-Abraham 1999). When a tissue develops hypoxaemia from a cardiac arrest, the endothelium of the blood vessels becomes leaky and a serous fluid accumulates in the extravascular space. Savenije *et al* (2002b) demonstrated that the accumulation of this fluid in the brain, as measured by the drop in impedance, is very rapid, and that electrical stunning without a cardiac arrest results in a more delayed accumulation of electrolytes in the extravascular space. The significance of these effects in practical terms is not clear, but it is a novel approach to studying the effect of electrical stunning on cell and brain function.

The stunning current recommended for ostriches has been 400 mA or 500 mA when using a 50 Hz AC head only system (Wotton & Sparrey 2002). In practice, currents are often higher than this. For example, the largest ostrich processor applies 400 to 800 mA for 8 to 10 s using 105 V / 50 Hz. Regurgitation during bleeding is a problem at some ostrich processing plants.

Harvesting foetuses

Occasionally there is concern about whether foetuses are conscious when removed from pregnant animals at slaughter. In some countries invasive procedures, such as cardiac puncture for serum collection and recovery of foetal tissues for the Asian health product market, are performed on the foetus as soon as the dam is eviscerated. Under normal circumstances, the foetus is not conscious when it is in the uterus (Mellor & Gregory 2003). In lambs, a relatively high oxygen tension is required to support consciousness, and the p_aO_2 of the foetus is below this level (28 mm Hg pressure) until the lamb starts breathing air. Provided the lamb does not start breathing when it is removed from the amniotic sac, it will not become conscious.

Fish and Crustaceans

The welfare issues associated with killing farmed fish has been reviewed by van de Vis *et al* (2003), and those linked to marine harvesting by Gregory (1998). There has been controversy amongst scientists as to whether fish can feel pain. Some take the view that fish probably cannot feel pain (Rose 2002), whereas others consider that elasmobranches have diminished ability to feel pain but teleost fish probably can (Sneddon *et al* 2002), but it is not clear which types of pain they can feel (Chervova 1997; Gregory 1999). In scientific terms, it is considered that a species can feel pain if it has eight physiological, behavioural and neuroanatomical features (Gregory, 2004). Few species have been examined for all eight criteria, and none of them are fish. In the absence of this information, judgements in the short term will have to be based on individual criteria that seem to be particularly convincing.

Righting behaviour is being used as an indicator of return of consciousness following stunning, and presumably a return of ability to feel pain (Robb *et al* 2002). However, in other species, and in other contexts, this criterion is falling from favour as a good indicator of the interface between consciousness and unconsciousness. It is a cerebellar reflex which can occur in subconscious states, at least in arboreal species such as the Australian brushtail possum (Littin 2004). The difference in value attached to this criterion needs to be reconciled.

The minimum current necessary to stun eels is 600 mA (Lambooij *et al* 2002a). This recommendation was based on the induction of an epileptiform EEG in the brain, and it applies to head only stunning, which is not a feasible method in large scale eel farms (Robb *et al* 2002). Lambooij *et al* (2002b) tested the more practical alternative of passing current through a tank of eels in water, and concluded that a satisfactory amp/dm^2 was 0.64. However the eels were prone to recovering before they died, and so it was recommended that the current should be followed by a second lower but longer-lasting current plus partial deoxygenation of the water using nitrogen. This additional procedure ensured that there was no recovery. When eels were killed in this way, their meat was less prone to oxidative rancidity, and was firmer and redder in comparison with the traditional method of placing the live eels in a bin with crushed salt (Morzel & van de Vis 2003). An in-line semi-automated rotary electrical stunning unit has been developed by Silsoe Research Institute (Lines *et al* 2003).

Concussion seems to be the stunning method that has the least effect on post-mortem muscle metabolism in fish (Ruff *et al* 2002). Linked to this, rigor sets in later, and there is reduced risk of meat gaping. Electrical stunning suffers from the disadvantage that it can result in blood spots (van de Vis *et al* 2003), but in trout this defect can be controlled by using a high frequency (1000 Hz) current (Lines *et al* 2003).

Iso-eugenol (Aqui-S) continues to be used for pre-slaughter sedation of salmon in the aquaculture industry in New Zealand, in spite of concerns by Japanese consumers about flavour residues. Aqui-S and clove oil have been trialled successfully in crabs (*Pseudocarcinus gigas*) destined for human consumption.

The methods used in some Asian restaurants for killing rock lobsters have been receiving media attention. A range of methods are used, including those shown in Figure 1. They are 'drowning' in freshwater, boiling, head spiking, chest spiking, splitting, tailing, and they may or may not be preceded by chilling or freezing. Most lobsters are split longitudinally and the carapace is used in presenting the meat, and they may or may not have been chilled, given a head or chest spike, or tailed before splitting. It is recommended that the lobsters are chilled to less than 4 °C before they are killed (Lowe and Gregory 1999).

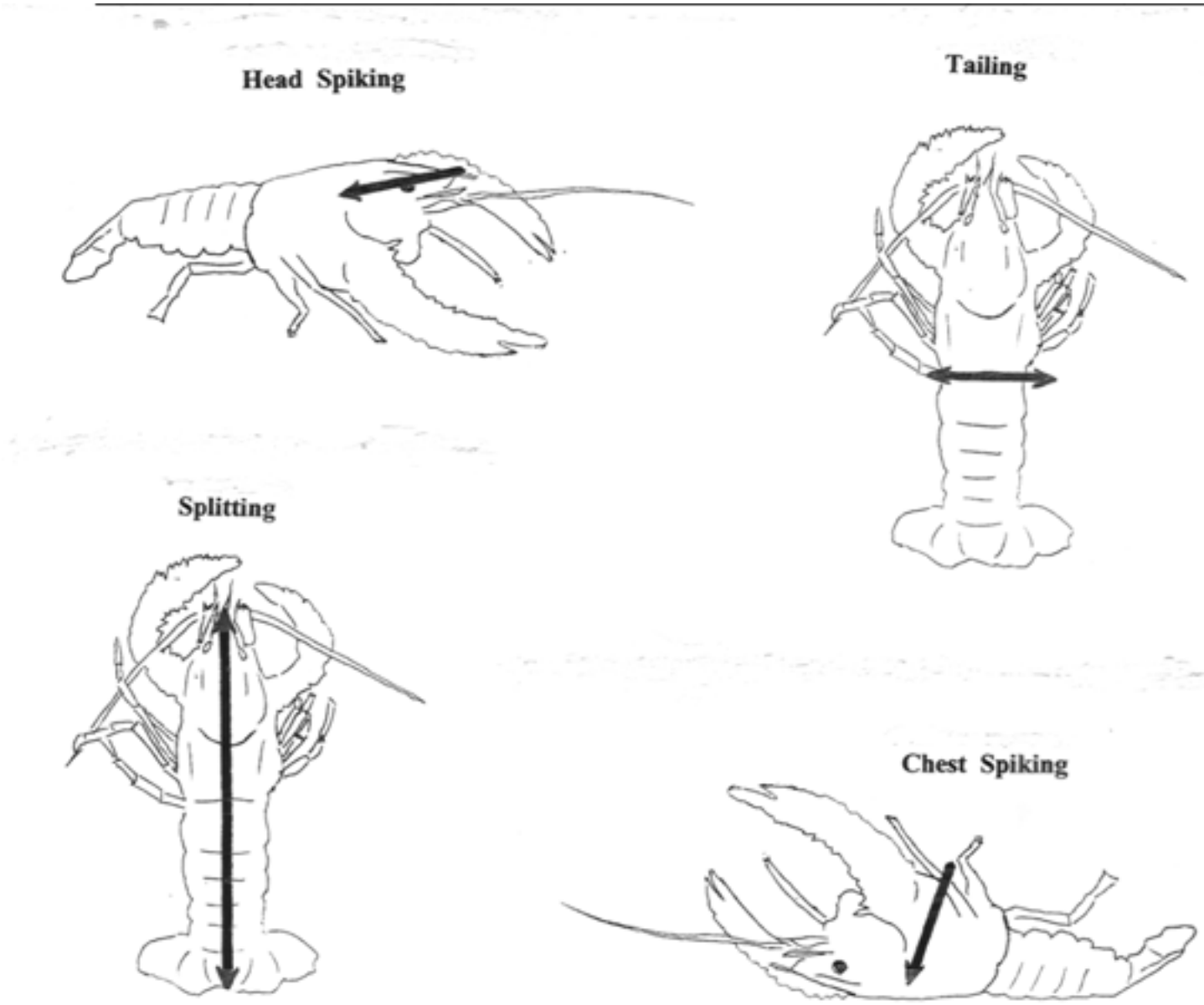

Figure 1. Approaches used in restaurants for killing rock lobsters with a knife or cleaver.

Electrical stunning monitors

Electrical stunning monitoring systems have become more sophisticated in recent years (Berry *et al* 2002; Gregory 2001). The advantage of stunning monitors compared to visual inspection of animals, or looking at the needle of an ammeter connected to the circuit, is that they can identify problems such as pre-stun shocks, interruptions in current flow at the start of stunning, and slow ramping up of the current as it is delivered. A system developed by Silsoe Research Institute for poultry waterbath stunners uses a data logger which is fitted between the shackle and an individual bird. It records rms current, peak current, voltage and duration of current flow for that bird, and the AC and DC measurements are used for determining true rms current.

Blood splash and bleeding efficiency

Four theories have been put forward to explain the cause of the blood capillary rupture that leads to blood splash, but none of these have been proven. Firstly, it could be due to counteracting muscle contractions during stunning causing localised tearing of the capillary bed (Gregory 1998). However, haemorrhages can be induced electrically in tissues such as the brain, which does not possess any skeletal muscle (Hassin 1933). If this applies more broadly, it is unlikely that localised striated muscle contraction is the only cause of vessel rupture. Secondly, Shaw *et al* (1971) suggested that one of the contributing factors may be arteriolar dilatation. This was based on the finding that, in rats, propranolol reduced and phentolamine increased the extent of blood splash. The effects of these drugs would not necessarily be limited to the arterial side of the circulation, as venous dilatation could also play a role (Vanhoutte *et al* 1981). Presumably, it is engorgement of the capillary bed which encourages rupture of vessels when placed under pressure. Thirdly, the blood vessels may be unduly fragile. Histological examination of blood splash in meat has shown that the vessels that burst are on the venous side of the capillary bed, which has less elastic walls than the arterial side, and so are weaker and more prone to damage. Blood splash is common in animals that have died from anticoagulant poisoning and these animals have raised capillary fragility (Littin 2004; Fulton & Berman 1964). It has been noted that lambs in a flock that had a high prevalence of blood splash had poor blood coagulation in terms of prothrombin time (Restall 1980/81), and it was suggested that the common link might be ingestion of excessive amounts of coumarins from pasture. The raised susceptibility to blood splash in unweaned lambs might be a low Vitamin K status because of their milk-based diet. Fourthly, during intense generalised muscle body contractions, such as those during electrical stunning, the venous and arterial systems experience severe external pressure. Squeezing of the veins results in large rises in venous pressure, which is transmitted to the capillary system at sites that can be some distance from the contractions. The venules in the capillary bed probably burst where they are weakest, or where venous pressure is particularly high. This referral of pressure, causing distant petechial haemorrhage, has been seen in other contexts when sudden intense pressures have been applied to veins (French & Callender 1962). Engorgement of the venous circulation would presumably exaggerate this effect.

It is not clear whether blood splash could be due to a direct effect of electrical current on blood vessels, but it seems unlikely. Electrical stimulation of tissues, even at low currents (e.g. 20 μA), will promote extravasation of blood cells from the capillary bed, but this is an inflammatory response and is slower than the time available under slaughtering conditions (Nanmark *et al* 1985). Leakage permitted by electroporation would only occur in the track of an applied current, but since current pathways are not properly understood it is not possible to conclude whether this mechanism is important.

It is well recognised in the broiler processing industry that high frequency currents result in fewer birds with engorged wing veins and wing haemorrhages in comparison with low frequencies (50 or 60 Hz). This has recently been confirmed for turkeys (Wilkins & Wotton 2002), and it was found that the effects of high frequency electrical stunning on breast meat pH fall and quality were minor. High frequency electrical stunning resulted in fewer carcases with broken coracoid and furculum bones, and a lower prevalence of haemorrhages in the meat at these sites. Bleeding efficiency is greater with high frequencies, and the prevalence of cardiac arrest at stunning is lower (Mouchonière *et al* 1999).

Bloody pygostyles can be an unsightly blemish in poultry. McNeal *et al* (2003) found that one way of reducing this problem was to decapitate the birds after stunning, instead of using the normal neck cutting

method. Decapitation after electrical stunning also had the advantage that the body lost physical activity sooner after cutting. Evidently, disruption of neurotransmission through the spinal cord led to earlier termination of the convulsions. Gregory *et al* (1999) found in a limited trial that when the prevalence of blood spots in breast muscle was high, it could be reduced by performing a ventral neck cut instead of the conventional dorsal cut. Head only stunning results in breast muscle haemorrhages at the humerus-coracoid joint, whereas with whole body stunning the haemorrhages tend to be in the middle of the muscle (Hillebrand *et al* 1996).

Bleeding efficiency and bleeding rate at sticking are influenced by the factors listed in Table 1. A cardiac arrest at the start of bleeding will slow the rate of blood loss, and in some situations it can result in less blood loss, but this is not an inevitable consequence of inducing a cardiac arrest during electrical stunning (Gregory 1998). Velarde *et al* (2003) showed that lambs that were hoisted and bled without being stunned, released less blood from the sticking wound than lambs that were electrically stunned (250 V, 50 Hz, 3 s), hoisted and then stuck. A likely explanation is that the muscle contractions associated with electrical stunning forced blood away from skeletal muscle towards the vessels in the thorax and abdomen. The implication is that bleeding efficiency in animals slaughtered without stunning is poorer by comparison with animals bled following electrical stunning. The role of severing the vagus nerves at sticking on subsequent bleeding efficiency and residual blood in the carcass has not been examined, but it is known that vagal severance affects the distribution of blood flow in different organs and it reduces the blood pressure and cardiac output responses during haemorrhage (Schertel *et al* 1991). This could have implications for different sticking methods.

Head to back stunning has fallen from favour since its successful introduction in the 1980s. It is not compatible with Halal market requirements and so it is no longer widely used for lambs in New Zealand or Australia. There are still some pig abattoirs using head to back stunning, and unlike the situation in lamb, it does not seem to reduce or prevent blood splash in pigs. Channon *et al* (2003) reported that the haemorrhages following head to back stunning in pigs were not always due to broken vertebrae.

Table 1. Factors affecting bleeding rate or bleeding efficiency at sticking

• Blood vessels that are severed
• Size and patency of the sticking wound
• Cardiac arrest at stunning
• Orientation of the carcass - positioned horizontally or vertically
• Vasodilatation or vasoconstriction in the capillary bed
• Tonic muscle contractions squeezing blood capillaries and vessels
• Clonic activity causing movement of blood towards the sticking wound

Religious slaughter

The welfare issues during slaughter without stunning include the stress of restraint, whether the cut is painful, and whether the animal experiences undue distress whilst it is bleeding out. Inverted restraint has been replaced by upright restraint in some countries, and it is worth considering the evidence behind this change. Over the years, six methods have been used for restraining cattle during Shechita. They are casting with a rope, hoisting by a hindleg, restraint in a straddle conveyor or restraining (V-shaped) conveyor, half inversion in a rotary pen, full inversion in a rotary pen, and restraint whilst standing upright. Koorts (1991) compared the prevalence and severity of struggling in over 1,500 cattle that were either inverted in a casting pen and subjected to Shechita, or were held in the same pen and stunned whilst in an upright position. The prevalence and severity of struggling was graded subjectively by a panel from the first attempt at loading the casting pen either up to the time the animal was stunned, or up to the time of Shechita. The system using stunning was quicker and involved less struggling (Table 2). 4% of the animals due to be killed by Shechita escaped from the casting pen, either because they were oversize or because they were frantic. 9% of the cattle subjected to stunning had to be restunned. In 15% of the cattle, more than one attempt was made at tipping them to the inverted position. A comparable study was conducted by Dunn (1990). The behaviour

of cattle in a casting pen was compared with that in an upright pen. The duration of struggling was shorter lasting in the upright pen (1 s *vs.* 11 s, $p<0.001$), the number of vocalisations was higher, and serum cortisol concentrations were greater. Van Oers (1987) found that when head restraint was applied after an animal was inverted, there was more vigorous struggling in comparison with head restraint before inversion. Studies on blood gas tensions indicate that the changes likely to occur during inversion for Shechita are not severe enough to cause serious respiratory embarrassment (Wagner *et al* 1990). Two disadvantages in using upright restraint are that the cut has to be made upwards instead of downwards, and this can be more awkward. Secondly, the Shochet is more likely to get covered with blood because of his position relative to the cut.

Table 2. Average time spent in the casting pen and prevalence of stressed behaviour before stunning or Shechita. After Koorts (1991)

Category	**Average time spent in the casting pen before stunning or Shechita s**				**Prevalence of stressed behaviour %**	
	Secular	*n*	*Shechita*	*n*	*Secular*	*Shechita*
Calm	7	1,085	33	511	69.4	31.4
Nervous	14	418	62	868	26.7	53.3
Wild	22	58	81	208	3.7	12.8
Frantic	35	2	99	41	0.1	2.5

The likely physiological events occurring when a neck is cut in the unanaesthetised state have been discussed by Gregory (2004). In summary, when the neck is cut with a knife there will be direct activation of neurones by the blade as it transects the nerves. This produces an intense but brief injury discharge in the afferent nerves. Thereafter the cut end of the nerves is depolarised and unable to respond to further stimuli. The afferent pathways that are severed serve a range of functions including pain, cold, heat, kinaesthesia, itch, and stretching or distortion of the skin. The sensations produced during the injury discharge is likely to be an amalgam of all such inputs, and the overall effect is likely to be a sense of shock, comparable to an electric shock. There is no reason to assume that one sensation (such as pain or cold) will over-ride all the other sensory input when the nerves are being cut. Subsequently, undamaged nerve endings in the neck wound could respond if stimulated or disturbed, and so the way the wound is managed before consciousness is lost, could be important in determining whether there is pain. Wound management has not been studied in any detail during Shechita.

Barnett & Cronin (in press) have performed an important study on time to loss of consciousness in 41 broilers during Shechita. The birds were taken from the shochet as soon as the cut was made, and placed on their feet on the floor. The time to loss of posture was recorded, and in this situation, this should be a good indicator of loss of balance associated with impaired consciousness. On average the time to loss of posture was 14 sec, and the range was 8 to 26 sec. The implication is that some birds could be fully conscious for up to 26 sec following the cut.

Gas stunning

In some countries, poultry waterbath electrical stunners are being replaced by gas stunning units. Gas stunning is being promoted because it results in less blood spots in the meat and fewer haemorrhages on the surface of the carcass. Five gas stunning methods are being used commercially and they differ in the gas composition. Two methods are used in the UK; 60% argon + 30% CO_2 and less than 2% O_2 using Ar in air. The anoxic method (less than 2% O_2 is falling from favour because of the severity of the carcass convulsions and the cost of Ar. In mainland Europe a mixture of 40% CO_2 + 30% O_2 + 30% N_2 is used for induction of

unconsciousness, followed by 80% CO_2 + 20% N_2 for killing. This method is being promoted by Stork PMT. In Japan and Italy, 40% or more CO_2 is applied for over a minute. In the Italian system the birds are lowered into a well containing a gradually increasing CO_2 concentration, with a final concentration of 60% or more CO_2. In the Japanese system the birds are suspended on shackles which are conveyed through the CO_2 unit. The argon-CO_2 method produces greater physical activity as the bird dies in comparison with the Stork method (Gerritzen *et al* 2000), and this is reflected in a slightly faster rate of post-mortem muscle glycolysis in breast muscle (Savenije *et al* 2002a). Whether the accelerated glycolysis is a disadvantage depends on how the carcasses are managed. If they are promptly chilled, it should not be a problem, but where this is not possible, it could result in firmer, if not tougher, breast meat.

There is some debate at the moment as to which of these gas mixtures is most humane. The primary concern is about breathlessness before loss of consciousness. It is recognised that in the human, CO_2 induces a sense of breathlessness with dypnoea. Whereas, hypoxia is not a potent stimulus of breathlessness and dypnoea (Manning and Schwartzstein 1995). During gas stunning the EEG can be divided into three phases. In phase 1 there is normal activity which lasts for 5 to 10 s. In phase 2 the amplitude of the EEG is reduced, and in some birds there is an increase in frequency. It is during this phase that consciousness starts to fail. In phase 3 the EEG is suppressed. Gasping in a standing bird during phases 1 and 2 is a concern from the animal welfare perspective as it indicates breathlessness whilst the bird is conscious. Coenen *et al* (2000) did not observe any intense gasping during phase 1 when comparing 40% CO_2 + 30% O_2 + 30% N_2 with 40% CO_2 + 15% O_2 + 45% N_2. During phase 2, there was less gasping in the higher O_2 mixture. The birds collapsed towards the end of phase 2, and there were no differences between the treatments in time to collapse. This work is not conclusive, because of the limited number of birds in each treatment (four), but as a preliminary finding it raised the possibility that there could be welfare advantages in supplementing CO_2 with O_2.

Some of the work by McIntyre *et al* (in preparation) supports the view that there could be advantages in supplementing CO_2 with O_2. They found that there was less gasping following a ten second exposure to 40% CO_2 + 30% O_2 + 30% N_2 in comparison with 40% CO_2 in air. However, there was no difference in the prevalence of gasping between 40% CO_2 + 30% O_2 + 30% N_2 and 40% CO_2 + 60% N_2. In other words, there was less gasping following 40% CO_2 in N_2 in comparison with 40 % CO_2 in air. More importantly, they found that 25% CO_2 in either air or N_2 resulted in considerable gasping in comparison with any of the 40% CO_2 mixtures. The time to collapse and loss of consciousness is longer with lower concentrations of CO_2.

The work by Raj and by Lambooij indicate that there are welfare advantages from using very low levels of oxygen, or a low (30%) CO_2 in combination with hypoxia, in comparison with 40% CO_2 + 30% O_2 + 30% N_2. Lambooij *et al* (1999) showed that before physical collapse, the birds subjected to 40% CO_2 + 30% O_2 + 30% N_2 showed 9 gasps per bird, birds subjected to 30% CO_2 + 60% Ar showed 3 gasps per bird, and those inhaling 90% Ar in air were almost gasp-free (all the treatments were significantly different from each other, $p < 0.05$). In addition the times to physical collapse were 32, 17 and 16 s respectively, and the 40% CO_2 + 30% O_2 + 30% N_2 treatment was significantly longer than the other treatments ($p< 0.05$). This strongly suggests that breathlessness is a greater problem with the 40% CO_2 + 30% O_2 + 30% N_2 method in comparison with the UK method (30% CO_2 + 60% Ar).

Raj *et al* (1998) examined the rate of induction during 40% CO_2 + 30% O_2 + 30% N_2 and 30% CO_2 + 60% Ar, in terms of the time to EEG suppression and the time to loss of somatosensory evoked potentials in the EEG. The 40% CO_2 + 30% O_2 + 30% N_2 mixture was slower acting than the 30% CO_2 + 60% Ar method. Whether this is important depends on the relative unpleasantness of the two systems. Since Coenen *et al* (2000) observed more intense gasping during the relatively long phase 2 with the 40% CO_2 + 30% O_2 + 30% N_2 mixture compared with 30% CO_2 + 60% Ar, it is thought that the CO_2-Ar method could be more humane. Here again the number of birds in each treatment was very small. Further work is needed using larger numbers of birds, before we conclude which method is more humane.

Headshaking is sometimes used when monitoring the effects of gas stunning, but it is a confusing indicator. In other situations, chickens perform headshaking when they have an irritation such as ear mites, or when consciousness is beginning to be clouded during an intravenous infusion of pentobarbitone. In the context of gas stunning, it is not clear whether headshaking is an indicator of irritation or failing consciousness. The

time to loss of posture (physical collapse) is a better indicator of failing consciousness (Mohan Raj and Gregory 1990a). It has been found to coincide with the onset of a high amplitude low frequency waveform in the EEG during anoxic stunning, and with the onset of suppression in the EEG during 31% CO_2 + 2% O_2 stunning in hens (Mohan Raj *et al* 1991; 1992). Convulsions are not usually a cause for concern from the welfare perspective as somatosensory evoked responses are lost and a high amplitude low frequency EEG waveform has set in by the time they occur (Mohan Raj *et al* 1990; Forslid 1987). The convulsions occur when somatomotor activity in the brain is no longer being controlled by activity from higher centres, and so they are a sign of neurological impairment.

Practical experience indicates that the convulsions associated with the 60% argon + 30% CO_2 cause more wing damage than the Stork system. The argon-CO_2 system is also more expensive in terms of gas cost, and it has been claimed that the engineering associated with this system introduces a longer delay in exposure to the final stunning concentration of the gas, and this could slow the onset of unconsciousness.

Practical experience has also shown that pork quality defects can be reduced by using gas stunning in place of electrical stunning. This was confirmed by Channon *et al* (2003) in a study which compared 1.3 A for 4 s with 90% CO_2 for 103 s. It was found that the prevalence of PSE meat and blood splash were lower with gas stunning. The reduction in PSE meat, however, was not reproducible between studies. Anoxic stunning using Ar has been evaluated in pigs. It produced very severe carcass convulsions, but the kicking can be reduced by adding CO_2 to the mixture (Raj, 1999). The concentration of CO_2 that suppresses convulsions can be critical (Mohan Raj and Gregory 1990b)

CONCLUSIONS

Based on the information examined when preparing this review, it is recommended that the following should be considered as future aims. The effect of captive bolt stunning on the presence of BSE prion in beef needs to be examined. If the prion is present in meat, and it is decided to use electrical stunning in place of the captive bolt, the next requirement will be to develop methods for preventing the blood splash that is caused by electrical stunning. We need to determine which of the main gas stunning systems used for poultry are acceptable from the humane standpoint. Work is in-hand in the UK, which should help meet this aim, and once that work has been published, it should be possible to reach a decision. Further development and extension is required on fish stunning methods.

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Figures on Pages 13–21

EFFECTS OF HEAT ON MEAT PROTEINS– IMPLICATIONS ON STRUCTURE AND QUALITY OF MEAT PRODUCTS

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Abstract

Globular and fibrous proteins are compared with regard to structural behaviour on heating, where the former expands and the latter contracts. The meat protein composition and structure is briefly described. The behaviour of the different meat proteins on heating is discussed. Most of the sarcoplasmic proteins aggregate between 40 and 60°C, but for some of them the coagulation can extend up to 90°C. For myofibrillar proteins in solution unfolding starts at 30-32°C, followed by protein-protein association at 36-40°C and subsequent gelation at 45-50°C (conc.> 0.5 % by weight). At temperatures between 53 and 63°C the collagen denaturation occurs, followed by collagen fiber shrinkage. If the collagen fibers are not stabilised by heat–resistant intermolecular bonds, it dissolves and forms gelatin on further heating. The structural changes on cooking in whole meat and comminuted meat products, and the alterations in water-holding and texture of the meat product that it leads to are then discussed.

Introduction

Cooking of meat products is essential to achieve a palatable and safe product. The meat proteins, approximately 20 % of a muscle's weight, represent the main constituents that make up the structure of the meat product. They undergo substantial structural changes on heating and therefore the quality of the meat product, which is mainly governed by the meat structure, also changes drastically after cooking. This review will try to cover the aspects of structural changes on cooking for meat proteins *per se*, in whole meat and comminuted meat products, and the quality alterations of the meat product that it leads to. Earlier reviews in this area are among others Hamm, 1977, Offer, 1984 and Asghar et al., 1985.

Proteins in general

A protein is built up from a long polymer chain of amino acids, a polypeptide chain. The variable side chains give each protein chain its distinctive character. There are three general categories of side chains: nonpolar, polar but uncharged and charged polar (Dickerson and Geis, 1969).

The build-up of the polypeptide chain of the proteins is called the *primary structure* (Figure 1). This polypeptide chain tends to form specific conformations in solutions, the so called localised *secondary structures,* i.e. α-helix, β-pleated sheet or random coil (Figure 1). *Tertiary and quaternary structures* of proteins are the denomination of the three-dimensional structure and the association of protein entities in solution, respectively (Figure 1).

The stabilisation of these structures of a given protein system is dependent mostly on non-covalent forces, such as hydrogen bonding, van der Waal's forces, electrostatic and hydrophobic interactions. The formation of *globular* proteins is a typical example of how hydrophobic interactions stabilises this type of tertiary structure. There is a driving force for the nonpolar, hydrophobic side chains of a protein to be removed from an aqueous to a nonpolar environment. The protein gains an extra 4 kcal of free energy stabilisation for every

nonpolar side chain group buried in the interior of the globular protein, mainly as an entropy effect (Kauzmann, 1959). Among the meat proteins myoglobin, giving meat its colour, is a typical example of a globular protein (Figure 2). Another structural form of proteins, which is highly prevalent among meat proteins, is the *fibrous* form of proteins. Actin, myosin and collagen in meat are typical fibrous proteins. These proteins are built up from three main structures, namely the α-helix, the antiparallel β-pleated sheet and the triple helix. Myosin is α helical, whereas collagen uses the triple helix (Figure 2). To stabilise these structures hydrogen bonding is frequent (Dickerson and Geis, 1969).

What happen to these type of protein structures on heating? At increased temperatures the hydrophobic side chains, for entropy reasons, can more favourably stay in the aqueous environment and in the case of the compact globular proteins leads to an expansion and partial unfolding. Furthermore, a free energy gain can be achieved by the association of two partially unfolded proteins, thereby shielding the hydrophobic side chains from the aqueous environment.

If the degree of association of protein entities is too large, leading to less colloidal stability of the system, the *solubility* of the proteins is lowered, and a precipitate is formed. If, however, the three-dimensional association of the proteins occurs in such a way that the attractive and the repulsive forces are so well balanced that a three-dimensional network is formed, a gel has set. This gel binds the water in the former solution, mainly by capillary forces, being solid-like in its mechanical behaviour. The gels can in turn vary from being transparent, containing a network of strands of small cross-sections to opaque gels containing much coarser aggregated structures (Hermansson, 1986). For one of the latest review on thermally-induced gelling of globular proteins see Clark, 1998.

For the fibrous proteins the large amount of hydrogen bonds and electrostatic interactions that keep the stretched molecules in register in the large building blocks, where the fibrous proteins take part, are broken on heating. This results in the molecules having a greater freedom to form any random configuration, as driven by entropy. Since the proteins are relatively stretched in the fibrous form fibrous proteins contract on cooking in contrast to globular proteins which expand.

Meat proteins, composition and structure

The muscle consists of 75 % water, 20 % protein, 3 % fat and 2 % soluble non-protein substances. Out of the latter 2 %, metals and vitamins constitute 3%, non-protein nitrogen 45 %, carbohydrates 34 % and inorganic compounds 18 %. The proteins can be divided into three groups; myofibrillar, sarcoplasmic and stromal proteins. The myofibrillar proteins constitute between 50 to 55 % of the total protein content, while the sarcoplasmic proteins account for approximately 30 to 34 %. The remaining 10 to 15 % of the proteins is the stromal fraction, frequently referred to as the connective tissue proteins.

The myofibrillar proteins are further divided into three subclasses: the myofilamentous fibrous proteins myosin and actin building up the myofibrillar structure, the regulatory proteins including the tropomyosin-troponin complex, α- and β-actinin, M-protein and C-protein and ultimately the scaffold proteins, such as titin, nebulin, desmin, vimentin and synemin, supporting the whole myofibrillar structure. Titin is a massive protein with a molecular weight of around 1 million Dalton.

The sarcoplasmic proteins are the soluble proteins of the sarcoplasm, to which belong most of the enzymes of the glycolytic pathway, creatine kinase and myoglobin. About 100 different proteins are known to be present in the sarcoplasmic fraction and they are globular proteins of relatively low molecular weight ranging from 17000 (Myoglobin) to 92500 (Phophorylase b).

The structures built up by the connective tissue proteins starts with an external covering sheet of connective tissue, the epimysium, around the whole muscle. This layer of connective tissue binds the individual bundles of muscle fibres into place and also binds groups of muscles together. The muscle fiber, which is the muscle cell, can vary in diameter from 10 to 100 μm and have a length of up to 30 cm. The cell has a membrane, called the sarcolemma, and is also surrounded by another type of connective tissue called the endomysium. The fibers are collected into fiber bundles, where the third type of connective tissue (perimysium) envelopes the fiber bundle (Ashgar and Pearson, 1980).

The connective tissue proteins collagen, reticulin and elastin are all fibrous proteins. Collagen, a glycoprotein, is the main structural component of the connective tissues (55-95 % of the dry matter content) and is composed of tropocollagen monomers about 2800Å long and 14-15Å in diameter with a molecular weight of 300,000. These tropocollagen molecules aggregate to form either extended fibers in the epimysium and perimysium or as a structural matrix in the endomysium. Collagen exists in several different genetic forms (I-V), which are present in muscle. Bailey et al., 1979 used immmunoflourescence to show that type I is present in the epimysium, types I and III are present in the perimysium and types III, IV and V collagen are present in the endomysium.

The muscle fibers constitute 75-92% of the total muscle volume and it holds long, thread-like structures, the myofibrils, wherein the sarcomere, the smallest contractile unit, is lined up.

The structural build-up of the sarcomere can be overviewed in Figure 4. The diameter of the myofibrils is about 1 µm and the length of a sarcomere is about 2.2 µm in a resting muscle.

According to Figure 3 the sarcomere is built from two ”building blocks”, that consist of a thick filament, extending over the A-band and a thin filament, extending from the Z-line towards the A-band in the H-zone. The thick filaments are composed of myosin. There are 200-400 molecules of myosin in each thick filament, with each being 1.5µm long and 130 Å in diameter(Knight and Trinick, 1987). By tryptic digestion, the myosin filament can be split into a heavy head, called heavy meromyosin (HMM), and a tail called light meromyosin (LMM). The water-insoluble LMM fraction has a molecular weight of 150,000 and is composed of either a double or triple-stranded α-helical structure. The head region consists of two globular units, each about 70 Å in diameter with 45 % α-helical content (Knight and Trinick, 1987).

The second major myofibrillar protein is actin. The fibrous actin (F-actin) is formed from longitudinal polymerization of globular actin (G-form, M_W 47,000 Da). In solution at low ionic strength actin exists in the monomeric globular form. When the ionic strength is raised, the monomers are polymerized into the fibrous structure, consisting of a double, twisted helix with a diameter of 70 Å (Figure 4, Ashgar and Pearson, 1980).

Behaviour of meat proteins in heating

Conformational changes of the proteins occurring on heating are usually called denaturation. The cooking temperature, where conformation changes occur is commonly called denaturation temperature and has been mostly investigated using differential scanning calorimetry (DSC). The unfolding of the proteins (the loss of helical structure) can also be followed by optical rotary dispersion (ORD) and circular dichroism (CD). Another away to follow the unfolding of the proteins is to measure the surface hydrophobicity of the proteins, using a flourescent probe 8-anilino-1-naphtalene sulfonate (ANS). The next step in the structural changes to occur on heating are the protein-protein interactions, resulting in the aggregation of proteins. These processes are mainly studied by turbidity measurements and loss in protein solubility. The gel forming ability and the type of gels formed by the proteins are usually studied, using some sort of mechanical and micro-structural measurements.

Sarcoplasmic proteins

According to the review by Hamm, 1977 most of the researchers have found that most sarcoplasmic proteins (i.e. those muscle proteins soluble in water or at low ionic strength) coagulate or aggregate between 40 and 60°C. Davey and Gilbert, 1974 found for the bull neck muscle that the heat coagulation of the sarcoplasmic proteins could extend up to 90°C. They were also the first to suggest that the sarcoplasmic proteins might have a role in the consistency of cooked meat in such a way that the heat-induced aggregated sarcoplasmic proteins can form a gel in between the structural meat elements and thereby link them together. In our investigations on tenderness of meat cooked to different temperatures (Tornberg et al., 1997) our measurements suggest that might well be the case. The mechanism by which this will operate will further be elaborated on later in this review.

Of special interest among the sarcoplasmic proteins is the myoglobin, as it is the carrier of the colour of meat. Myoglobin aggregates at about 65°C in meat, whereas in pure solution it denatures at a higher temperature (Draudt, 1969).

Another interesting aspect of the sarcoplasmic proteins is the tenderizing effect these enzymes can have, using low temperature long time heating (heating rate of 0.1°C/min) on beef muscles. Laakkonnen et al., 1970 have shown that collagenase could remain active in the meat at cooking temperatures < 60 °C, whereas at faster heating and reaching a higher end temperature of 70-80°C they were inactivated. They also showed that a heating time of at least six hours was needed to achieve a substantial lowering of the shear force, i.e. a tenderizing effect. Over the same time most of the water losses between 25 and 30 % (w/w) had occurred.

Myofibrillar proteins

Changes in secondary and tertiary structure

With regard to changes in secondary structure on heating of myosin Morita and Yasui, 1991 have for example measured the change in helix content (circular dichroism) and surface hydrophobicity (ANS) of LMM, i.e. the tail portion of the myosin molecule, at pH 6 and 0.6 M KCL. The helix content of LMM began to decrease at about 30°C and attained a minimum at 70°C. Simultaneously, heating to 65°C progressively increased the surface hydrophobicity, whereas at higher temperatures it decreased again. The decrease in hydrophobicity observed at the higher temperatures suggests that part of the hydrophobic residues take part in protein-protein interactions leading to a network formation of aggregates, a gel.

The advantage of the DSC method is that it can be used in complex mixtures and at high concentrations of proteins, which are the situation occurring in meat. A typical curve (Figure 4) from thermal transitions found in a muscle is composed of three major transition zones A, B and C. The first transition displays its maximum between 54 and 58°C and has been attributed to myosin (Martens and Vold, 1976, Wright et al., 1977). The second transition, which occurs between 65 and 67°C, was assigned to collagen (Martens and Vold, 1976, Stabursvik and Martens, 1980) and to sarcoplasmic proteins (Wright et al., 1977). The third transition has been assigned to actin and is found between 80 and 83°C (Wright et al., 1977). For the second transition it has also been shown that both isolated actomyosin and myosin and its sub-units undergo transitions in the same temperature range (Wright and Wilding, 1984). Recently, the thermal denaturation of titin from pork and beef has been investigated , using DSC. Denaturation was characterised by a single DSC peak at 78.4 and 75.6°C for beef and pork titin, respectively (Pospiech et al., 2002).

Ziegler and Acton, 1984 reviewed and summarised conformational changes of natural actomyosin. In the 30-35°C range, native tropomyosin is dissociated from the F-actin backbone, while at 38°C the F-actin superhelix dissociates into single chains. The myosin light chain sub units dissociate from the heavy chains at about 40°C, followed by conformational changes in the head and the hinge regions of the myosin molecules. Dissociation of the actin-myosin complex occurs at 45-50°C and light meromyosin (LMM) undergoes a helix-to-coil transformation at 50-55°C. Above 70°C major conformational changes in globular actin takes place. However, the exact temperatures for these changes is influenced by such factors as ionic strength, pH, heating rate, muscle source and type.

Changes in quaternary structure

Xiong and Brekke, 1990 have studied salt-soluble proteins (SSP, the myofibrillar proteins) from pre- and postrigor chicken on heating at 0.6 M NaCl, pH 6, using ANS, turbidity and rigidity measurements for unfolding(hydrophobicity), protein-protein association and gelation, respectively (Figure 5 a-d). Despite some differences between breast and leg muscles and between pre- and postrigor SSP samples, a general trend was seen in all samples that protein- protein association beginning at 36-40 °C was preceded by protein unfolding at 30-32°C. Gelation at 45-50°C was initiated after interactions of proteins had taken place. They further stated from these investigations that the hydrophobicity was not dependent on the type of muscle (white, breast and red, leg) nor on muscle rigor state, but rather the protein-protein association varied due to these variables, thereby forming different type of gels. According to Figure 5b the optical density of the SSP solution increased drastically above 40°C and after 45°C the increase was less. There are no differences between pre- and post-rigor meat, which could be due to the fact that actomyosin complex dissociate at 40-45°C. However, breast SSP showed a great difference from leg SSP in aggregation behaviour. But when gels are formed the penetration force is the highest for the breast post-rigor SSP (Figure 5c). Comparing the water-holding of these different gels, as visualised in Figure 5d, the best is achieved by breast, pre-rigor.

High penetration force for gels usually reflect highly aggregated junction points in the gel, as Hermansson, 1982 has shown for blood plasma gels, and good water holding gels suggest a fine-stranded gel with a higher amount of junction points per volume unit. This suggests that breast prerigor SSP form more the last type of gel, as revealed by figure 8b,d, having a lower degree of aggregation and better water-holding, whereas the leg SSP forms the former type of gel, having a high degree of aggregation and a higher penetration force.

As shown above the denaturation of myofibrillar proteins in solution usually results in gel formation, because especially myosin is unique in the sense that it form gels at very low concentration of 0.5 % by weight (Hermansson and Langton, 1988). For comparison sarcoplasmic proteins need about 3 % by weight to gel (Hamm and Grabowska, 1978). When purified myosin is heated, the firmness of the gel reaches its maximum at 45°C at pH 5.5 or at 60°C at pH 6 (Sharp and Offer, 1992). If actin is present in the solution a firmer gel is obtained (Yasui et al., 1980). Ionic strength and pH are important factors since they determine if the myosin exists in monomeric form or as filaments. At ionic strengths >0.3 and at neutral pH, the myosin molecules are dispersed as monomers, forming a coarse network with large pores. At lower ionic strength the myosin molecules are assembled in filaments, resembling the natural thick filaments in the muscle. During heating a firmer gel is formed, especially if the filaments are very long. Such a gel consists of a finer and more uniform network, with smaller pores (Sharp and Offer, 1992).

The gel formation of myosin occurs in two steps, in two separate temperature regions on heating. The first part of the reaction occurs between 30-50°C and the second step at temperatures above 50°C.The first step involves aggregation of the globular head of myosin. Sharp and Offer, 1992 have studied heating of purified myosin at different constant temperature for 30 minutes in an electron microscope. They found that after heating at 30°C in 30 minutes the appearance of the myosin molecule had not changed. After heating at 35°C, the presence of native myosin molecules with two heads was still dominating, but other types of new structure had been formed, such as two myosin molecules that had aggregated by dimerisation of their heads. After heating at 40°C there were no native myosin molecules left and the only monomers present had coalesced heads. Heating to 50°C resulted in further aggregation. At this temperature it was hard to distinguish between the individual tails. Heating at 50-60°C lead to the formation of large globular aggregates. No tails where seen after these temperatures. The second stage involved structural changes in the helix-structure of myosin leading to network formation, where hydrophobic groups interact with each other.

Connective tissue proteins

At temperatures between 53 and 63 °C the collagen denaturation occurs according to DSC measurements (Martens et al., 1982), which probably involves first the breakage of hydrogen bonds loosing up the fibrillar structure and then the contraction of the collagen molecule. If unrestrained, collagen fibers shrink to one-quarter of its resting length on heating to temperatures between 60 and 70°C. If the collagen fibres then are not stabilised by heat-resistant intermolecular bonds, it dissolves and forms gelatine on further heating. The presence of heat-stable bonds means that intermolecular linkages are retained at these temperatures and a proportion of the fibre matrix does not dissolve (Light et al., 1985). In young animals the epimysium contains primarily of thermally labile cross-links, the perimysium a mixture of thermally labile and stable cross-links and the endomysium of thermally stable cross-links (Sims and Bailey, 1981). As the animal age increases the thermally labile increasingly converts into thermally stable cross-links (Shimokomaki et al., 1972). Higher levels of heat-stable cross-links lead to the development of greater tension in the connective tissue during cooking (Sims and Bailey, 1981).

Wu et al., 1985 have followed the structural alterations, using scanning electron microscopy, of the epimysium, perimysium and endomysium from Bovine *sternomandi bularis* caused be heating to 60 and 80°C for 1 hour. The epimysium did not show large alterations after cooking, whereas the perimysium and endomysium became granular at 60°C and start to gelatinise at 80°C. There are also differences in solubilisation for the different type of collagens, where type I is more easily solubilised on heating than type III (Burson and Hunt, 1986).

Structure and qulity of meat products on cooking

Different quality aspects of meat peoducts

Important quality aspects of meat can embrace quality factors, such as fat- and water-holding, appearance, and the eating quality of meat products. The concept of the eating quality implies texture/tenderness, juiciness, flavour and aroma. In this review we will confine ourselves to quality aspects such as water-holding and texture of meat products

Besides whole meat, meat products, such as hamburgers and emulsion sausages, will be referred to in this review. The procedure of manufacturing hamburger patties normally includes mincing, blending and forming, after which the patties are either frozen or fried and then frozen. Frankfurters and similar sausages are made by chopping meat, with the addition of water and NaCl, in a bowl chopper into a fine meat homogenate, in which pork fat and is further dispersed and emulsified. Heat treatment of the stuffed sausage batter is performed in a smoke-chamber. The microstructure of the different meat products, such as a transverse cut of whole meat (A), a beef burger (B) and an emulsion sausage (C) can be compared in Figure 6. A hamburger is composed of more or less intact meat fibers and fiber bundles up to 50 - 70 % but randomly distributed compared to the well-defined anisotropic structure of the whole muscle. In the emulsion sausages, however, meat protein network formation constitutes the major part of the structure, as shown by Andersson et al. (2000).

Structural changes of meat products during cooking

Whole meat

The structural events during cooking of the different proteins in their structural environment of the meat have been investigated by among others by Cheng and Parrish, 1976; Jones et al., 1977 and Bendall and Restall, 1983. During heating, the different meat proteins denature as described above and they cause meat structural changes, such as the destruction of cell membranes (Rowe, 1989), transversal and longitudinal shrinkage of meat fibers, the aggregation and gel formation of sarcoplasmic proteins and the shrinkage and the solubilisation of the connective tissue.

Transverse shrinkage to the fibre axis occurs mainly at 40-60°C, which widens the gap already present at rigor between the fibers and their surrounding endomysium. There is, however, a controversy regarding these observations. Davey & Gilbert (1974) for example found no change in the cross-sectional area on cooking of the neck muscle, whereas Bendall & Restall (1983) found that the transverse shrinkage of both fibres and fibre bundles of M. psoas major starts at about 40°C. There is also a disagreement between the results presented in the literature with regard to the temperature, where the longitudinal shrinkage of the fibre starts. Offer, 1984 and Bendall & Restall, 1983 have observed that fibres do not shorten until 60°C, whereas Hostetler & Landman, 1968 have reported that both sarcomere and fibre shortening usually begin at temperatures of 40-50°C. At 60-70°C the connective tissue network and the muscle fibres co-operatively shrink longitudinally, the extent of shrinkage increasing with temperature. This is mainly based on the fact that the intra-muscular collagen (mainly perimysium) shrinks longitudinally at 64°C (Mohr & Bendall, 1969).

We have in one of our investigations (Tornberg et al., 1997) followed the structural changes in the different protein systems of the meat during cooking. Firstly, we separated the water phase from whole meat and studied the aggregation of the sarcoplasmic proteins, recording the change in absorbance in a spectrometer (Figure 7 a). From this figure it can be deduced that the increase in absorbance starts at about 40°C and is more or less terminated at 60°C. This is in accordance with the behaviour of the sarcoplasmic proteins on heating as discussed earlier. Secondly, the shrinkage during cooking of the separated connective tissue per se and fibre shrinkage, both transversally and longitudinally, have been followed under the light microscope during cooking. The micrographs taken continuously during heating were quantified with an image analysing system (Figure 7 b-d).

The transverse shrinkage of the fibre starts at 35-40°C and then increases almost linearly as a function of temperature. The total shrinkage at 80°C can, though, according to Figure 7 b, vary from 3 up to 14 % area. The shrinkage of the connective tissue per se starts at 60°C, where after at around 65°C it contracts more intensively. However, the amount of shrinkage varies substantially from about 7 % area up to 19 % area. The

discrepancy in results could, among other things, be due to large biological variation within a muscle and between different muscles. These observations are similar to those observed by Bendall and Restall, 1983.

Comminuted meat products

Comminution in combination with salt addition and subsequent heating, which are processes used in the making of hamburgers and emulsion sausages, drastically alter the structure of the meat system. On cooking hamburgers the higher occurrence of whole fibers and pieces of fibers causes more shrinkage as compared to emulsion sausages. In the latter product, higher amounts of myofibrillar proteins are extracted, which on heating create a dense protein network, a gel, that holds water efficiently by capillary forces.

The quality of that protein network is influenced by a number of factors interacting in a complicated way as visualised in Figure 8. The amount of myofibrillar proteins which are extracted into the water-phase during comminution and blending is generally considered to be the most important factor for the quality of the meat network. Moreover, the type of gel matrix formed is related to the disperse or aggregated state of the protein prior to gelation. The complex meat system consists of, not only dissolved proteins, but also insoluble components like meat fibers, connective tissue and fat. The amount and state of these components have a large impact on the gelation properties. The properties of the gel are also influenced by the heating process.

Water-holding of meat products on cooking

Whole meat

Considering the structural basis of water-holding in whole meat, the work of Offer *et al*. cannot be overlooked (Offer, 1984, Offer and Knight, 1988 and Offer et al., 1989). It is important to point out that the structural origin of water-holding in whole meat and highly comminuted products is different. In the former it is the shrinkage and swelling of myofibrils that is the crucial factor (Offer & Knight, 1988), whereas the ability of meat proteins to form different types of gel comes more into play in comminuted meat products (Hermansson, 1986).

Water loss from whole raw meat can be obtained by evaporation from the surface and as exudate, when a muscle is cut. This exudate, a solution of sarcoplasmic proteins, is drained from the cut surface of the meat by gravity, if the viscosity of the water is low enough and the capillary forces do not retain it. Offer *et al.* (1989) have confirmed that this drip loss arises predominantly from the longitudinal channels through the meat between the fiber bundles. The main question then arisis, in order to be able to control and understand changes in water-holding, is how water is accumulated and lost in those channels.

Most of the water in the living muscle is held within the myofibrils (≈ 80 %), in the spaces between the thick and thin filaments (Offer *et al.,* 1989). Any large changes in the distribution of water within the meat structure, by necessity, originate from changes in this spacing. Lateral shrinkage of the filament lattice is brought about by a pH-fall closer to the isoelectric point, rigor contraction and myosin denaturation (Offer and Knight, 1988). There will only be changes in the water distribution, if the myofibrils change in volume. The fact that fiber and fiber bundles shrink when their constituent myofibrils shrink, has been shown by Offer and Cousins (1992), thereby giving rise to the two extracellular compartments around fibers and fiber bundles.

Cooking induces structural changes, which decrease the water-holding capacity of the meat. The review by Offer (1984) summarises the structural changes occurring on cooking as follows: When the transverse shrinkage to the fibre axis occurs mainly at 40-60°C this widens the gap already present at rigor between the fibers and their surrounding endomysium. At 60-70°C the connective tissue network and the muscle fibres co-operatively shrink longitudinally, the extent of shrinkage increasing with temperature. This longitudinal shrinkage causes the great water loss that is obtained on cooking. It is then presumed that water is expelled by the pressure exerted by the shrinking connective tissue on the aqueous solution in the extracellular void.

A convenient way of studying water holding in meat is to investigate the multi-exponential decay of the relaxation time, T_2, of water protons in the muscle, using the ^{1}H-pulse-NMR (Nuclear Magnetic Resonance). Two dominating, discernible relaxation processes have mostly been observed, where the major fraction (≈ 80 %) of the muscle water has a T_2 (called T_{23}) between 35-50 ms, while the rest of the water relaxes in the range of 100-150 ms (called T_{22}). The percentage of water relaxing with the shortest relaxation time can be considered as mainly held by the myofibrils, since a very high fraction of the water is occupied by the myofibrils. Tornberg and Larsson (1986) have further shown, by comparing the percentage

of water having a T_2 = 100-150 ms with the percentage of area around the fiber bundles (evaluated by light microscopy), that the latter could be predicted with an 80 % probability using the pulse-NMR method.

Studies of the water distribution on cooking of meat, using ^{1}H-pulse-NMR, have been done by Fjelkner-Modig and Tornberg, 1986; Tornberg and Larsson, 1986 and Borisova and Oreshkin, 1992, Tornberg et al., 1993; Micklander et al. 2002 and Bertram et al., 2004. It was noted both by Fjelkner-Modig & Tornberg, 1986, Tornberg and Larsson, 1986 and Tornberg et al., 1993 that the T_2 for the major fraction of the water (T23) in both beef and pork was lowered on cooking from around 40 ms to around 30 ms. This suggests a more aggregated, dense protein structure in the myofibrils on heating, which is in accordance with the denaturation behaviour of the proteins as discussed earlier.. For the cooked meat samples, the percentage water of T_{22} and extracellular space around fiber bundles, as evaluated by light microscopy, was compared at the different cooking temperatures (Figure 9). The amount of water around fiber bundles increases up to 50°C, in comparison with the raw meat, which seems to be in accordance with the transverse shrinkage of fibers and fiber bundles. Above 50°C this widened gap diminishes, again up to 70°C, probably mainly due to the shrinkage of the connective tissue. The increase in extracellular space from 70 to 90°C is more difficult to comprehend but, according to the light micrographs a swelling of the perimysium seems to occur at these cooking temperatures.

Comminuted meat products

On comminution of meat and salt addition, leading to the extraction of meat proteins, which occurs when producing hamburger patties and/or emulsion sausages more of the structure consists of a protein gel network after heat treatment. This is especially the case for the highly comminuted emulsion sausages(4 % salt on meat basis), which is reflected in the water-holding of this meat product, being superior to both hamburgers (0.4 % salt) and whole meat, reflected in Figure 15. In this figure the cooking loss of the whole meat, the hamburger and the emulsion sausage of beef *M. biceps femoris* can be compared at different cooking temperatures (The heating gradient was 1.5°C/minute up to 60°C, thereafter 0.7°C/ minute was used until the final temperature was reached in the centre of the meat sample). The temperature dependence on cooking loss for the whole meat shown in this Figure, where the highest increase in water loss is achieved in the temperature region of 60 to 80 °C, is similar to other investigators (Davey and Gilbert, 1974; Bouton et al., 1976; Bendall and Restall, 1983; Honikel, 1987). It is further interesting to note that although the hamburgers have been comminuted, the cooking losses are almost as large as for the whole meat. This is probably due to the more prevalent shrinkage of whole fibers and pieces of fibers, causing larger water losses in the hamburgers as compared to emulsion sausages. Because in the latter product, the higher amounts of myofibrillar proteins extracted create a dense protein network that holds more water (in combination with 4 % added potato starch, which is usually added in Swedish emulsion sausages).

In one of our latest study on beefburgers patties was fried on a double-sided pan fryer to a mid-temperature of 72°C, having different pan temperatures (100, 150 and 175°C). Kovascne et al., 2004 found that the water losses, sometimes reaching up to 60 % of the initial water content, never went below 80 % drip loss. This means that the pressure driven water loss, due to meat protein contraction, is a substantially more important mechanism governing the water loss on frying of beefburgers, than the evaporation losses occurring at the surface crust formed at the higher frying temperatures of 150 and 175 °C.

Textural properties of meat products on cooking

Whole meat

Meat texture can be evaluated by both sensory and instrumental methods. Numerous studies have been conducted in the area of meat cookery, but especially attempts to relate sensory evaluated tenderness with mechanical and structural changes in the meat during cooking are scarce (Martens et al., 1982, Josell and Tornberg, 1994 and Tornberg et al., 1997). Textural properties of meat with emphasis on the instrumental methods have been reviewed by amongst others Purslow (1991), Lepetit and Culioli (1994) and Harris and Shorthose (1988). In this review only the cooking temperature dependence of texture properties of meat will be dealt with.

During the mastication of meat, deformation and fracture of the samples takes place. The mechanical forces acting on meat can include shear, compressive and tensile forces and they should be defined in the mechanical test in use. As meat is a composite, it is important to study in which structural elements failure takes place, and where cracks propagate, to be able to understand its mechanical properties. Tensile tests on cooked strips of both beef (Bouton & Harris, 1972; Munro, 1983; Purslow, 1985) and pork (Josell and Tornberg, 1994; Mutungi et al., 1995; Christensen et al., 2000) have been used.

For the assessment of the texture of whole meat, the empirical method of the Warner-Bratzler shear device is the most widely used (Bratzler, 1932). The most commonly used configuration is the one in which the shearing plane is perpendicular to the muscle fibres. Tensile, shear and compression forces operate in this type of test. The W-B-technique is, however, the instrumental technique that usually yields the best correlation with sensory panel scores for meat toughness.

When relating the sensory properties of the meat with the mechanical, structural traits Josell and Tornberg, 1994 and Tornberg et al., 1997 used in the former case *M. longissimus* from pork and in the latter case whole and minced meat from beef (M. *biceps femoris*). From the former investigation the sensory evaluated tenderness of pork loin increases substantially from 50 to 65°C, where after it decreases again to 80°C (Figure 11). In this study also the temperature dependence of longitudinal and transversal fracture stress and strain were recorded, using tensile measurements(Figure 12 a and b). As shown by Purslow (1985) for beef the longitudinal rupture of the fibers require higher stresses than transversal rupture, which was also the case for pork. The increase in tenderness in the temperature interval 50-65°C was observed as decrease in the transversal fracture stress and in the transversal and longitudinal fracture strain (Figure 12 a, b). The elastic modulus of the meat registered both at small and large deformations increased with cooking temperature from 50 up to 80°C (Figure 12 d).

Bouton and Harris, 1981 showed, using W-B shear peak values, that there is a decrease between 50 and 65°C, which they attributed to a weakening of the collagenous connective tissue, due to denaturation. This phenomenon could also be the reason for the fracture stress to diminish from 50 to 65°C in our investigation. However, we are more inclined to suggest another hypothesis to explain the observed phenomena. In the tensile measurements the strain at fracture tells us how much the meat yields on tearing and this yielding would increase between 50 and 65°C, if the weakening of the perimysium connective tissue would be the dominating mechanism in the tensile measurements. However, the opposite is observed, i.e. from 50 to 65°C a decrease in fracture strain from 70 to 30 % is achieved (Figure 12 b). Concomitantly, the elastic modulus of the whole meat system increases (Figure 12 c, d), which means that the meat converts from a viscoelastic to a more or less elastic material.

We suggest that the reason for the toughness observed for the raw meat up to about 50°C is based on the fact that the applied stress during mastication is reduced by viscous flow in the fluid-filled channels in between fibres and fibre bundles. This situation is, however, improved from 50 to 65°C by the formation of a gel of aggregated sarcoplasmic proteins gluing the fibres and fibre bundles together. The viscous flow becomes then lower as the elasticity of the meat increases in that temperature region. That means there is a higher probability of the applied stress being transferred within the material to the crack without any viscous dissipation of energy and there propagating it. The piece of meat is then more easily fractured in the mouth and mastication is facilitated and tenderness improved. Above 65°C elasticity acts adversely and impairs the tenderness. For a fully brittle fracture in a linearly elastic material (which we suppose cooked meat above 65°C behaves like) higher elastic modulus gives rise to larger tensile stresses to extend a crack (Jowitt, 1979), i.e. a tougher meat. We suggests that the contraction of the connective tissue, mainly occurring after 65°C as seen in Figure 10, gives rise to an increase in the elasticity of the meat by forming a much denser material in the temperature region of 65 to 80°C and thereby a tougher meat.

Comparison between whole and minced meat

These findings are further substantiated, when comparing the rheological properties of whole and minced meat (M.*biceps femoris*, beef) in relation to sensory and structural characteristics (Tornberg et al., 1997). The sensory evaluation (Figure 13) showed that, for the whole meat, the toughness decreased drastically from 55 to 60°C, thereafter increased again up to 80°C. For the minced meat, however, the hardness increased over the whole temperature range and was significantly lower than the toughness of the whole meat at cooking

temperatures below 60°C. The rheological properties of the whole meat, cut transversally to the fibre direction, and the minced meat were also recorded, using a low deformation dynamic shear test, and the results can be seen in Figure 14. The storage modulus increases steeply with the temperature from 50 to 65°C both for the whole and the minced meat, where after it levels off to values for the modulus of 80 kPa and 70 kPa for the whole and minced meat, respectively. The phase angle, which is the quotient between the loss and the storage modulus, decreases already from 35-40°C down to a low plateau round 65-70°C. These results tell us that the spacial arrangement of the fibers is of utmost importance for the textural behaviour of the meat. In the whole meat the crack that fractures the meat has to pass over the fluid filled channels at temperatures below 55 °C (for the case of biceps femoris) and therefore the fracture energy will dissipate as viscous flow and is therefore anticipated as tough. For the minced meat, however, the structure is no longer anisotropic and the more or less disintegrated muscle fiber and bundles are now randomly distributed in the batter and the crack then preferentially goes through the extracted myofibrillar mass that holds the fiber together in the batter. The fracture stress to propagate a crack in that material is much less than the stress needed to pass through a meat fibre. As the temperature increases from 45 to 65°C in the beef burger batter the extracted proteins form a gel and after 65°C the contraction of the connective tissue and the substantial loss of water contribute to a denser product. All these structural changes give rise to an increased elasticity of the minced meat (Figure 14), and also an enhanced sensorial perceived toughness (Figure 13).

If this increased toughness of the minced meat on heating was beneficial for the overall impression of it was not investigated in that study. For an emulsion sausage, however, where different textures were produced by using varying recipes, salt concentrations and pH (Andersson et al., 1997), the overall acceptability of the emulsion sausage was mostly governed by the properties of the protein network. This was best characterised by the fracture strain in the tensile test used. When comparing the stress-strain curves obtained for aggregated and non-aggregated sausages, the latter were characterised by having larger fracture strains and they were also rated the highest overall acceptability. Even the elastic modulus (G') was significantly higher for non-aggregated sausage (31.5 kPa) compared to the aggregated (24.9 kPa). Evidently, a dense, elastic meat protein gel is sensorially preferred to a more brittle, grainy protein gel in an emulsion sausage.

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Figures

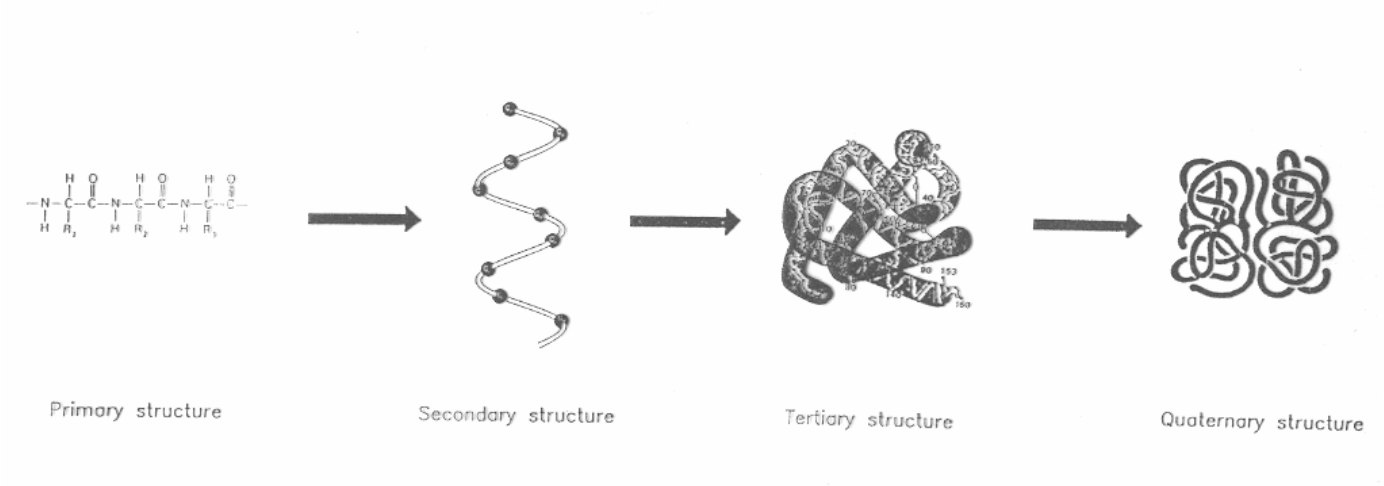
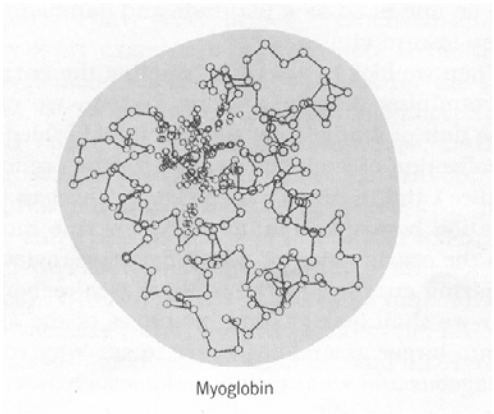


Figure 1: The different type of structure of proteins in solution. From Tornberg et al., 1990.



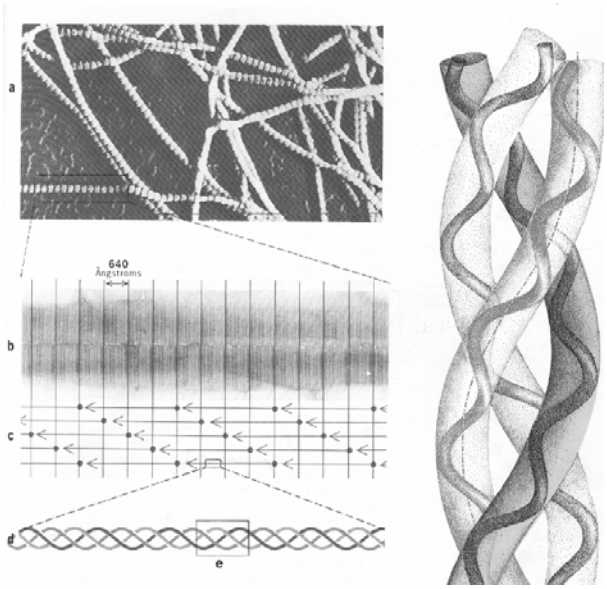


Figure 2: Example of meat proteins being: 1) globular: Myoglobin; 2) fibrous: The build-up of a collagen fibril (a) from the tropocollagen molecule(e). From Dickerson & Geis, 1969.

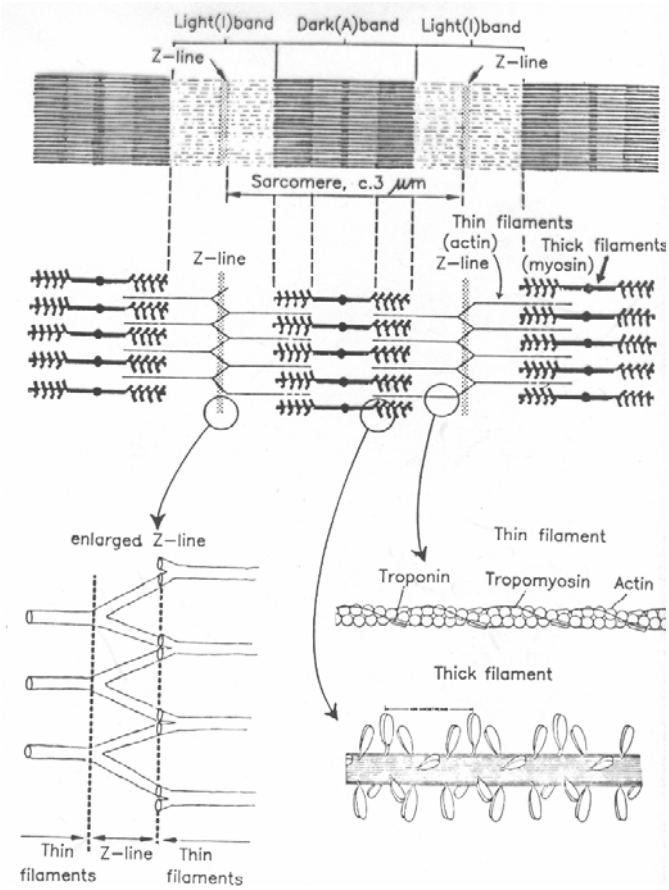


Figure 3: The structural build-up of the sarcomere, the thin and thick filaments. From Tornberg et al., 1990

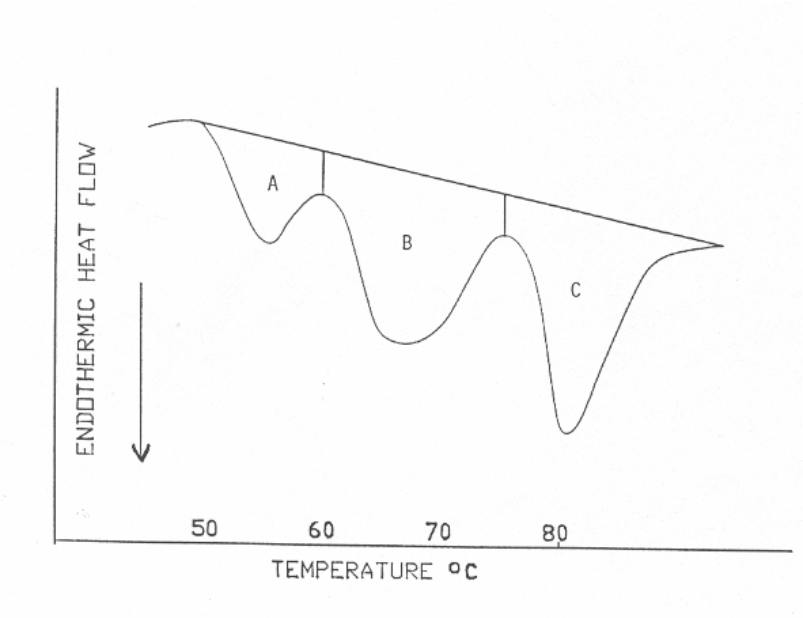


Figure 4: A typical thermal curve of muscle is composed of three major zones: A: Myosin subunits; B: Sarcoplasmic proteins and collagen, and C: Actin. From Findlay et al., 1986.

a) b)

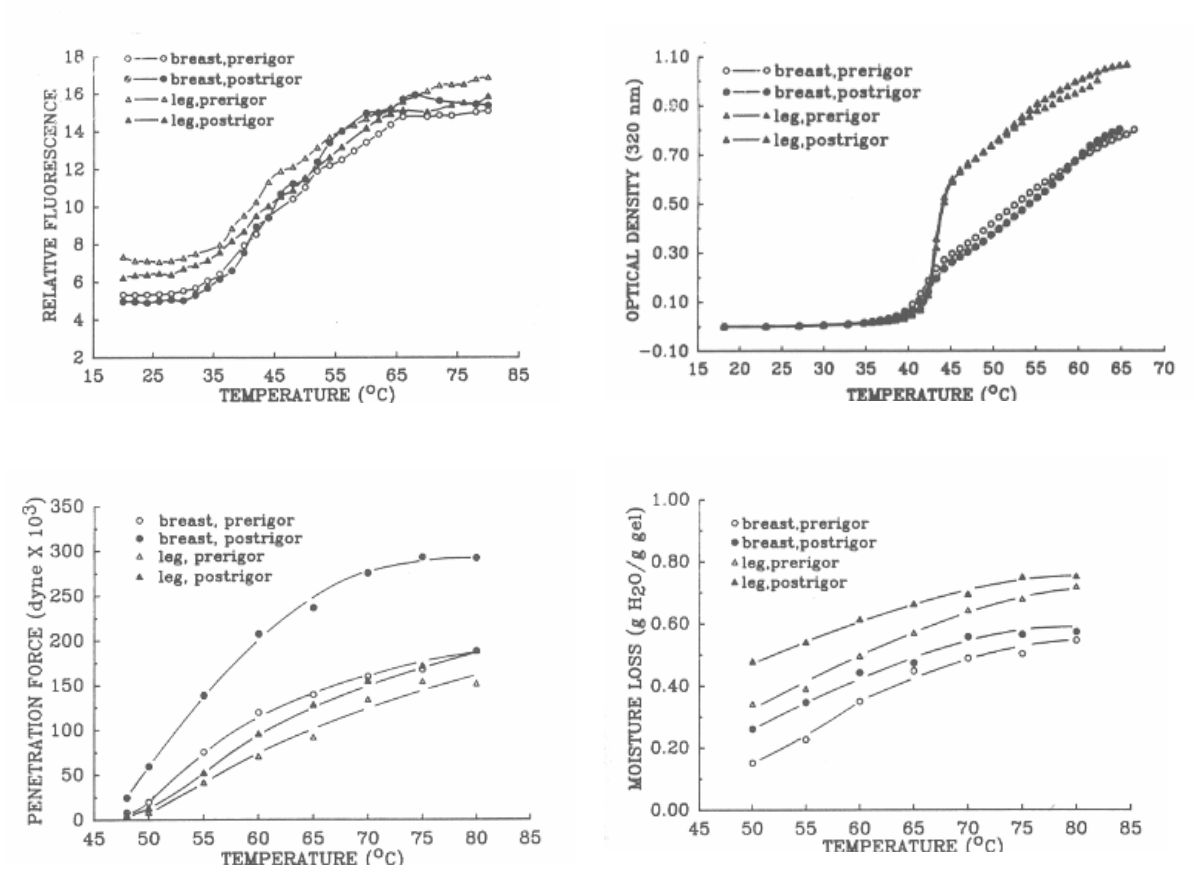


Figure 5: Salt-soluble proteins(SSP) prepared from pre- and postrigor chicken muscles as a function temperature. SSP were suspended in 0.6 M NaCl, pH 6.0. Heating rate = 1°C/min. a) ANS-protein flourescence intensity. b) Protein-protein interactions measured as turbidity. c) Gel strength as penetration force. d) Water loss. From Xiong and Brekke, 1990.

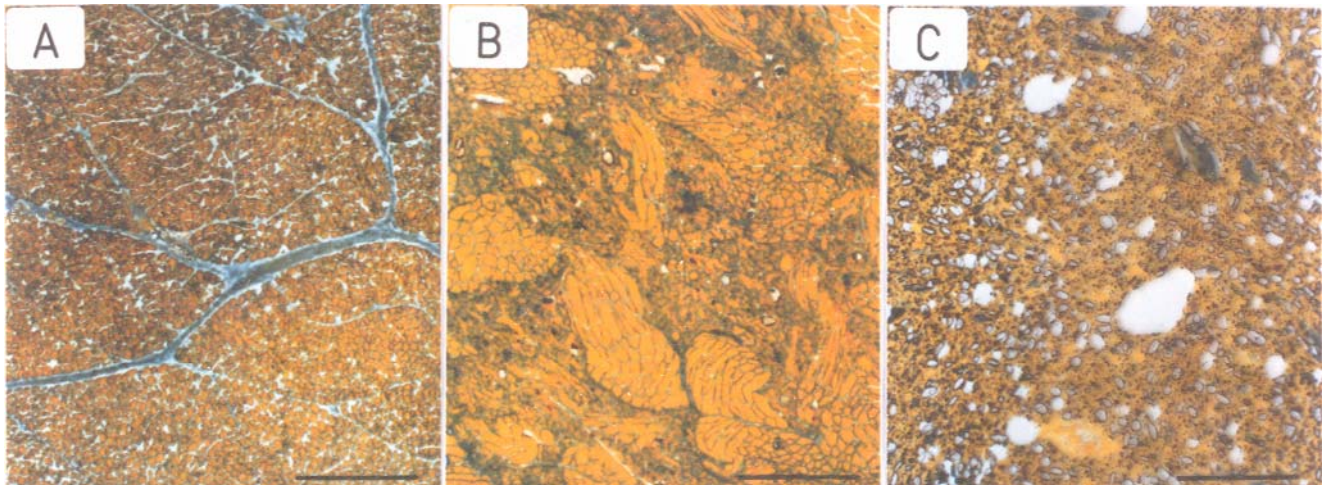


Figure 6: Microstructure of three meat products, using light microscopy. A: A transverse cut of a cutlet. B: Beef burger. C: Emulsion sausage. The bar represents 1 mm.

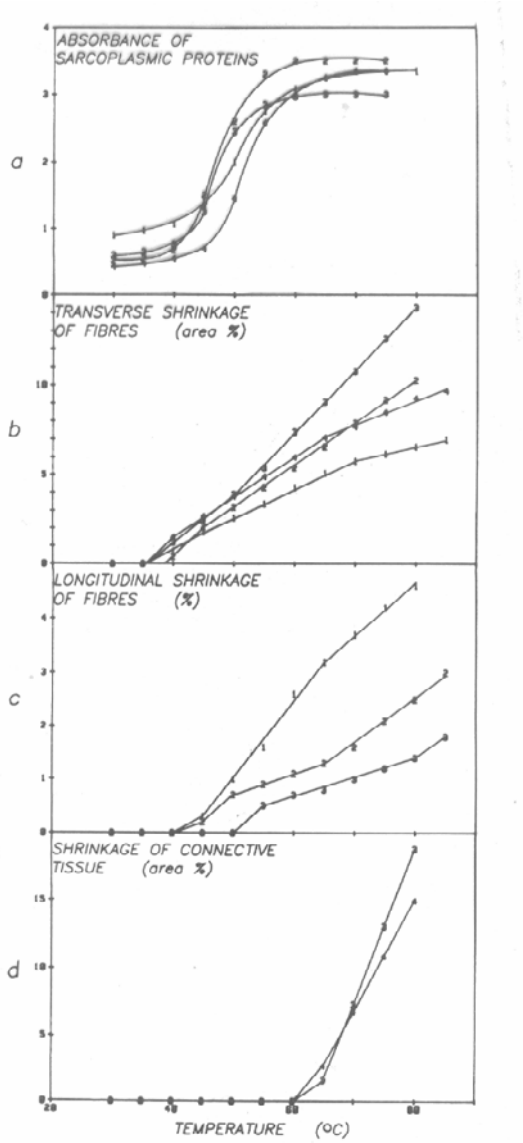


Figure 7: Effect of cooking temperature on the absorbance of the sarcoplasmic fraction (a), the transverse (b) and longitudinal (c) shrinkage of fibres and the shrinkage of connective tissue (d) prepared from four different animals, using beef *M. biceps femoris*. From Tornberg et al., 1997.

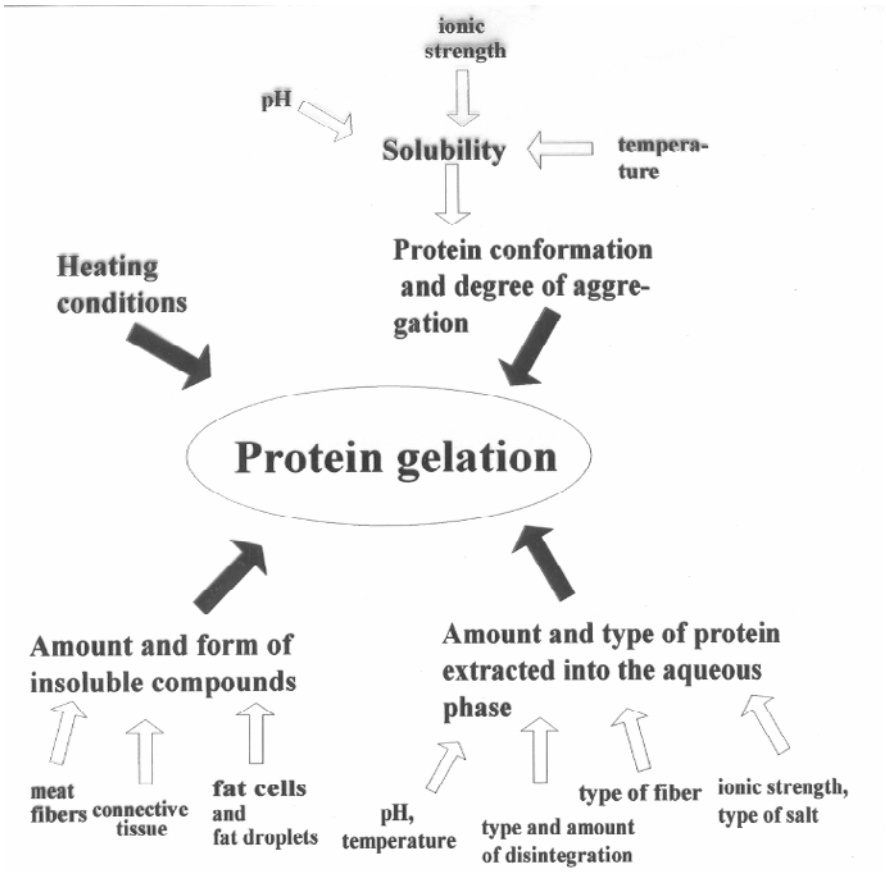


Figure 8: Factors affecting protein gelation, when cooking a meat batter. From Andersson et al., 1997.

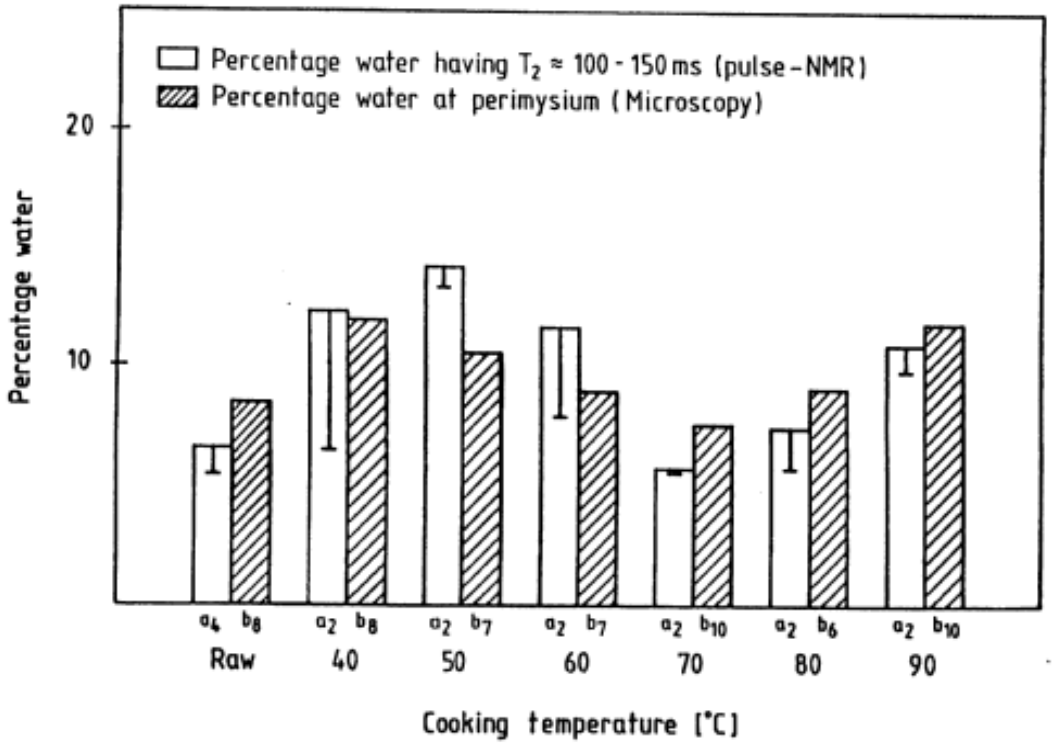
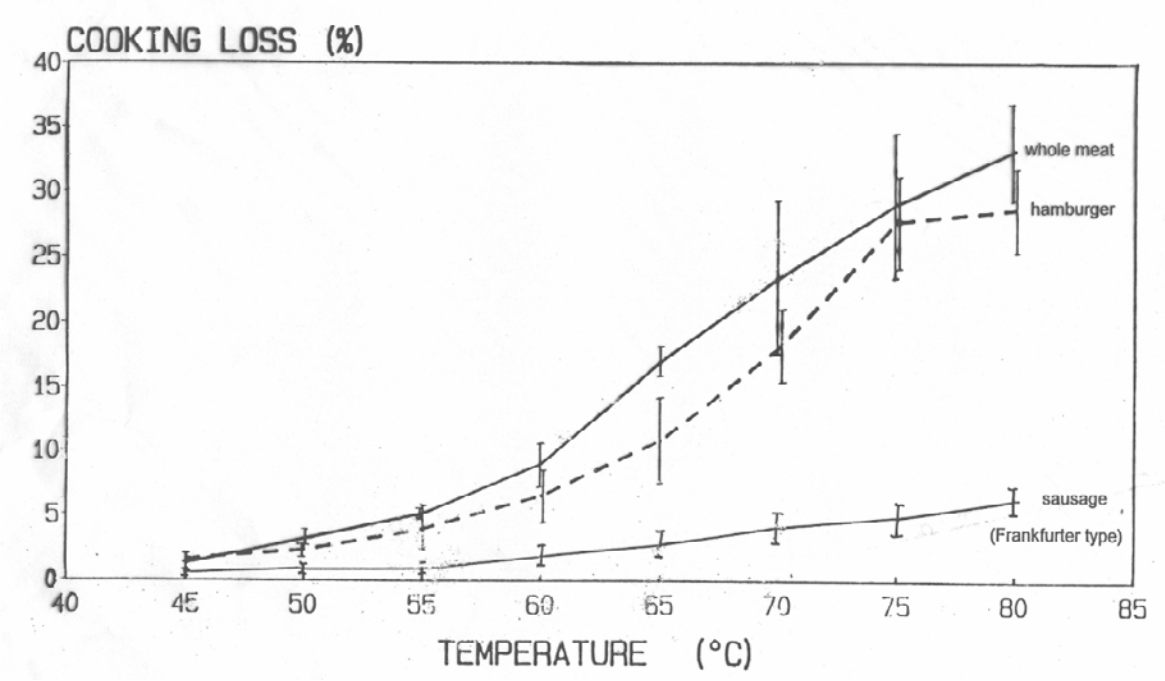


Figure 9: Percentage water having a T_{22} of 100-150 ms, using proton pulse NMR, and percentage water around fiber bundles (at perimysium), as determined with light microscopy, as a function of



cooking temperature (a_n: n = number of samples; b_n: n = number of photos).

Figure10: Cooking losses (%) as a function of cooking temperature for whole meat, hamburger and an emulsion sausage. The meat raw material used in all cases was *M. biceps femoris.*

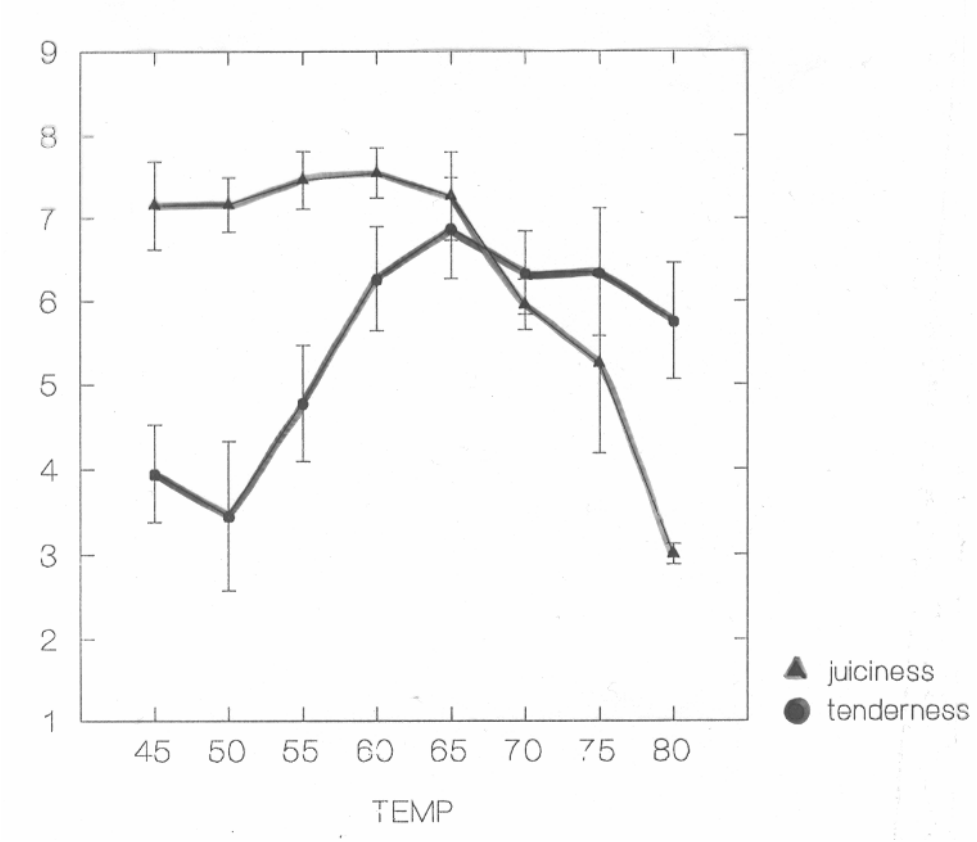


Figure 11: Sensory evaluated tenderness and juiciness for pork *M. longissimus dorsi* versus cooking temperature. From Josell and Tornberg, 1994.

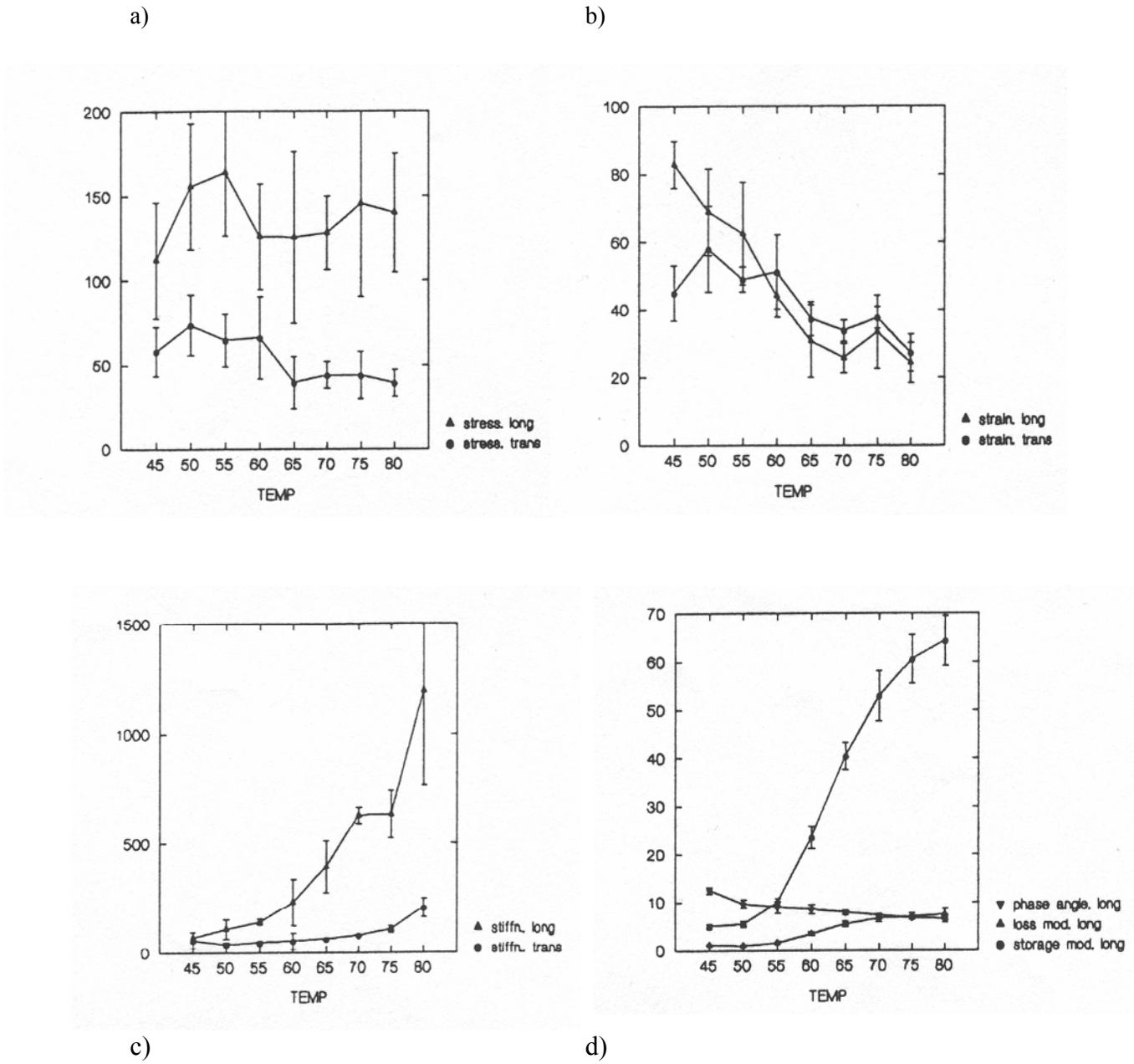


Figure 12: Different mechanical properties for pork *M. longissimus dorsi* versus cooking temperature. a) Longitudinal and transversal fracture stress (kPa). b) Longitudinal and transversal fracture strain (%). c) Longitudinal and transversal initial stiffness (kPa). d) Longitudinal storage and loss moduli (kPa). From Josell and Tornberg, 1994.

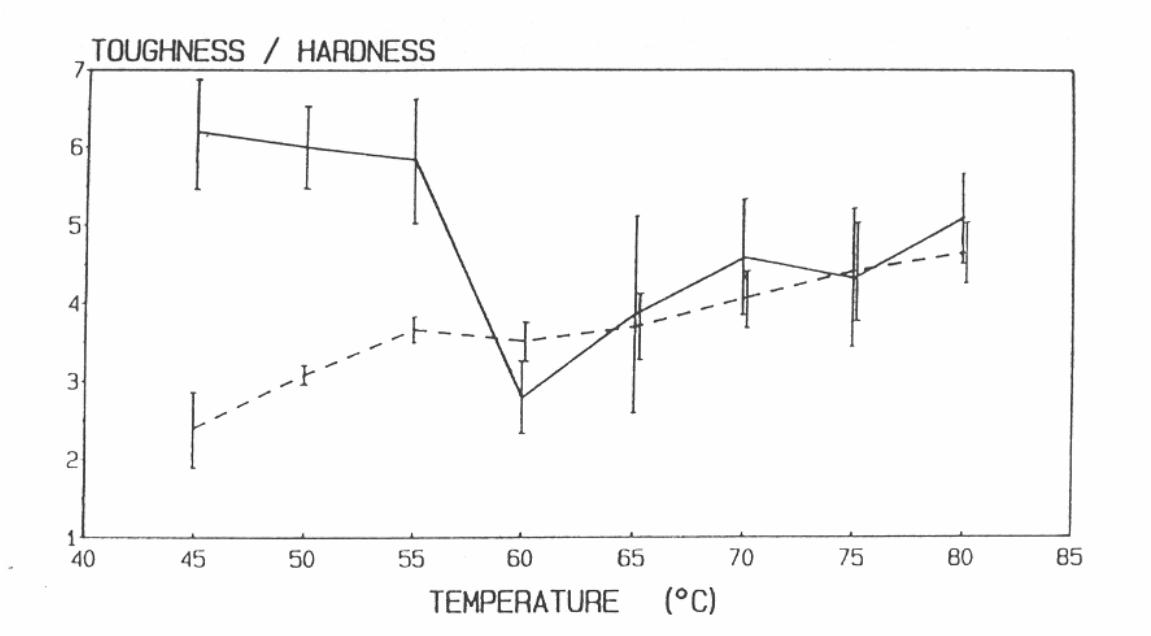


Figure 13: Toughness of whole meat (—) and hardness of minced patties (---), made out of beef *M. biceps femoris,* as a function of cooking temperature. The bars give the standard deviation. From Tornberg et al., 1997.

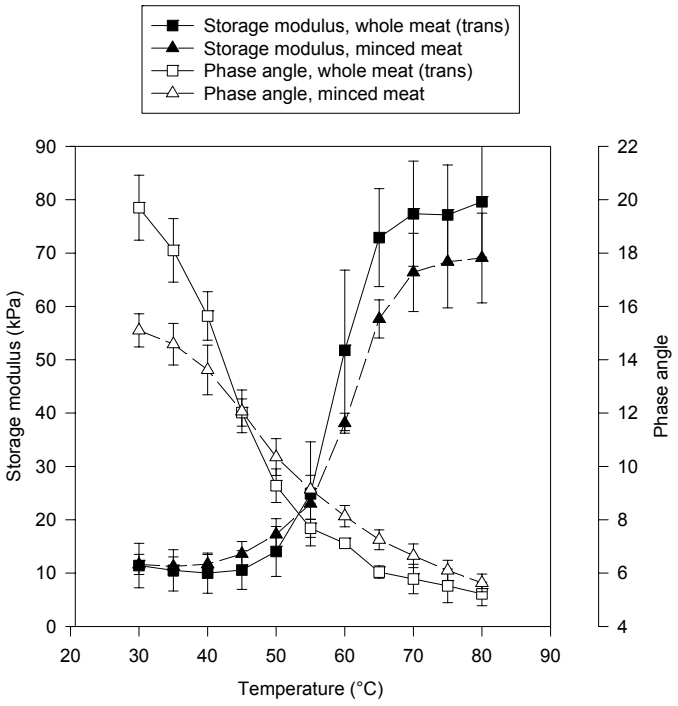


Figure 14: Storage modulus (kPa) and phase angle (degrees) for whole meat (■, □) and for minced meat (σ , Δ), respectively, made out of beef *M. biceps femoris,* as a function of cooking temperature. The bars give standard deviation. From Tornberg et al., 1997.

PHYSICOCHEMICAL PROPERTIES OF MEAT BATTERS WITH ADDED WALNUT: EFFECT OF SALT LEVELS

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Background

Epidemiological studies show that frequent consumption of nuts in general, and walnuts in particular, correlates inversely with myocardial infarction or death by vascular ischaemic disease (Sabaté, 1993). This effect has been associated with the peculiar blend of nutrients and phytochemical compounds found in walnuts, which exert beneficial effects on serum lipid profiles and other risk factors that can cause or exacerbate cardiovascular diseases .

One of the various strategies that have been adopted to achieve healthier meat products is the reformulation of meat derivatives to incorporate ingredients like walnut that can confer potential heart-healthy benefits. Meat products have been formulated with added walnuts, resulting in products with acceptable physicochemical and sensory properties (Jiménez Colmenero *et al*., 2003; Carballo, *et al*., 2003; Cofrades *et al*., 2004).

Since high salt intake has been related to high blood pressure and a considerable proportion of dietary salt comes from meat and meat products, there is growing interest among consumers and processors in reducing the use of salt (minimizing sodium) in meat processing. However, reducing salt limits the extractability of proteins and alters the pattern of denaturation and thermal aggregation of the major muscle protein (Trout & Schmidt, 1986), thus influencing the characteristics of meat products. No data are available on how salt level affects meat batter physicochemical properties formulated with added walnut.

Objectives

The purpose of this study was to determine how the percentage of NaCl (1.5 and 2.5 %) influences the effect of added walnut (0 and 20 %) on physicochemical properties (texture, binding properties) of raw and cooked meat batters.

Materials and methods

Pork meat and walnut (particle size approx. 12 μm) were used to prepare four different meat batters. Two control lots (C) were prepared with 85% meat, 0.18 % sodium tripolyphosphate, water, and salt in two concentrations: 1.5 % (LS) and 2.5 % (NS). Two samples with added walnut (W) were also prepared, in which 20 % of the meat was replaced by an equal percentage of walnut. The ingredients were homogenized and ground in a chilled cutter (2 °C) (Stephan Universal Machine UM5, Stephan u. Sóhne GmbH & Co., Hameln, Germany). Mixing time was standardized to 5 min and the final temperature was below 10 °C in all cases. The batters (60 ± 0.5 g) were placed in jars (diam = 33 mm), and some were heated to an internal temperature of 70 °C. After tempering for 1 hr, weight loss (WL) was determined and expressed as % initial sample weight. Determinations were carried out in quadruplicate.

Protein, fat, moisture, ash and pH of the raw samples (non-heated) were determined as in Jiménez Colmenero *et al*., (2003). Penetration tests were carried out (6 determinations) on raw samples in their containers once they attained ambient temperature (20-22 °C). The tests were performed with a 5 mm diameter cylindrical stainless steel plunger attached to a 50 N cell connected to the crosshead of a TA-XT2 Texture Analyser (Texture Technologies Corp., Scarsdale, NY). Force-deformation curves were obtained at 0.8 mm/s crosshead speed. Gel strength (GS) (J) was estimated as the force-deformation area after penetration to 3 mm. Texture Profile Analysis (TPA) was performed as described by Bourne (1978). Six cores (diam = 30 mm, height = 20 mm) from cooked samples were axially compressed to 30 % of their original height. Force-time deformation curves were derived with a 250 N load cell applied at a crosshead speed of 0.8 mm/s.

Two-way analysis of variance by F test and least squares differences by Statgraphics 2.1 (STSC Inc., Rockville, MD) were used to compare mean values and to identify significant differences ($P<0.05$) among treatments (added walnut and salt levels).

Results and discussion

Addition of walnut increased ($P<0.05$) fat (C 2.6 %, W 14.5 %) and decreased ($P<0.05$) moisture (C 75.7 %, W 62.5) contents in meat batters. Ash proportions were lower ($P<0.05$) in LS (2.4 %) than in NS (3.6 %). The pH values of meat batters increased ($P<0.05$) with added walnut (C 5.6, W 6.0) .

Higher levels of salt translated into higher ($P<0.05$) GS values in raw meat batters (Figure 1). The difference was only significant with 1.5 % NaCl, indicating that the effect of walnut addition on GS was influenced by the salt concentration. In meat batters containing 2.5 % NaCl, weight losses were lower than 4 %, indicating good water and fat binding properties. Values were similar in LS sample containing 20 % of added walnut (Figure 2).

Some textural parameters (e.g., Hd and Cw) of heated samples varied according to the salt concentration, but not significantly (Table 1). There has been conflicting reports on the effect of salt on texture, and it has been suggested that these have to do with the diversity of factors that can influence thermal gelation processes (Jiménez-Colmenero et al., 1998). When walnut was added, gel/emulsion structures exhibited lower ($P<0.05$) values of Hd, Sp, Ch and Cw (Table 1). The changes induced by walnut were possibly due to a number factors Walnut addition caused an increase in the fat level (and a decrease in the percentage of moisture) of the meat batters. Contrary to the results of this experiment (Table 1), it has been reported that a higher protein/moisture ratio produces harder structures (Carballo et al., 1996). On the other hand, while protein contents were comparable (19.4 %), the addition of walnut entailed a decrease in the amount of meat protein in favour of globular (walnut) proteins, which generally tends to interfere with muscle protein interactions (Lee, Wu and Okama, 1992). This limited the formation of a thermal gel matrix system, resulting in softer structures (Table 1) with good water and fat binding properties (Figure 2).

Conclusions

Walnut affected the properties of meat batters. Their presence limited the gel forming ability of protein matrixes so that meat batters were softer and less cohesive, but they did maintain good water and fat binding properties. Salt levels did not influence the effect of walnut on texture (TPA parameters) and weight loss. These results suggest that addition of walnut may be a way not only of promoting the various bioactive compounds existing in walnut but also of reducing sodium content in meat products of this kind. Such characteristics are highly appreciated by the growing number of consumers currently interested in functional foods.

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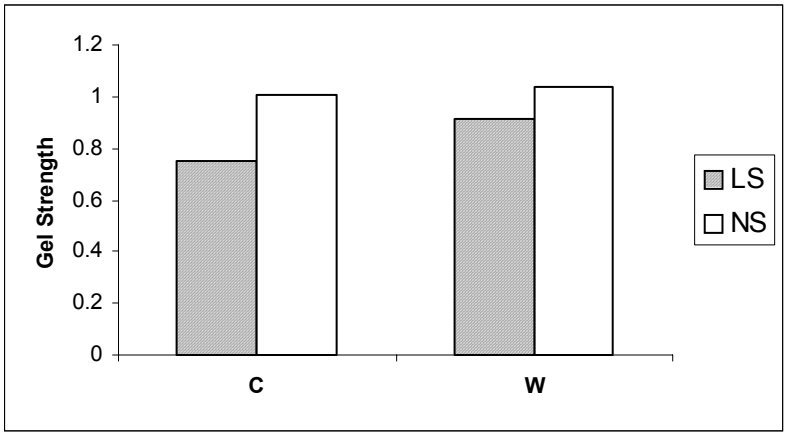
Acknowledgments. This research was supported under project AGL2001-2398-C03-01. Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I). Ministerio de Ciencia y Tecnología. Thanks are due to "La Morella Nuts, S. A." for supplying the walnuts.



Figures 1. Gel strength (J x 10^{-3}) of raw meat batters control (C) and with 20 % of added walnut (W) as a function of salt levels 1.5 % (LS) and 2.5 % (NS). Different letters for added walnut and different number for salt levels indicate significant differences ($P<0.05$).

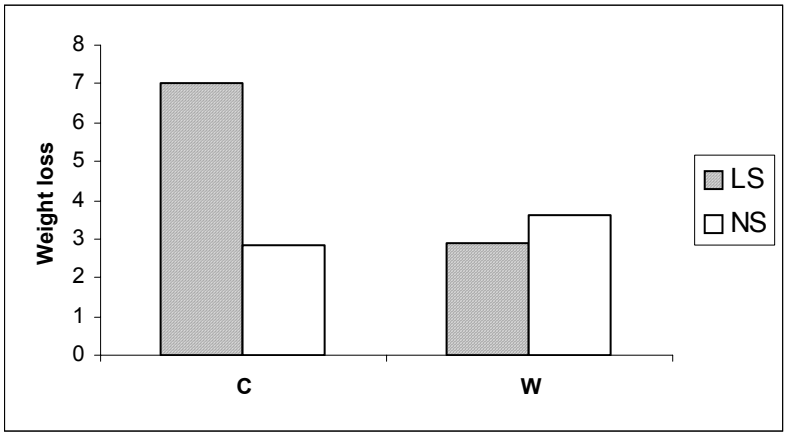


Figure 2. Weight loss (%) of meat batters control (C) and with 20 % of added walnut (W) as a function of salt levels 1.5 % (LS) and 2.5 % (NS). Different letters for added walnut and different number for salt levels indicate significant differences ($P<0.05$).

Table 1. Texture profile analysis of cooked meat batter control (C) and with 20 % of added walnut (W) as a function of salt levels 1.5 % (LS) and 2.5 % (NS).

	Hardness (N) Hd		Springiness (mm) Sp		Cohesiviness		Chewiness (Nxmm) Cw	
Samples	LS	NS	LS	NS	LS	NS	LS	NS
C	$60.37^{a}{}_{1}$	$62.87^{a}{}_{1}$	$5.37^{a}{}_{1}$	$5.33^{a}{}_{1}$	$0.515^{a}{}_{1}$	$0.514^{a}{}_{1}$	$166.85^{a}{}_{1}$	$172.65^{a}{}_{1}$
W	$48.18^{b}{}_{1}$	$50.29^{b}{}_{1}$	$5.13^{a}{}_{1}$	$5.08^{b}{}_{1}$	$0.487^{b}{}_{1}$	$0.484^{b}{}_{1}$	$120.67^{b}{}_{1}$	$123.88^{b}{}_{1}$
SEM	1.22		0.06		0.003		3.98	

Different letters in the same column and different numbers in the same row indicate significant differences ($P<0.05$). SEM = Standard error of the mean.

EFFECTS OF BLENDING CHICKEN BY-PRODUCTS WITH PALM FAT OXIDATION AND SENSORY PROPERTIES OF CHICKEN BOLOGNA DURING FROZEN STORAGE (-18°C)

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Background

Traditional meat products are commonly associated with high fats and cholesterol. As cholesterol and unhealthy PUFA / SFA ratios are adversely related to coronary heart disease (Muguerza et. al 2001), it is only prudent for meat producers in the food industry to offer less cholesterol / less fats processed meats to the consumer. Some meat processing methods such as pre-emulsion, blending vacuum chopping and tumbling of poultry by-product such as mechanical deboned chicken meat (MDCM), spent hen meat and chicken trimming have been efficiently utilized as raw materials in the production of emulsion-type, value added, meat products (Seri Chempaka et. al 1996). Palm fats were successfully incorporated in some meat product formulations on a direct weight per weight substitution with animal fats (Babji et. al 2001).

Objectives

The purpose of this work was to study the utilization of chicken by-products and palm shortening on the TBA value and sensory properties of chicken bologna stored as frozen (-18°C).

Materials and methods

Chicken bologna was produced using 45% mechanical deboned chicken meat, 30% spent hen meat and 15% chicken trimming. Five chicken bologna formulations containing 10% of various fats were studied. Chicken fat was used as the control formulation (C), red palm fat (T1), 1:1 combination of chicken fat and red palm fat (T2), white palm fat (T3) and 1:1 combination of chicken fat and white palm fat (T4).

Thiobarbituric Acid (TBA) Test

The development of oxidative rancidity was measured by distillation method of Tarladgis et al. (1960).

Sensory Evaluation

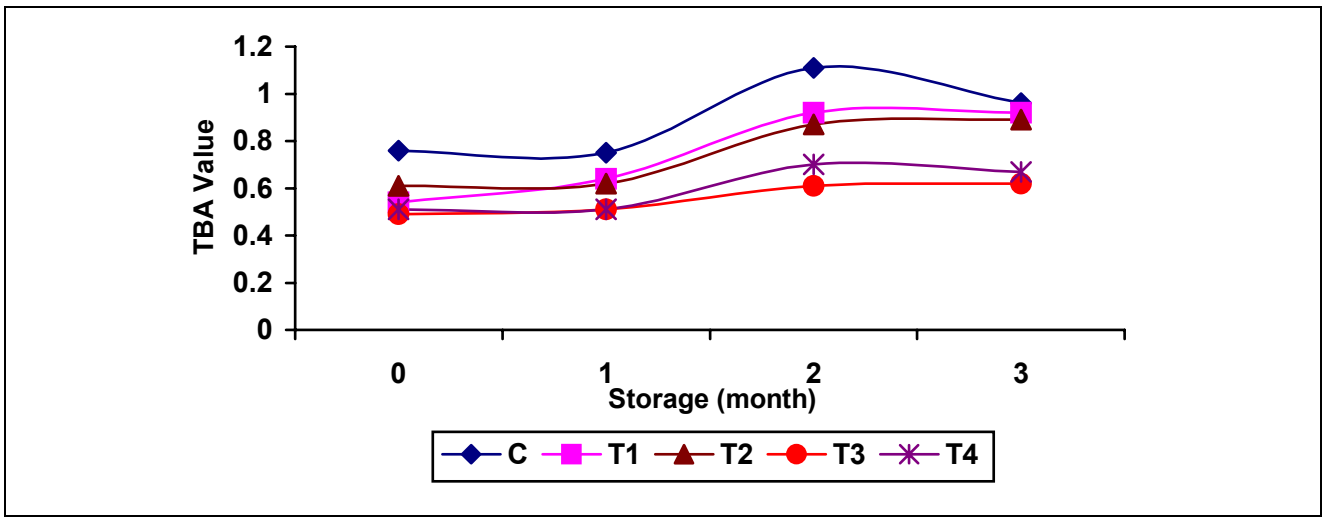
Sensory evaluation was carried out by panel consisting of students and staff of the Department of Food Science and Nutrition, Universiti Kebangsaan Malaysia; 60 panelists altogether. The sensory technique adopted was hedonic test using a seven-point scale. Attributes evaluated included colour, aroma, hardness, springiness, juiciness, oiliness, chicken flavour, off-flavour and overall acceptability. Samples were presented in sample cups coded with 3 digit random numbers and were presented to panelists using random numbers of permutation. Sensory evaluation was conducted in a Sensory Evaluation Laboratory equipped with six isolated booths (School of Chemical Science and Food Technology, Universiti Kebangsaan Malaysia).

Data were subjected to analyses of variance (ANOVA), using the SAS package software (SAS, 1985).

Results and discussion

Thiobarbituric Acid Value (TBA)

Over the entire storage period (3 months), the TBA values of chicken bologna prepared with chicken fat (CF) was higher than those with red palm fat (RPF) and palm fat (PF) (Figure 1). It was clearly shown that the incorporation of red palm fat (RPF) and palm fat (PF) had reduced the TBA values of bologna compared to chicken fat (CF). Substitution of palm fat (PF) in formulation T3 and T4 gave significant differences ($P<0.05$) for the TBA value, when compared to the control formulation (C). Whereas, no significant difference was observed in TBA value between control formulation (C), T1 and T2 ($P>0.05$). Generally, the TBA values of chicken bologna decreased after 3 months of frozen storage ($P<0.05$).



(C= 100%CF; T1=100%RPF; T2=50%CF+50%RPF; T3=100%PF; T4=50%CF+50%PF)
CF : Chicken Fat **RPF** :Red Palm Fat **PF** : Palm Fat

Figure 1: Thiobarbituric Acid Values of Chicken Bologna Stored at -18°C for 3 months.

Sensory Evaluation

Mean scores for various sensory attributes are shown in Table 1. Storage as frozen did not significantly affect ($P>0.05$) consumer acceptance for attributes such as colour, aroma, hardness, springiness, juiciness, oiliness, chicken flavour, off-flavour and overall acceptance for the chicken bologna. Consumer test showed no significant differences ($P>0.05$) between formulation for attributes such as colour, hardness, springiness and oiliness. It was observed that bologna prepared with red palm fat, namely, T1 and T2, received very low sensory score and these scores were significantly lower than control formulation (C) in the chicken flavour rating at the 3 months of storage ($P<0.05$). Bologna prepared with CF was most accepted by the consumer both in chicken flavour and off flavour attributes. However, there were no significant differences ($P>0.05$) between formulations in terms of off flavour attributes at 3 months of storage. The control formulation (100%CF) had the highest scores for the overall acceptance. The study showed that substitution of palm fat (PF) into formulation T3 and T4 in terms of overall acceptance was not significantly different from that of the control formulation (100% CF). Whereas the incorporation of red palm fat (RPF) into chicken bologna, T1 and T2 showed an overall acceptance scores that were significantly different ($P<0.05$) compared to the control formulation (C). The overall acceptance scores were highly correlated to the chicken flavour ($r = 0.89$, $P<0.05$) and springiness ($r = 0.98$, $P<0.05$).

Table 1: Means for Various Sensory Attributes of Chicken Bologna Stored at -18°C for 3 months.

Attributes	Storage Time (Month)	Treatment				
		C	T1	T2	T3	T4
Colour	0	$^{x}4.20^{a}$	$^{x}3.97^{a}$	$^{x}4.47^{a}$	$^{x}4.08^{a}$	$^{x}4.25^{a}$
	3	$^{x}4.25^{a}$	$^{x}4.07^{a}$	$^{x}4.30^{a}$	$^{x}4.45^{a}$	$^{x}4.18^{a}$
Aroma	0	$^{x}4.65^{a}$	$^{x}4.18^{ab}$	$^{x}4.42^{ab}$	$^{x}4.17^{b}$	$^{x}4.23^{ab}$
	3	$^{x}4.30^{a}$	$^{x}4.23^{a}$	$^{x}4.42^{a}$	$^{x}4.58^{a}$	$^{x}4.17^{a}$
Hardness	0	$^{x}4.55^{a}$	$^{x}4.55^{a}$	$^{x}4.35^{a}$	$^{x}4.33^{a}$	$^{x}4.62^{a}$
	3	$^{x}4.60^{a}$	$^{x}4.32^{a}$	$^{x}4.18^{a}$	$^{x}4.42^{a}$	$^{x}4.52^{a}$
Springiness	0	$^{x}4.55^{a}$	$^{x}4.17^{a}$	$^{x}4.27^{a}$	$^{x}4.38^{a}$	$^{x}4.42^{a}$
	3	$^{x}4.40^{a}$	$^{x}4.28^{a}$	$^{x}4.30^{a}$	$^{x}4.35^{a}$	$^{x}4.55^{a}$
Juiciness	0	$^{x}4.65^{a}$	$^{x}4.20^{a}$	$^{x}4.35^{a}$	$^{x}4.25^{a}$	$^{x}4.22^{a}$
	3	$^{x}4.63^{a}$	$^{x}4.05^{b}$	$^{x}4.25^{ab}$	$^{x}4.23^{ab}$	$^{x}4.35^{ab}$
Oilliness	0	$^{x}4.55^{a}$	$^{x}4.20^{a}$	$^{x}4.23^{a}$	$^{x}4.25^{a}$	$^{x}4.40^{a}$
	3	$^{x}4.72^{a}$	$^{x}4.25^{a}$	$^{x}4.32^{a}$	$^{x}4.32^{a}$	$^{x}4.37^{a}$
Chicken Flavour	0	$^{x}4.75^{a}$	$^{x}4.37^{ab}$	$^{x}4.27^{b}$	$^{x}4.55^{ab}$	$^{x}4.50^{ab}$
	3	$^{x}4.68^{a}$	$^{x}4.02^{b}$	$^{x}4.12^{b}$	$^{x}4.43^{ab}$	$^{x}4.48^{ab}$
Off-Flavour	0	$^{x}4.32^{a}$	$^{x}3.80^{b}$	$^{x}3.95^{ab}$	$^{x}4.03^{ab}$	$^{x}3.83^{ab}$
	3	$^{x}4.08^{a}$	$^{x}3.75^{a}$	$^{x}3.97^{a}$	$^{x}3.92^{a}$	$^{x}4.03^{a}$
Overall Acceptability	0	$^{x}4.68^{a}$	$^{x}4.20^{b}$	$^{x}4.08^{b}$	$^{x}4.50^{ab}$	$^{x}4.45^{ab}$
	3	$^{x}4.77^{a}$	$^{x}4.08^{b}$	$^{x}4.27^{b}$	$^{x}4.32^{ab}$	$^{x}4.47^{ab}$

$^{a-b}$Mean values within the same row bearing different superscripts differ significantly (P<0.05)
$^{x-z}$Mean values within the same column bearing different superscripts differ significantly (P<0.05)
C = (100% CF), **T1** = (100%RPF), **T2** = (50%CF + 50% RPF), **T3** = (100% PF),
T4 = (50%CF + 50% PF)

Conclusions

This study pointed out the potential of palm oil product, especially palm fat (PF), to be used as fat sources in the production of comminuted meat products. The results also indicated that incorporation of palm fat did not adverely affect the sensory rating attributes of bolognas. In the storage study, bologna containing red palm fat (RPF) and palm fat (PF) showed lower TBA values than bologna with 100% chicken fat. This study indicated that addition of red palm fat and palm oil resulted in chicken bologna with better sensory properties and lower TBA values, when combined with MDCM, spent hen meat and chicken trimming.

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EFFECT OF ADDING TUMERIC AND LEMON GRASS DURING MARINATION ON THE QUALITY OF ROASTED CHICKEN

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Background

Today's consumer is looking for easy-to-prepare, juicy and tasty products. Marination is an excellent way to turn ordinary and less value meat cuts into succulent food products. Marinating not only adds value to cheaper cuts, but also creates new products with specific taste profile: Rhizome *Cucurma longa*, tumeric have been reported to exhibit antioxidative activity (Toda et al 1985), while Uhl (2000) noted antimicrobial effect of lemon grass, *Cymbopogon citratus* used in marinations of meat and roast chicken.

Objectives

This study monitored the antioxidative and sensory properties of tumeric and lemon grass extracts in marinated roasted chicken.

Materials and methods

Freshly slaughtered broilers and hen carcasses were deboned and the breast meat was separated and kept frozen at -18ºC until used for marination. The formulations were indicated in Table 1.

Table 1. Formulation of five treatments of marinades for boiler meat and spent hen meat.

Ingredients	C1 (%)	C2 (%)	C3 (%)	C4 (%)	C5 (%)
Salt	8.00	8.00	8.00	8.00	8.00
Sodium Nitrite	0.08	0.08	0.08	0.08	0.08
Sodium Erythrobate	0.30	0.30	0.30	0.30	0.30
Sodium tripolyphosphate	1.50	1.50	1.50	1.50	1.50
Sugar	5.50	5.50	5.50	5.50	5.50
Citric acid	0.60	0.60	0.60	0.60	0.60
Curry powder	5.00	5.00	5.00	5.00	5.00
Meat tenderizer	-	3.00	3.00	3.00	3.00
Tumeric	-	-	3.00	-	1.50
Lemon grass	-	-	-	3.00	1.50
Water	79.02	76.02	73.02	73.02	73.02
Total	100.00	100.00	100.00	100.00	100.00

Marination involved soaking of chicken breast meat (200g) with skin in treated marinades at 4ºC for 0, 8 and 24 hours. After marination, the samples were kept at 4ºC for 24 hours, then roasted using a microwave oven.

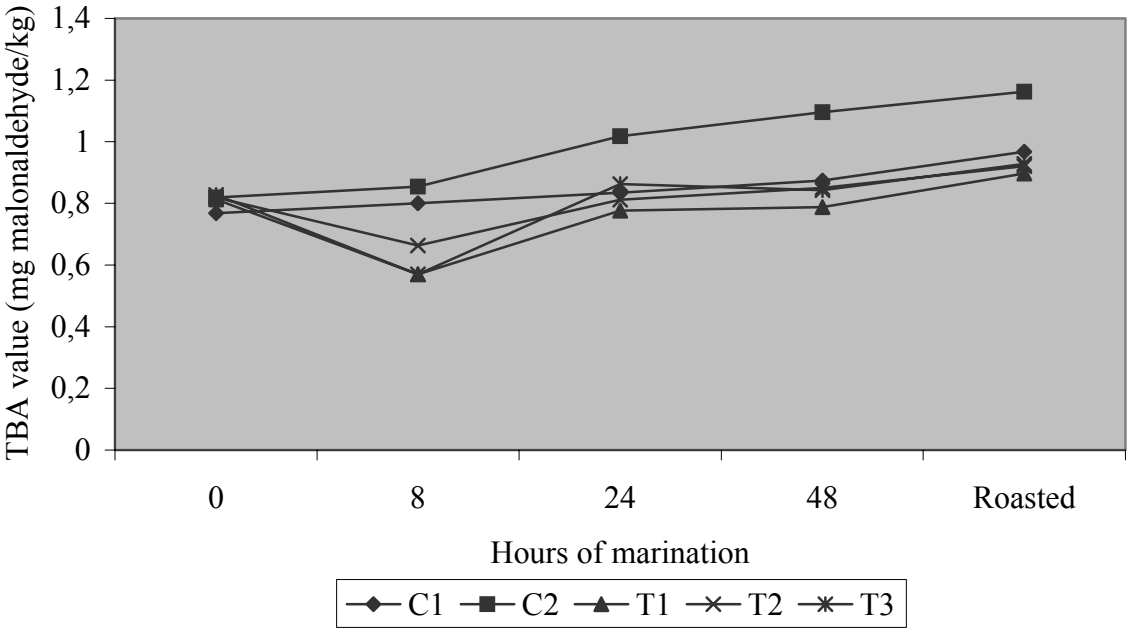
TBA value was determined using the method by Tarladgis et al (1960).

Sensory evaluation was carried out using the Hedonic Scale of 1= lowest, and 7= highest for scoring various attributes. A panel of 50 people was used to evaluate color, aroma, taste, juiciness, tenderness and overall acceptance of the samples.

Statistical analyses were done on the results using SAS, 1985 with Duncan test to differenciate among treatment at $P< 0.05$.

Results and discussion

Results of TBA test indicated a significant (P<0.05) decrease for marinated chicken treated with tumeric, lemon grass and combinaion of tumeric and lemon grass for spent hen, particularly after 8 hours of marination (Figure 1). The two control samples of spent hen and broiler meat had increased TBA values throughout the 48 hours of marination. The antioxidative effect was probably contributed by natural compounds such as cucurmin and citrolene present in tumeric and lemon grass respectively (Priyadarsini, 1997)



C1: Control (Broiler Meat)
C2: Control (Spent hen)
T1: Spent hen + tumeric
T2: Spent hen + Lemon grass
T3: Spent hen + tumeric: lemon grass (1:1)

Figure 1. Changes in TBA values (mg malonaldehyde/kg) for five formulations of marinated chicken meat at various time of marination and roasting.

Table 2 showed the sensory scores of color, odour, taste, texture, juiciness and overall acceptance of four treatment of marinated spent hen meat and two treatments of unmarinated broiler and spent hen meat respectively. Addition of tumeric and lemon grass and combination of these spices resulted in increase in the overall acceptance of roasted spent hen compared to control spent hen. However, the control broiler chicken was softer and more juicy compared to marinated spent hen meat. Marination with tumeric, lemon grass and combination of these two spices increased consumer acceptance for the softness and juiciness of spent hen meat when compared to the control spent hen meat. There were significant differences (P<0.05) for softness for lemon grass and combination of lemon grass and tumeric, when compared to the control non marinated spent hen meat. The problem of toughness with spent hen meat still remained, due to inherent accumulation of connective tissue in older bird. (Roland et al, 1981).

Table 2. Sensory evaluation using hedonic scale of 1= most dislike and 7= most acceptable of five marinated chicken breast meat.

	Formulation				
Attributes	C1	C2	T1	T2	T3
Color	4.20^{AB}	4.60^{AB}	4.72^{A}	4.24^{AB}	4.08^{B}
Odor	4.66^{A}	4.32^{A}	4.18^{A}	4.52^{A}	4.44^{A}
Taste	4.44^{A}	3.82^{B}	3.98^{AB}	4.14^{AB}	3.98^{AB}
Softness	4.62^{A}	3.00^{C}	3.36^{BC}	3.64^{B}	3.90^{B}
Juiciness	4.34^{A}	3.34^{B}	3.74^{B}	3.66^{B}	3.84^{AB}
Overall acceptance	4.52^{A}	3.62^{B}	3.94^{B}	4.12^{AB}	4.10^{AB}

Mean score n=50
$^{A-D}$: Means with different superscript for row are significantly different ($p<0.05$).
C1- Control (Broiler Meat)
C2-Control (Spent Hen)
T1-Spent Hen + tumeric
T2-Spent Hen + Lemon grass
T3-Spent Hen + combination of lemon grass and tumeric (1:1)

Conclusions

Tumeric and lemon grass added to marinades were effective in reducing oxidation in hen meat. Addition of tumeric and lemon grass and their combination in marinades also resulted in improving consumer acceptance of the roasted spent hen breast meat.

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EFFECTS OF COOKING ON CAROTENE AND FREE FATTY ACID CONTENTS OF CHICKEN BURGER INCORPORATED WITH PALM FATS

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Background

Oxidative rancidity is one of the limiting feature of prolonged storage of fresh meats at frozen temperatures as lipid peroxidation contributed towards the nutritional deterioration of such products. Processing techniques prior to freezing burgers involved comminuting the meat, disrupting the tissue membranes and dislodgement of iron from heme compounds, thus exposing the fats to oxygen and prooxidants such as sodium chloride and free iron. Heating/cooking release iron from heme compounds and disrupt the cellular integrity of muscle food, accelerating the process of oxidation. As chicken fat is highly unsaturated, studies of chicken fat being replaced with palm fat (PF) were done (Babji et al 2000, Babji et al 2001) to delay oxidation. Palm fats are a natural source and provider of vitamins A and E. However, the retention of carotene after cooking in processed meats with PF substitution had not been studied.

Objectives

The purpose of this study is to evaluate the changes in peroxide value (PV), free fatty acid (FA), thiobarbituric acid (TBA) and carotene content of raw and cooked chicken burgers incorporated with red palm fat, palm fat and control.

Materials and methods

Palm stearin (PS) and red palm stearin (RPS) burger were compared with control chicken fat burgers (C) at a fixed level of fats (15%). The burgers at 70 g each were cooked on an open flat pan by flipping every 3 minutes until the final internal temperature of 70^0C was reached. The duration of heat treatments were three and ten minutes. The fats from the samples were extracted at the 1, 2 and 3 months of storage at -18^0C. Chloroform/methanol lipid extraction of the samples was done, based on the method of Folch et al., (1957). Test done were on the free fatty acid content (PORIM 1995) and thiobarbituric acid values (TBA) as described by Tarladgis et al (1960). Carotene content analysis was carried out using the method of Hart and Scott (1995). Data were subjected to analysis of variance (ANOVA), using the SAS software package (SAS 1985).

Results and discussion

Free fatty acid (FA) was the highest in burgers with chicken fat (C), followed by palm stearin (PS) and red palm stearin (RPS) treated chicken burgers (Table 1). Prolonged heating resulted in increasing values of FA, peroxide (PV) and thiobarbituric acid (TBA) (Table 2). On the first month of storage (-18^0C), carotene content of RPS burgers were at 298 ppm (raw) and 86 ppm (10 min of cooking). After the third month of storage, the carotene content were significantly reduced to 180 and 38 ppm (raw and 10 min of cooking) respectively. The Red Palm Stearin (RPS) treatment had the lowest FA, PV and TBA values throughout the 3 months of shelf life study.

Table 1. Free fatty acid and carotene content of chicken burger at 1, 2 and 3 months of storage at -18^{0}C.

		Free Fatty Acid (%)			Carotene content (ppm)		
Treatment		Month 1	Month 2	Month 3	Month 1	Month 2	Month 3
Raw	C	0.33 a	0.14 c	0.33 a	0 b	0 b	0 b
	PS	0.29 b	0.43 a	0.29 b	0 b	0 b	0 b
	RPS	0.15 c	0.29 b	0.15 c	298 a	194 a	180 a
Heat Treated (3mins)	C	0.44 a	0.24 c	0.44 a	0 b	0 b	0 b
	PS	0.31 b	0.59 a	0.31 b	0 b	0 b	0 b
	RPS	0.27 c	0.44 b	0.27 c	96 a	86 a	87 a
Heat Treated (10 mins)	C	0.60 a	0.80 a	0.60 a	0 b	0 b	0 b
	PS	0.24 b	0.60 b	0.24 b	0 b	0 b	0 b
	RPS	0.25 b	0.42 b	0.25 b	86 a	67 a	38 a

Means within the same column (a-c) with different small letters are significantly different (P<0.05)

Table 2. Peroxide value and thiobarbituric acid value of chicken burger at 1, 2 and 3 months of storage at -18^{0}C.

		Peroxide value			TBA value		
Treatment		Month 1	Month 2	Month 3	Month 1	Month 2	Month 3
Raw	C	13.28 a	24.35 b	26.77 a	0.68 a	0.69 a	0.77 a
	PS	10.05 b	25.50 a	19.20 c	0.59 b	0.60 b	0.64 b
	RPS	9.30 c	22.35 c	21.44 b	0.32 c	0.37 c	0.40 c
Heat Treated (3mins)	C	14.98 a	28.63 a	34.54 a	1.08 a	2.00 a	1.29 a
	PS	9.31 c	23.43 b	25.37 b	0.96 b	0.96 b	1.24 b
	RPS	10.28 b	15.77 c	24.88 c	0.41 c	0.42 c	0.47 c
Heat Treated (10 mins)	C	15.06 a	47.43 a	46.13 a	1.79 a	1.85 a	1.88 a
	PS	13.19 b	33.78 b	29.80 b	0.98 b	1.30 b	1.71 b
	RPS	11.53 c	25.73 c	26.56 c	0.48 c	0.52 c	0.60 c

Means within the same column (a-c) with different small letters are significantly different (P<0.05)

Conclusions

Substitution of 15% chicken fat with palm stearin and red palm stearin improved the products' shelf life quality with decreased values of free fatty acids, peroxide and thiobarbituric acid values. The presence of carotene in RPS treatments further enhanced the oxidative stability of the products.

Acknowledgement

The authors appreciated the fundings from the Ministry of Science Malaysia through the IRPA grant scheme and from the Malaysian Palm Oil Board, Universiti Kebangsaan Malaysia and the assistance provided by Carotino Ptd. Ltd. and Cargills Specialty Fats & Oils Carotino Ptd. Ltd. towards the research of this paper.

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THE EFFECT OF SODIUM CHLORIDE, SOYBEAN PROTEIN AND STARCH ON GEL PROPERTIES AND COLOR OF BEEF SURIMI

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Background

The quality of meat surimi products is determined by the functional properties of muscle protein, which are water-holding capacity, gel and emulsification properties. These functional properties are based on the solubility of protein and can also determine the binding properties and physical stability of meat surimi.
Myofibril protein, which is composed of myosin and actin, is not only the major protein in muscle, but also the main component of extractable protein. Myosin is insoluble at normal ion strength (0.15 to 0.20M) in muscle of live animals, while it can dissolve at higher ion strength and proper pH. Adding sodium chloride and phosphate can increase the ion strength range from 0.3 to 0.6M. As a result, myosin dissolved can be extracted effectively. Furthermore, the increased pH is also helpful for myosin extraction. Stanley (1994) pointed out that increasing ion strength to a specific extent would improve gel properties and water-holding capacity of beef significantly. Siegal (1979) reported that phosphate and sodium chloride could improve cohesiveness of myosin thus, improve gel properties. Liu et al. (1996) studied the influence of ion strength on heat gel properties of myofibril protein of chicken myosin. The result indicated that the gel formed by protein solution with low ion strength (0.2M KCl, pH6.0) is very soft, while hardness of gel increased gradually along with the increasing ion strength. LesioÂw et al(2001) reported mechanism of rheological changes in poultry myobrillar proteins during gelation.

Non-meat proteins have been widely used in surimi-based meat products to improve gelation strength. The most commonly used were beef plasma protein (BPP), soybean protein, egg white protein, and whey protein concentration (Porter, 1993; Park, 1994). Soybean protein plays an important role in increasing fat usage factor and modifying the texture and emulsification properties of meat products. Soybean protein concentration or soybean protein isolate are usually added to meat surimi in the form of powder or emulsion. These merits make it widely used in meat industry in these days. Although the emulsification properties of soybean protein have been well documented, there is little study on its influence on beef gel properties.
Starch worked as a thickening ingredient in meat production. It is widely used in both traditional Chinese meat products and Western meat products. Natural starch contains two kinds of starch, namely straight-chain starch and branch-chain starch. Generally speaking, the more branch-chain starch added to the product, the better the thickening function is, the greater the gel strength is, and vice versa. Zhou (2003) compared the properties of sausages with added potato starch, cassava starch, wax cornstarch, and cornstarch. It suggested that the palatability, color, springiness and texture of sausages with added potato starch were the best. Adding starch in the production of meat surimi can improve water-holding capacity and modify texture of final products. This is mainly because starch can adsorb water, swell and form gel in the process of heating. According to earlier study, the gelation temperature of starch particles was higher than the denaturation temperature of meat proteins. As a result, meat proteins had been denatured and formed matrix when starch particles began to gelatinize. Starch particles captured weaker-binding water and immobilized it to improve the strength of matrix thus, enhanced the water-holding capacity. At the same time, starch particles swelled, moistened as it adsorbed water. Therefore, starch can improve elasticity and texture of final products by binding minced meat and stuffing the holes between it. Furthermore, adding starch particles can reduce fat loss and increase yield of final product by binding melted lipid in the process of heating as well (Kong, 1996).

Objectives

The objective of this research was to determine the effect of varying concentrations of the additives, such as sodium chloride, soybean protein and four starches (cornstarch, mung bean starch, potato starch and modified starch) on the quality of a beef based meat surimi product. Gel properties and color were measured as indicators of quality.

Materials and methods

The basic formula of the beef sausage was as follows, 1kg of loin from 3 years old local Mongolian cattle, 30g sodium chloride, 100g potato starch, 50g soybean protein, 10g carrageenan, 4g monosodium glutamate, 10g sugar, 3g pepper, 20g garlic, 10g ginger powder.

The sodium chloride, soybean protein and starch content of the product were modified by the following changes to the basic formula. The product was prepared with 1%, 2%, 4% and 5% (w/w) sodium chloride per kg of raw beef in stead of the basic formula content of 3%. The product was prepared with 1%, 2%, 3% and 4% (w/w) soybean protein per kg of raw beef in stead of the basic formula content of 5%. The product was prepared with 5%, 15% and 20% (w/w) of starch per kg of raw beef in stead of the basic formula content of 10%. Additionally, product was prepared with mung bean, modified starch and cornstarch replacing potato starch at all four concentrations. Sausages produced following the basic formula but without corresponding materials in the specific experiment, such as sodium chloride, soybean protein and starch, were taken as controls.

Raw beef was cut into pieces and minced after removing connective tissue. Then the minced meat was cured with sodium chloride at 4□ for 24h. The cured meat was chopped and blended with additives and filled into nature casing. Then the sausages was cooled to 4□ after cooking at 90□ for 30min. Sausages produced by this procedure were taken as samples in this research.

Samples were prepared for texture analysis by removing the product casing and into cylinders of 3cm length. Texture analysis was performed using a TA.XT2i/25 texture analyser, supplied by Stable Micro System Company, England. The sample was penetrated twice with a 50 mm diameter probe and double apex profiles were generated automatically for each sample. Distance, time and force measured by the texture analyser were used to calculate the parameters of hardness, springiness and cohesiveness of the meat surimi products.

The color of samples was measured using a WSC-S color difference instrument, supplied by Shanghai Physical Optical Instrument Factoryi, China. Samples were prepared for color measurements by removing the casing and cutting to form a 1cm long cylinder. The sample was then placed into the sample case and the brightness (L*) and redness (a*) values determined.

Data were analyzed by one way analysis of variance. When significant differences among treatments were detected, treatment means were compared using the least significant difference method. The comparison error was 0.05.

Results and discussion

The effect of sodium chloride on gel properties of beef surimi was shown in table 1. The hardness, springiness and cohesiveness increased from 1890.06, 0.649, 0.359 to 2937.10, 0.722 and 0.471 respectively along with the amount of sodium chloride increasing. It had been well documented that the solubility of myofibril protein, cohesiveness and amount of actomyosin, which was formed by the connection of myosin and actin, would increase with the amount of sodium chloride increasing from 1.7 percent to 11.2 percent. As a result, gel strength increased as well. Although in theory, the more sodium chloride added, the more salt-soluble protein extracted, it was unacceptable for consumer to add so much sodium chloride in meat product. So the amount of sodium chloride in meat products is usually no more than 3.5 percent of meat weight, or the products will be too salty for consumption.

The effect of sodium chloride on the color of beef surimi was presented in table 2. Brightness of beef surimi decreased significantly (from 46.72 to 42.70) with the amount of sodium chloride increase, while the effect of sodium chloride on redness was not significant. So increasing the amount of sodium chloride added to beef surimi would decrease brightness of final products.

The stability of gel is mainly determined by the amount of functional proteins. So it is necessary to add some functional proteins for the purpose of strengthening the gel in meat surimi products. As the optimum pH for the formation of soybean protein gel is in the range of that of meat products, it is a suitable functional protein additive in the production of meat surimi. Soybean protein can form gel before myofibril shrinkage in the

cooking process of sausage and other meat surimi products. It can also form a compact film surrounding muscle tissue. As a result, there is less water as well as water-soluble vitamin and minerals loss during cooking process.The effect of soybean protein on beef gel properties was shown in table 3. Hardness of gel increased from 1955.02 to 2890.66, while springiness reduced from 0.829 to 0.779 with the amount of soybean protein isolate increasing. There was no significant change in cohesiveness (from 0.513 to 0.467). Soybean protein was able to disperse fat, form matrix, concrete and gelate while heating, thus increasing gel strength of product after cooking. But the more soybean protein added, the more solute in final products. High content of soybean protein may compete limited water with meat proteins and thus reduce springiness of the final products.

The effect of soybean protein on the color of beef surimi products was shown in table 4. Brightness of beef sausages decreased significantly(from 40.91 to 38.72) with increasing amount of soybean protein($P < 0.05$). But the influence of soybean protein on redness was not significant.

The effect of different kinds of starch on gel strength of beef surimi is shown in table 5. The gel strength of beef surimi made with various kinds of starch increased compared with that of control. The more starch added, the greater the gel strength is. The main reason is that starch particles absorbed water and swelled, thereby increased water-holding capacity and enhanced the strength of matrix during heating. Starch can bind proteins, fat and water together and make beef surimi more viscous. The capacity of starch increasing gel strength varies from kinds to kinds (Ma L. et al., 1996). The capacity of mung bean starch and modified starch is the largest because mung bean starch has a higher content of branch-chain starch and higher water binding capacity. So it could swell to a relatively large volume (Wu et al., 1987); the swelled starch particles would bring about greater stress on beef protein. The most obvious characteristic of modified starch is that hydroxymethyl group and hydroxyethyl group endow modified starch with excellent hydrophilic and lipophilic properties, thus improve the quality of surimi-based meat products. As the branched chain starch content of cornstarch was relatively lower than others, the gel formed by it was relatively weaker (Okada, 1985).

The effect of different starches on gel springiness of beef surimi was shown in table 6. The springiness decreased gradually compared with that of control when the amount of starch added to beef surimi increased gradually. The extent to which the springiness of beef surimi decreased varies from kinds to kinds as well. The springiness of beef surimi made with mung bean starch was the best, while that of beef surimi with cornstarch was the worst.

The effect of different starches on gel cohesiveness of beef surimi is shown in table 7. Adding starch decreased the gel cohesiveness of beef surimi in comparison with that of control. The gel cohesiveness decreased with gradually increasing amount of starch added to beef surimi. The effect of different starches on cohesiveness varied from kinds to kinds. Cohesiveness of beef surimi made with modified starch is the best, while that of beef surimi with cornstarch and potato starch was relatively worse.

The effect of different starches on the brightness (L^* value) and redness (a* value) of beef surimi products was shown in table 8 and 9.Adding starch decreased the L^* value and a* value of beef surimi in comparison with that of control. With the gradually increasing amount of starch added to beef surimi, the L^* value and a* value decreased gradually. The effect of different starches on L^* value made some differences, among them, the lightness of beef surimi made with mung bean starch and potato starch was better, and that of with cornstarch and modified starch was lower. On the other hand, the effect of different starches on the a* value made some differences, but the difference was insignificant ($P > 0.05$).

Conclusions

Adding sodium chloride and soybean protein had a great influence on the gel properties and color of beef surimi products. The hardness, springiness, cohesiveness of beef surimi gel increased, while brightness decreased with the amount of sodium chloride increasing. But there is no significant influence on redness ($P > 0.05$). The gel hardness increased gradually with increasing amount of soybean protein added to beef surimi, but cohesiveness and a* value changed little, and L^* value decreased significantly($P < 0.05$). Adding various starches increased gel strength of beef surimi products; on the other hand, gel springiness and

cohesiveness decreased. The effect of different kinds of starch on gel properties of final products varied greatly. The mung bean starch was the best as meat additive, while cornstarch is the worst for that use. Adding starch decreased the *L** value of beef surimi products, and the more starch added to beef surimi, the more *L** value decreased. The effect of starch on *L** value varied from kinds to kinds. The effect of beef surimi made with mung bean starch and potato starch was better, while that of cornstarch and modified starch was worse. No matter what kind of starch added, a* value of beef surimi products decreased.

Thanks for the Sustentation Fund offend by Heilongjiang provincial Science and Technology Bureau

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Table 1. Effect of sodium chloride on gel properties of beef

sodium chloride	Hardness	Springiness	Cohesiveness
Control (no sodium chloride)	1890.06±113.38^{d}	0.649±0.033^{c}	0.359±0.036^{b}
1%	2175.92±126.69^{c}	0.651±0.046^{c}	0.362±0.031^{b}
2%	2342.12±82.59^{c}	0.653±0.032^{c}	0.354±0.024^{b}
3%	2558.16±84.51^{b}	0.703±0.032^{bc}	0.428±0.024^{a}
4%	2760.84±123.27^{a}	0.769±0.069^{ab}	0.474±0.054^{a}
5%	2937.10±89.94^{a}	0.722±0.032^{a}	0.471±0.028^{a}

a, b, c Means in the same row with same superscripts letter do not differ($P > 0.05$), with different superscripts letter differ ($P < 0.05$).

Table 2. Effect of sodium chloride on colour properties of beef

Salt	Brightness	Redness
Control (no sodium chloride)	46.72±0.91^{a}	11.74±0.28^{a}
1%	44.29±0.73^{b}	11.79±0.41^{a}
2%	43.42±0.54^{bc}	11.89±0.31^{a}
3%	42.93±0.74^{c}	11.52±0.40^{a}
4%	41.77±1.37^{c}	11.36±0.37^{a}
5%	42.70±0.37^{c}	11.39±0.25^{a}

a, b, c Means in the same row with same superscripts letter do not differ($P > 0.05$), with different superscripts letter differ ($P < 0.05$).

Table 3. Effect of soybean protein on gel characteristics of beef

Soybean protein	Hardness	Springiness	Cohesiveness
Control(no sodium chloride)	1955.02±102.71^{c}	0.829±0.028^{a}	0.513±0.051
1%	2153.94±218.12^{c}	0.847±0.054^{a}	0.467±0.043
2%	2480.18±112.16^{b}	0.823±0.055^{a}	0.479±0.034
3%	2776.60±164.32^{a}	0.860±0.037^{a}	0.513±0.036
4%	2722.20±252.92^{a}	0.784±0.062^{b}	0.474±0.046
5%	2890.66±188.83^{a}	0.779±0.082	0.467±0.074

a, b, c Means in the same line with same superscripts letter do not differ($P > 0.05$), with different superscripts letter differ ($P < 0.05$).

Table 4. Effect of soybean protein on colour characteristics of beef

Soybean protein	Brightness (L^*)	Redness (a*)
Control (no sodium chloride)	40.91±0.55^{a}	11.73±0.58
1%	40.70±0.59^{a}	12.03±0.17
2%	40.28±0.49^{ab}	11.19±0.47
3%	39.48±0.53^{b}	11.65±0.49
4%	39.61±0.48^{b}	11.49±0.51
5%	38.72±0.51^{b}	11.39±0.35

a, b, c Means in the same line with same superscripts letter do not differ($P > 0.05$), with different superscripts letter differ ($P < 0.05$).

Table 5. Effect of different starches on hardness of beef

	mung bean	Potato	Modified	Corn
Control(no starch)	1055.00±127.75^{d}	1055.00±127.75^{d}	1055.00±127.75^{c}	1055.00±127.75^{d}
5%	1196.80±113.45$^{d\ xy}$	1276.70±109.58$^{c\ x}$	1148.36±65.73$^{c\ y}$	1027.6±54.48$^{d\ z}$
10%	1655.48±70.94$^{c\ x}$	1558.52±97.92$^{b\ xy}$	1633.66±139.12$^{b\ x}$	1450.92±140.65$^{c\ y}$
15%	2151.82±111.59$^{b\ x}$	2109.82±63.21$^{a\ x}$	2245.22±243.67$^{a\ x}$	1843.36±141.57$^{b\ y}$
20%	2725.56±148.06$^{a\ x}$	2173.90±53.78$^{a\ z}$	2441.20±106.55$^{a\ y}$	2067.74±156.38$^{a\ z}$

a, b, c Means in the same line with same superscripts letter do not differ($P > 0.05$), with different superscripts letter differ ($P < 0.05$), x, y，zMeans in the same row with same superscripts letter do not differ($P > 0.05$), with different superscripts letter differ ($P < 0.05$).

Table 6. Effect of starch on springiness of beef

	mung bean	Potato	Modified	Corn
Control(no starch)	0.865±0.015^{a}	0.865±0.015^{a}	0.865±0.015^{a}	0.865±0.015^{a}
5%	0.778±0.042$^{b\ x}$	0.745±0.042$^{b\ xy}$	0.821±0.036$^{a\ x}$	0.731±0.047$^{b\ y}$
10%	0.705±0.037^{c}	0.703±0.082^{b}	0.712±0.105^{b}	0.672±0.061^{bc}
15%	0.687±0.058$^{c\ xy}$	0.708±0.035$^{b\ x}$	0.642±0.053$^{c\ y}$	0.623±0.056$^{cd\ y}$
20%	0.639±0.050$^{c\ x}$	0.631±0.088$^{c\ x}$	0.623±0.012$^{c\ xy}$	0.605±0.086$^{d\ y}$

a, b, c Means in the same line with same superscripts letter do not differ($P > 0.05$), with different superscripts letter differ ($P < 0.05$), x, y，zMeans in the same row with same superscripts letter do not differ($P > 0.05$), with different superscripts letter differ ($P < 0.05$).

Table 7. Effect of starch on cohesiveness of beef

	mung bean	Potato	Modified	Corn
Control	0.372±0.044^{a}	0.372±0.044^{a}	0.372±0.044^{a}	0.372±0.044^{a}
5%	0.353±0.014$^{a\ xy}$	0.333±0.052$^{a\ xy}$	0.396±0.079$^{a\ x}$	0.327±0.022$^{ab\ y}$
10%	0.367±0.027$^{a\ x}$	0.323±0.015$^{ab\ y}$	0.326±0.035$^{b\ x}$	0.319±0.033$^{b\ y}$
15%	0.305±0.026$^{b\ xy}$	0.281±0.022$^{b\ y}$	0.320±0.024$^{b\ x}$	0.287±0.009$^{b\ y}$
20%	0.288±0.040$^{b\ x}$	0.231±0.036$^{c\ y}$	0.312±0.009$^{b\ x}$	0.232±0.049$^{c\ y}$

a, b, c Means in the same line with same superscripts letter do not differ($P > 0.05$), with different superscripts letter differ ($P < 0.05$), x, y，z Means in the same row with same superscripts letter do not differ($P > 0.05$), with different superscripts letter differ ($P < 0.05$).

Table 8. Effect of starch on brightness value of beef

	mung bean	Potato	Modified	Corn
Control	40.16±1.02^{a}	40.16±1.02^{a}	40.16±1.02^{a}	40.16±1.02^{a}
5%	40.47±0.74^{a}	39.60±0.71^{ab}	38.16±0.54^{b}	40.31±0.68^{a}
10%	40.05±0.61$^{a\ x}$	39.30±0.49$^{ab\ x}$	37.46±0.51$^{b\ y}$	38.24±1.16$^{b\ y}$
15%	39.15±0.66$^{ab\ x}$	38.66±0.81$^{bc\ x}$	35.67±1.25$^{c\ y}$	36.81±0.75$^{b\ y}$
20%	38.00±0.72$^{b\ x}$	37.80±0.62$^{c\ x}$	33.68±0.58$^{d\ y}$	34.95±1.22$^{c\ y}$

a, b, c Means in the same line with same superscripts letter do not differ($P > 0.05$), with different superscripts letter differ ($P < 0.05$), x, y，zMeans in the same row with same superscripts letter do not differ($P > 0.05$), with different superscripts letter differ ($P < 0.05$).

Table 9. Affect of starch on red value of beef

	mung bean	Potato	Modified	Corn
Control	13.63±1.06^{a}	13.63±1.06^{a}	13.63±1.06^{a}	13.63±1.06^{a}
5%	12.62±0.49^{ab}	13.28±0.57^{ab}	13.49±0.20^{a}	13.69±0.27^{a}
10%	12.53±1.20^{ab}	12.33±0.78^{b}	13.12±0.39^{ab}	13.68±0.55^{a}
15%	12.12±0.83^{b}	12.29±0.95^{b}	12.72±1.28^{b}	13.11±0.54^{ab}
20%	11.57±0.65^{b}	11.93±1.12^{b}	12.11±1.07^{b}	12.87±0.71^{b}

a, b, c Means in the same line with same superscripts letter do not differ($P > 0.05$), with different superscripts letter differ ($P < 0.05$).

RESIDUAL LEVELS OF THREE PRESERVATIVES AND ECONOMICAL VIABILITY OF SURFACE TREATMENTS APPLIED IN PORTUGUESE SMOKED DRY SAUSAGES

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Background

In Portugal, as in the E.U., benzoates, sorbates and the esters of *p*-hydroxy-benzoic acid (parabens) may only be used in surface treatments of dry sausages casings. Legislated values are *Quantum satis* (without specified maximum level, these addictives should be added in agreement to the good manufacture practices, using the necessary amount to reach the proposed objectives) however, there is no reference about residual values in the final product (Decreto-Lei nº274, 2000; Decreto-Lei nº363, 1998). Residual levels vary with the method of application, time length of exposure, salt concentration, type of food, porosity of the food, shape and size of the food, and handling after exposure to the additive (Sofos, 1989). In this way, before a given application, tests should be conducted to determine appropriate solutions, exposure times, and handling of the necessary residual preservative levels in the product. In spray, dipping, or immersion uses, it is important to know the uptake, migration, diffusion, and residual levels of the preservative into the product. This will be useful in determining levels needed, time of exposure, expected inhibitory activity, and potential undesirable effects on product quality.

Objectives

In this contest, the main goals of this study were to investigate, in Portuguese smoked dry sausages, the residual amounts of potassium sorbate (PS), sodium benzoate (SB) and methyl *p*-hydroxybenzoate (MHB) applied as surface treatment and also, the viability of such treatments regarding the production costs.

Materials and methods

Samples studied for this project were "chouriços" type Alentejano (A) and Ribatejano (R) produced by a large factory in Portugal as outlined by Matos et al. (2003). In control chouriço type R samples, surface treatment applied in casings was only cleansing with water to remove the salt and then immersion in cold water until fill up. In chouriço type A control samples, first casings were dipped in a solution of 30 kg of water and 1.5 l of commercial vinegar for 10 minutes, secondly cleansing with water and finally immersion in tap water until filling. These were normal plant practices for natural casings. For both sausage types, in test samples, casings were surface treated with three different salts in seven different combinations (Table 1), after water washing. Casings were immersed for 15 minutes in these solutions until filling. The experiments were divided in four trials. Each trial was conducted in batters of 560 kg. In the first trial the surface treatments were performed with potassium sorbate (PS), sodium benzoate (SB), methyl *p*-hydroxybenzoate (MHB) and control (AC1) (Table 1) in chouriço type A. The second trial was also conducted in chouriço type A and the surface treatments with potassium sorbate and sodium benzoate (PS+SB), potassium sorbate and methyl *p*-hydroxybenzoate (PS+MHB), sodium benzoate and methyl *p*-hydroxybenzoate (SB+MHB), potassium sorbate, sodium benzoate and methyl *p*-hydroxybenzoate (PS+SB+MHB) and, control (AC2), were applied (Table 1). In the third trial were applied the treatments PS, SB, MHB and control (RC1) (Table 1) in chouriço type R and, in the fourth trial, the treatments PS+SB, PS+MHB, SB+MHB, PS+SB+MHB and control (RC2) also in Ribatejano sausage type (Table 1). From all experiments 3 samples of sausages were taken from each surface treatment and control. Sausage samples were not peeled to accomplish the chemical measurements. The determination of PS, SB and MHB residual values were performed by High Performance Liquid Chromatography (HPLC) using a NOVA PACK, part nº36975 (Waters). Solution A, with 2.5 kg KH_2PO_4 + 0.1% acetic acid solution, pH at 4.4 (reached with 20% KOH solution), was prepared for eluent composition. Eluent for HPLC was constituted by 85% solution A and 15% ethanol. Detector of UV λ at 217 nm, oven temperature was 35ºC at 1.0 ml/minute stream. Production costs concerned with the application of surface treatments in the natural casings in both types of sausages were determined according to the variable cost system and production costs were calculated as basic standard costs type (Rocha & Rúbio, 1999; Santos,

1998; Pereira & Franco, 1994). Calculations were based on real data achieved in a meat industry, under commercial conditions.

Results and discussion

Residual levels of PS, SB and MHB of sausages type Ribatejano and Alentejano are summarised in Table 2. Surface treatments production costs are presented in Tables 3 and 4 for chouriço type A and R, respectively. In Table 5 the totality of production costs for both types of sausages is shown. Values of methyl *p*-hydroxybenzoate in the accomplished analyses, were not detected (Table 2). The *p*-hydroxybenzoic esters may be linked to some extent to proteins, emulsifiers and other substrate constituents on account of their phenolic OH group (Lueck, 1980) and/or the non absorption of MHB by the casing during immersion time could be responsible for this MHB residual values absence. Residual levels of PS and of SB found in the product after immersion of sausage dry smoked natural casings for 15 min in 2.5% (w/v) solutions of these three preservatives in seven different combinations varied from 0.009 to 0.056% (w/w) in treatments with PS, SB, PS+MHB and SB+MHB and, from 0.021 to 0.030% (w/w) (addition of residual mean values obtained for both salts, PS and SB) in treatments with combination of salts PS+SB and PS+SB+MHB (Table 2).

For the same preservative, differences found between types of products may be related to the porosity of the natural casings used for each type of product (pork intestine salted for chouriço type R and beef dry casing for chouriço type A). In this way, it is very difficult to estimate expected values in the final product once the amount of addictive that will remain in the casing after dipping in salts solutions and which amount will migrate from the surface to the interior of the product are ignored.

Concerning eventually toxicological effects, Hossaini *et al.* (2000) in mouse uterotrophic assay found no oestrogenic response of methyl *p*-hydroxybenzoate and of propyl *p*-hydroxybenzoate at doses of 100 mg/kg body weight per day and for the ethyl congener even at 1000 mg/kg body weight per day. The authors concluded that parabens are not potent oestrogens *in vivo.* Binstok *et al.* (1998), in a meat system containing 200 mg of sorbates and 150 mg of nitrites, heated at 60^{o}C for 5 hours, found that a mutagenic compound, ethyl-nitrolic acid (ENA) can be formed. More research is required to determine the minimum ENA amount for genotoxic activity and for establishing its health implications. Sodium benzoate toxicity in rats F344 and mice B6C3F1 was investigated by Fujitani (1993) reporting that levels of 2.4% in male rats and 3% in male mice of SB administrated in the diet for 10 days were toxic. The cost of surface treatments applied in the casings can increase from 0.0006 € up to 0.0024 €, per kilogram of final product, in sausage type Alentejano and, from 0.0001 € up to 0.003 € in sausage type Ribatejano, depending on the salt combination used for surface treatment (Tables 3 and 4).

Conclusions

Regarding potential undesirable effects on product quality it would seem indispensable to accomplish more experiments to establish maximum legislated values for these preservatives applied as surface treatments to dry smoked sausage casings. Also, determination of the accurate amounts to be added, time of exposure, diffusion and migration must be performed in order to obtain the expected residual levels into the product. Surface treatments represent less than 0.5% of the production costs in both types of sausages. Nevertheless, treatment selection should be supported through a technical and economical decision well based once, meat industries, due to the specific technologies used and the smaller commercialization margins, must assure their competitiveness in a very demanding market.

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Table 1
Surface treatments applied in Alentejano and Ribatejano smoked dry sausages casings.

Combinations of salts	Preparation	Amount of salt added	Final concentration (w/v)
Control of Alentejano sausage (AC1 and AC2)	30 l of water + 1.5 l of commercial vinegar	-	–
Control of Ribatejano sausage (RC1 and RC2)	Clean with water and immersion in cold water	-	–
Potassium sorbate (PS) (SIGMA, S1751)	In 20 l of water at 35±5^{0}C	500 g	2.5%
Sodium benzoate (SB) (SIGMA, B3375)	In 20 l of water at 35±5^{0}C	500 g	2.5%
Methyl *p*-hydroxybenzoate (MHB). (SIGMA, H5501)	In 20 l of water at 35±5^{0}C	500 g	2.5%
Potassium sorbate and Sodium benzoate (PS+SB)	In 40 l of water at 35±5^{0}C	500 g of each salt	1.25% of each salt
Potassium sorbate + Methyl *p*-hydroxybenzoate (PS+MHB)	In 40 l of water at 35±5^{0}C	500 g of each salt	1.25% of each salt
Sodium benzoate + Methyl *p*-hydroxybenzoate (SB+MHB)	In 40 l of water at 35±5^{0}C	500 g of each salt	1.25% of each salt
Potassium sorbate + Sodium benzoate + Methyl *p*-hydroxybenzoate (PS+SB+MHB)	In 60 l of water at 35±5^{0}C	500 g of each salt	0.83% of each salt

Table 2
Residual mean values of potassium sorbate (PS), sodium benzoate (SB) and methyl p-hydroxybenzoate (MHB) in Alentejano (A) and Ribatejano (R) Portuguese types of sausages, after treatments. Standard deviation is shown in parenthesis, for n=3.

Treatments	PS (mg/kg)		SB (mg/kg)		MHB (mg/kg)	
	A	R	A	R	A	R
C1	-	-	-	-	-	-
PS	345.7 (14.2)	151.7 (14.6)	-	-	-	-
SB	-	-	560.3 (305.5)	233.3 (3.5)	-	-
MHB	-	-	-	-	nd	nd
C2	-	-	-	-	-	-
PS+SB	121.3 (85.6)	101.7 (13.2)	151.3 (57.7)	122.3 (31.2)	-	-
SB+MHB	-	-	144.3 (44.2)	131.3 (78.6)	nd	nd
PS+MHB	109.3 (54.1)	93.0 (5.3)	-	-	nd	nd
PS+SB+MHB	104.0 (5.2)	133.0 (11.3)	108.2 (5.3)	166.7 (12.4)	nd	nd

nd - not detected

Table 3
Production costs of surface treatments applied to beef dry casings in chouriço type Alentejano.

Components[a]	€ / kg	Surface treatments[b] (kg)							
		Control	PS	SB	MHB	PS+SB	PS+MHB	SB+MHB	PS+SB+ MHB
Water	0,005	30,000	20,000	20,000	20,000	20,000	20,000	20,000	30,000
Commercial vinegar	0,818	1,500							
Potassium sorbate	10,260	0,000	0,250			0,250	0,250		0,250
Sodium benzoate	1,915	0,000		0,250		0,250		0,250	0,250
Methyl *p*-hydroxybenzoate	9,477	0,000			0,250		0,250	0,250	0,250
Total value [c] (€)		1,377	2,665	0,579	2,469	3,144	5,034	2,948	5,563
Production cost per kg [d] (€)		0,0008	0,0015	0,0003	0,0014	0,0018	0,0029	0,0017	0,0032

[a]The labour costs were considered in Table 7.
[b]Surface treatments are described in Table 2.
[c]Total value (€) = Value (€ / kg) X Surface treatment (kg)
[d]Production cost per kg (€)= Total value of each surface treatment / kilograms of meat batter processed (1740,64 kg)

Table 4
Production costs of surface treatments applied to pork salted casings in chouriço type Ribatejano

Components[a]	€ / kg	Surface treatments[b] (kg)							
		Control	PS	SB	MHB	PS+SB	PS+MHB	SB+MHB	PS+SB+ MHB
Water	0,005	60,000	20,000	20,000	20,000	20,000	20,000	20,000	30,000
Commercial vinegar	0,818	0,000							
Potassium sorbate	10,260	0,000	0,250			0,250	0,250		0,250
Sodium benzoate	1,915	0,000		0,250		0,250		0,250	0,250
Methyl *p*-hydroxybenzoate	9,477	0,000			0,250		0,250	0,250	0,250
Total value [c] (€)		0,299	2,665	0,579	2,469	3,144	5,034	2,948	5,563
Production cost per kg [d] (€)		0,0002	0,0015	0,0003	0,0014	0,0018	0,0029	0,0017	0,0032

[a]The labour costs were considered in Table 7.
[b]CSurface treatments are described in Table 2.
[c]Total value (€) = Value (€ / kg) X Surface treatment (kg)
[d]Production cost per kg (€) = Total value of each surface treatment / kilograms of meat batter processed (1733,67 kg)

Table 5
Distribution of the production costs by components for both types of sausages (Alentejano and Ribatejano).

Chouriço Type Alentejano	€ / kg	Percentage (%) of the total cost
Raw meat material	2,697	66,18
Formulation ingredients.	0,239	5,86
Packaging material	0,754	18,50
Labour	0,305	7,48
Sanitation	0,020	0,49
Energy	0,060	1,48
TOTAL	**4,075**	**100**
Chouriço Type Ribatejano	**€ / kg**	**Percentage (%) of the total cost**
Raw meat material	2,629	64,58
Formulation ingredients.	0,286	7,02
Packaging material	0,762	18,72
Labour	0,314	7,71
Sanitation	0,020	0,49
Energy	0,060	1,47
TOTAL	**4,071**	**100**

STUDY ON SALT-INDUCED CHEMICAL CHANGES IN PORK MUSCLE BY FT-IR IMAGING

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Background

Processing methods like heating, freezing or salting are commonly used in muscle food preparation and all may lead to changes in texture. Texture is a complex property considered to be affected by muscle microstructure and composition, i.e. myofibrillar proteins, intramuscular connective tissue and fat distribution.

Therefore, there is a demand for techniques that enable us to gain more detailed knowledge about microstructural changes in these proteins.

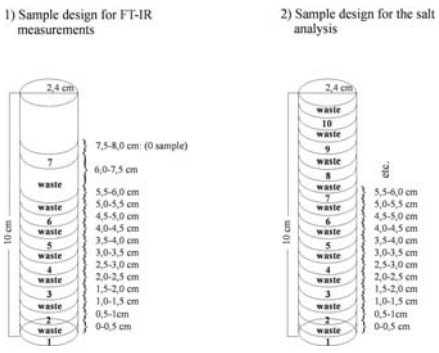
FT-IR-microspectroscopy and FT-IR imaging have been found to be a useful analytical tool so far mainly in biomedical science. Since it is a combination of spectroscopy and microscopy it allows to investigate those properties simultaneously and can provide information on chemical composition and morphology of complex tissues at the same time.
Most recently, a FT-IR imaging system was used to monitor denaturation induced by heating in beef muscle tissue (Kirschner et al. 2004).

Objective

The intention of this study was to investigate if FT-IR imaging can be used as a tool to monitor chemical changes such as denaturation processes in pork muscle tissue which occur during salting.

Materials and methods

Sample preparation



In this study samples from pork muscle (*m. semimembranosus)* with three different salt concentrations were investigated. The raw meat samples were placed in tubes (2.4 cm in diameter and 10 cm in height) that were kept in saturated brine (26% NaCl) so that the brine would diffuse into the samples resulting in salt gradient throughout the sample. After two weeks small blocks from regions of different salt concentrations were excised from the sample tube, frozen in liquid Nitrogen and stored at -80°C until use. For the FT-IR-imaging cryo-sections of 8 µm thickness (two sections each for low, medium and high salt concentration, i.e. from layer 1, layer 4 and layer 7 respectively) were prepared with a Leica cryostat CM3050 S (Leica microsystems, Nussloch, Germany) and mounted on infrared transparent CaF_2 slides.

Salt concentrations in the parallel sample (in the corresponding layers as shown above) determined by chemical Cl^--analysis were 1.6% for the low salt, 7.7% for the medium salt, and 15.4% for the high salt layer. In the following the samples will only be referred to as high, medium and low salt sample.

IR data collection and evaluation

An IR-microscope (IRscope II, BRUKER OPTICS, Germany) equipped with a 64 x 64 focal plane array detector (FPA) was used to collect 4096 spectra (a sample area of 270 µm x 270 µm which can be analysed simultaneously at a spatial resolution of 4 µm per pixel) on a pork muscle tissue sample. The analysis of the acquired IR images was done with in-house developed software in C++. The Microsoft C++ Developer

Studio was used to create Dynamic Link Libraries, that were linked to Dynamic Imager, an image analysis developer platform (Kirschner et al.2004). First, the image spectra where scatter-corrected using extended multiplicative signal correction (EMSC). EMSC is a pre-processing method that allows the separation of physical light-scattering effects (e.g. sample thickness) from chemical absorbance effects in spectra (Martens et al. 2003). Then, the raw spectra were submitted to a quality test, which among other things checks spectra for water vapour and signal to noise ratio. Spectra that did not pass the quality test were not used for further data analysis.

Results and discussion

The most prominent protein band in a FT-IR spectrum of muscle fibres is the Amide I band that appears in the frequency region of 1700-1600 cm^{-1}. It is mainly related to the carbonyl stretching vibration with minor contribution of C-N stretching and N-H bending vibrations. The Amide I vibration mainly depends on the secondary structure of the protein backbone and therefore is often used for secondary-structure analysis of proteins (Barth and Zscherp 2002).

Figure 1 displays five spectra across a randomly chosen fibre (spectrum 1 located at the periphery, with increasing numbers moving towards the centre of the fibre and 5 representing a spectrum located in the centre), which were extracted from the FT-IR image obtained from a cryo-section with high salt content. Spectra from an area around the edge of the fibre cell (Spectrum 1 and 2) exhibit a decrease in absorbance at 1654 cm^{-1} compared to those taken from inner parts of the fibre.
In order to gain a better resolution of the Amide I components derivation was applied, which is illustrated in Figure 2. The second derivatives of the five spectra shown in Figure 1 reveal the mentioned effect at 1654 cm^{-1} more clearly. The frequency region of 1648-1658 cm^{-1} is considered to correlate to α-helical protein structures (Barth and Zscherp 2002). The observed decrease at 1654 cm^{-1} might therefore indicate either an increase in denaturation with a shift from α-helix to β-sheet structure as observed by Kirschner et al. (2004) in heat-treated beef myofibre samples in the range of 45°C to 70°C, or it might be due to a salt-induced spatial diffusion of α-helix or β-sheet compounds within the fibre.

Figure 3 shows chemical images for three different salt concentrations. The chemical images were reconstructed from the spectra obtained from FDA-detection by employing the I_{1630}/I_{1654} band ratio, which gives a measure of the level of change: blue corresponds to a low while red corresponds to a high band ratio. Black domains in these images refer to spectra that did not pass the quality test, i.e. they mostly correspond to extracellular areas which can be seen when compared to photomicrographs. Figure 3c exhibits a fairly low band ratio indicated by predominantly dark blue colouration in the chemical image. Figure 3b reveals a lighter blue colour, which is caused by an increase in protein I_{1630}/I_{1654} band ratio. This effect is further increased in Figure 3a. Here, the higher degree of denaturation at the outer parts of the fibres is even more distinct than in Figure 3b. This supports the results presented in Figures 1 and 2, i.e. that the outer regions of the muscle fibre cells reveal a higher degree of either denaturation or an increase in β-sheet or decrease in α-helix structure respectively compared to the fibre centre. As seen from the photomicrograph at high salt content the fibre cell appear shrunken to high degree and thus the increase of extracellular space (Knight and Parsons 1988) is the reason for the significant increase in black pixel in the corresponding chemical image.

The possible reasons for the increase in the I_{1630}/I_{1654} band ratio at the fibre periphery may be as already mentioned a change in secondary structure for individual protein types or a possible diffusion effect that causes migration of protein structures. The latter may lead either to an increase in β-sheet-components or a decrease in α-helix which causes the higher I_{1630}/I_{1654} ratio in the outer parts of the fibre cells.

Conclusions

- The FT-IR imaging technique employing FPA-detectors might provide a useful tool for the detection of chemical changes like protein denaturation or diffusion effects. These changes can be followed in a spatially resolved manner.
- It was suggested that high salt contents may induce a higher degree of denaturation on the periphery compared to the centre of a muscle fibre cell.
- This method could possibly be used also to gain a better understanding in the mechanisms in salt diffusion in muscle tissue

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Figures

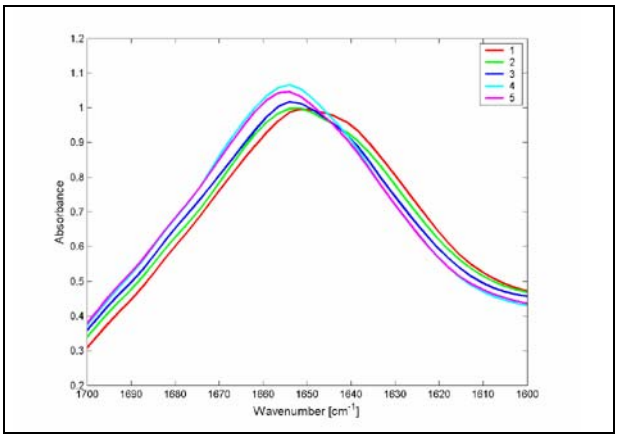

Fig.1: IR-spectra across a single myofibre from the high salt content sample shown in the frequency range 1700-1600 cm^{-1}; numbers 1-5 represent spectra from the edge towards the centre of the fibre cell

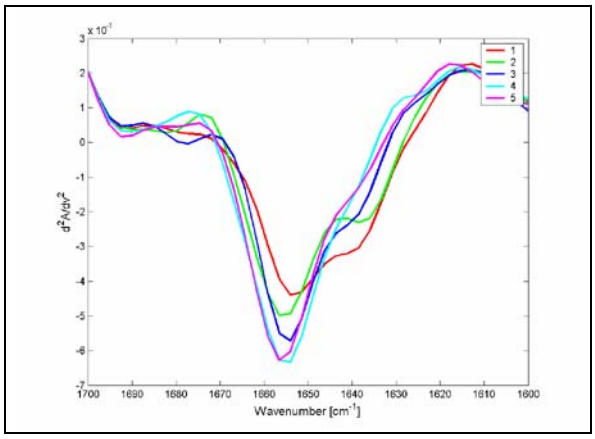

Fig.2: 2.Derivatives of IR-spectra from the high salt content sample displayed in the frequency range 1700-1600 cm^{-}; numbers 1-5 represent spectra from the edge towards the centre of the fibre cell

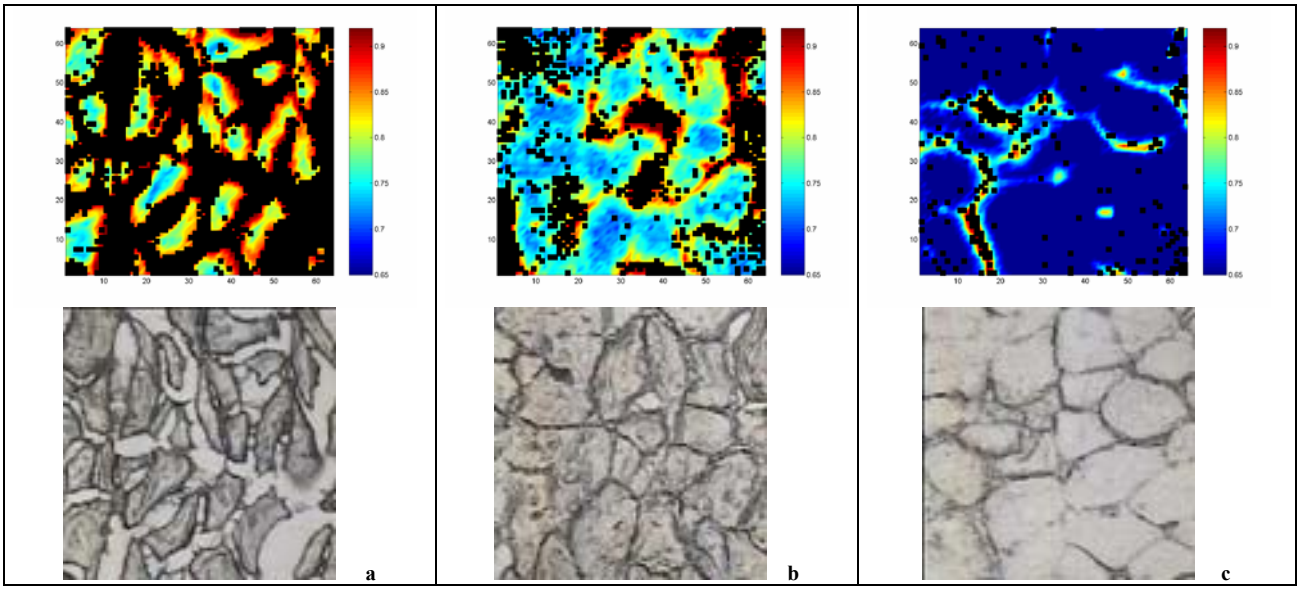

Fig.3: Chemical images (showing the I_{1630}/I_{1654} band ratio) obtained for 3 salt concentrations: high (a), medium (b), low (c) (from left to right). Corresponding photomicrographs are shown below the respective IR-image.

LOW VOLTAGE ELECTRICAL STIMULATION, HOT BONING AND HIGH TEMPERATURE CONDITIONING OF *LONGISSIMUS LUMBORUM* MUSCLE FROM *BOS INDICUS*: DRIP AND COOKING LOSS, WATER HOLDING CAPACITY AND COLOUR

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Background

The interest in the technologies to accelerate conversion of muscle into meat remains. It is well established that electrical stimulation increases the rate of post-mortem glycolysis, allowing the use of hot boning and preventing that the meat becomes though (Hwang et al., 2003). Several studies demonstrated the flexibility of boning unchilled carcasses (hot boning) improving processing efficiency, functional properties, flavour, colour, juiciness, drip, and cooking loss (Henrickson, 1975; Kastner et al., 1973; Jeremiah et al., 1997). In a previous work, Cardoso et al. (2002) studied the influence of hot boning, high temperature conditioning, low voltage electrical stimulation, and aging on the rate of glycolysis, tenderness, and shear values of *longissimus dorsi* (*lumborum*). In this work were measured the effects of the same variables on water holding capacity, drip and cooking losses, and colour of *longissimus lumborum*.

Objectives

The purpose of the study was to demonstrate that hot boned *l. lumborum* from electrically stimulated *Bos indicus* carcasses can have drip and cooking loss, water holding capacity and colour, similar to those from conventionally boned meat.

Materials and methods

Forty Nelore (*Bos indicus*) pasture-fed steers with 30-36 months of age and average slaughter weights of approximately 450 kg were slaughtered at four different occasions over a three month period. Animals were stunned and bled. The beginning of bleeding was time zero for all treatments. Low voltage electrical stimulation (LVES) with a JARVIS BV 80 stimulator [20 V (rms); 60 Hz; 0.25 amps; for 90s alternating 5s on, 1s off] was applied immediately after exsanguination. Cattle were slaughtered at the Meat Technology Center of Institute of Food Technology in Campinas. Carcasses had an average weight of 250 kg. The animals were randomly assigned to five treatments (**Figure 1**) and two replications for each slaughtering session.

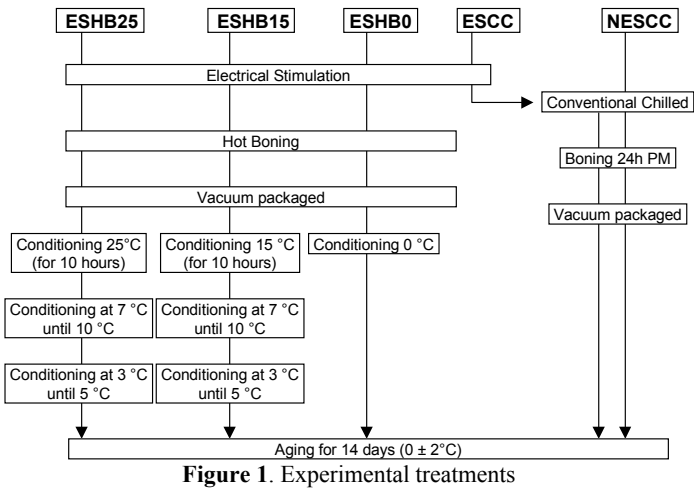

Figure 1. Experimental treatments

The *longissimus lumborum* (LL) was the muscle studied. The hot boned (HB) muscles were excised from the electrically stimulated carcasses after approximately 45 min post mortem (p.m) or conventionally chilled

(CC) and boned 24 h p.m. The LL muscle were cut in three pieces of the same size (10,16cm) and all pieces were vacuum packaged in CRYOVAC® shrinkable bags and placed into 536X235X162 mm cardboard boxes. Water holding capacity and drip loss: Muscles samples for water holding capacity (WHC) measurements, were 24 h p.m., at the 7th and 14th days p.m. For WHC determination, triplicate samples of 500 mg of muscle were placed on a filter paper (Whatmann n.2) and pressed between two plexiglass plates for 5 minutes at 500kg/cm2 on the ram of a laboratory press, following the procedure of Hoffmann et al. (1982). Results were expressed as the ratio between the areas of the liquid infiltrated (External Area=eA) and meat spot (Internal Area=iA) measured with a planimeter. The drip loss of the vacuum packaged cuts was determined by weighing the muscles before packaging and after unpacking on the 2nd, 7th and 14th days p.m. Results were expressed as percentages (%) of losses. Cooking and cooking loss: Three 2.54cm LL slices per treatment were cooked at 2nd, 7th and 14th days p.m.. The LL steaks were cooked according to AMSA (1995) guidelines, in an electrical grill SIRMAN PDL model with both plates heated, till their internal temperature reached 74°C. Temperatures were monitored using a digital thermometer NOVUS 51 with thermocouples (K type) inserted into the center of each steak. Cooking loss measurements were obtained by recording sample weights before and after cooking. Results were expressed as percentages (%) of losses. Color Analysis: Color readings were taken in the L* a* b* color space on the cross section of the LL muscle using a Minolta Spectrophotometer CM 508-d with D_{65} illuminant and 10° observer angle. Color was measured after deboning and on the 7th and 14th days p.m. with five replications. At 7 and 14 days p.m. the readings were made 30 min after unpacking and air exposure of the steaks. The mean of five replications was used as final result for reading and expressed in *L (lightness), a*(redness) and b* (yellowness) values. Statistical analysis: The results were statistically analyzed using the Statistical Analysis System program version 8.0. Analysis of variance was used to test for significance of slaughter period, treatment, ageing time and interaction treatment/ageing time effects. The mean values were compared pairwise with Duncan's Multiple Range. The significance level of 5% was used in all analysis.

Results and discussion

The drip loss, cooking loss and water holding capacity values are shown on Table 1. The drip losses of the LL were not significantly different ($p>0.05$) in relation to the treatments up to 7 days post mortem. In the 14th day of ageing the muscles of treatments ESHB15 and ESHB0 had significantly ($p<0.05$) lower values of drip losses: 1.08 and 1.22% respectively than those of the NESCC treatment (1.9%) but not significantly different ($p>0.05$) from the muscles of ESHB25 (1.35%) and ESCC (1.53%). However ageing had a significant ($p<0.05$) influence on drip losses with the higher values being reached with the higher values been reached after 7 days for all treatments except the ESHB25 one which had its highest values after 14 days of storage.

Table 1. Water holding capacity, drip and cooking losses of M. *longissimus lumborum* submitted postmortem to different treatments.

Measurement	TREATMENTS									
	ESHB25		ESHB15		ESHB0		ESCC		NESCC	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Drip loss (%)										
2 days p.m.	0.70^{y}	± 0.06	0.60^{y}	± 0.07	0.69^{y}	± 0.07	0.38^{y}	± 0.04	0.79^{y}	± 0.32
7 days p.m.	0.89^{y}	± 0.15	0.85^{xy}	± 0.11	0.98^{xy}	± 0.11	1.05^{x}	± 0.23	1.34^{xy}	± 0.28
14 days p.m.	$1.35^{ab;x}$	± 0.23	$1.08^{b;x}$	± 0.17	$1.22^{b;x}$	± 0.23	$1.53^{ab;x}$	± 0.35	$1.91^{a;x}$	± 0.23
Cooking loss (%)										
2 days p.m.	$26.27^{ab;x}$	± 1.23	$25.03^{b;x}$	± 0.79	$26.19^{ab;x}$	± 0.52	$29.40^{a;\ x}$	± 1.07	$26.90^{ab;x}$	± 1.48
7 days p.m.	28.38^{x}	± 0.54	27.50^{x}	± 1.10	27.91^{x}	± 0.33	28.67^{x}	± 0.98	28.35^{x}	± 0.65
14 days p.m.	27.04^{x}	± 0.97	27.82^{x}	± 0.06	27.80^{x}	± 1.19	28.79^{x}	± 0.89	27.63^{x}	± 0.88
WHC(eA/iA)**										
24 hours p.m.	$0.47^{b;x}$	± 0.02	$0.47^{b;x}$	± 0.02	$0.50^{ab;x}$	± 0.01	$0.51^{ab;x}$	± 0.02	$0.52^{a;x}$	± 0.02
7 days p.m.	$0.43^{c;x}$	± 0.02	$0.44^{bc;y}$	± 0.02	$0.51^{a;x}$	± 0.02	$0.46^{abc;y}$	± 0.01	$0.48^{ab;xy}$	± 0.02
14 days p.m.	$0.47^{ab;x}$	± 0.01	$0.40^{c;z}$	± 0.01	$0.48^{a;x}$	± 0.03	$0.43^{bc;y}$	± 0.01	$0.44^{abc;y}$	± 0.02

*SEM=Standard Error of Mean, n=8 replications for treatments; Same superscript letters in the same row or column among means (^{a,b,c}:rows=treatments; ^{x,y,z}:columns=ageing) indicate no significant difference by Duncan test ($p < 0.05$)

These results indicated that the drip loss is not affected greatly by the exposition of the meat immediately post-mortem to high temperatures (15-25ºC), and nor by the electrical stimulation, contrary to the results of Smith (1985), and agreeing with the results of West (1982) who found that drip loses of non-stimulated vacuum-packed *l. dorsi* stored at 0 ± 2ºC was 2.7% against 3.0 and 3.1% for low and high voltage

electrically stimulated muscles. George et al., (1980) reported drip losses for vacuum packed LL stored at 1ºC for two days p.m. of 2.2% for stimulated muscle, and 2.5% for non-stimulated muscle. These reported values were much larger than those observed in the present study. Butchers et al. (1998) reported drip losses for LL in the range of 0.65 - 1.30% when kept 1ºC for 48 h, showing statistical evidence that the electrical stimulation increased the drip losses, contrary to the results of the present work.

After 2 days of aging significant differences ($p<0.05$) in total cooking losses due to different treatments were found. The muscles from the ESCC presented significantly higher losses (29.40%) than the ones from the ESHB15 (25.03%) treatment but both treatments showed no differences in relation to the other treatments. After 7 and 14 days of storage no significant differences ($p>0.05$) among treatments were found regarding cooking losses, that ranged from 27.50 to 28.79%. These results indicate that the treatments studied have no great effect on cooking loss.

Water holding capacity (WHC) in this work is the ability of meat to retain its own water. Twenty four hours post mortem the LL of the ESHB25 and ESHB15 treatments had the lower means for WHC values (0.47) that were only significantly different ($p<0.05$) from the value determined for the meat of the NESCC treatment (0.52). After 14 days of storage at 0 ± 2ºC the lower means for WHC were of treatments ESHB15, ESCC and NESCC, respectively 0.40, 0.43, 0.44. the storage time reduced significantly ($p<0.05$) the WHC means for the treatments ESHB15, ESCC and NESCC but had no influence on the WHC values for ESHB25 (0.47) and ESHB0 (0.48) treatments.

Meat colour is the primary criterion by which consumers evaluate meat quality and acceptability. Consumers prefer bright-red fresh meat. In Table 2 is presented the means of the L*, a* and b* color parameters of LL measured after boning, at 7 and 14 days post mortem. Immediately after hot boning treatments (ESHB25, ESHB15 and ESHB0) had significantly lower L* values than those measured on ESCC and NESCC muscles. The ESCC L* values were also significantly higher than those of the NESCC muscles. After 7 days of ageing the means of L* values of ESCC muscles had significantly higher (34.77) than the values of the muscles of the other treatments with the exception of the ESHB25 (32.30). The L* values of ESCC treatment also showed the greatest variability as indicated by a standard error of mean (SE) that was more than double the values observed for the other treatments. The means L* value of muscles of ESHB0 treatment were the lowest (28.72) but was not significantly different from the others treatments. After 14 days of ageing the muscles of ESCC treatment presented the highest means L* value (34.38) and the ESHB0 the lowest value (30.85) both not significantly different ($p>0.05$) from the other treatments. Ageing time caused a significant increase in L* values for all treatments except for the ESCC muscles that showed no significant increase during the ageing period. For the LL of the ESHB25 and ESHB0 treatments there were significant increases in L* values from values observed immediately after boning and those measured after 7 days of ageing, but not significant increases from 7 to 14 days of ageing. For the ESHB15 muscles the L* values increased from boning to the 14th day of ageing. The NESCC muscles showed L* values increase from the 7th to the 14th day of ageing.

Table 2. L*, a* and b* values of M. *longissimus lumborum* submitted postmortem to different treatments

Measurement	TREATMENTS									
	ESHB25		ESHB15		ESHB0		ESCC		NESCC	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
L*										
After boning	$24.34^{c;y}$	± 0.50	$25.16^{c;z}$	± 0.67	$23.13^{c;y}$	± 0.36	$31.22^{a;x}$	± 0.84	$28.68^{b;y}$	± 1.46
7 days p.m.	$32.30^{ab;x}$	± 0.74	$30.66^{b;y}$	± 0.66	$28.72^{b;x}$	± 0.85	$34.77^{a;x}$	± 2.09	$30.81^{b;xy}$	± 1.00
14 days p.m.	$33.82^{ab;x}$	± 0.71	$33.14^{ab;x}$	± 0.91	$30.85^{b;x}$	± 1.16	$34.38^{a;x}$	± 0.37	$32.83^{ab;x}$	± 1.26
a*										
After boning	$9.85^{c;y}$	± 0.39	$9.77^{c;y}$	± 0.30	$10.69^{bc;y}$	± 0.52	$11.99^{ab;y}$	± 0.21	$12.21^{a;y}$	± 0.75
7 days p.m.	16.03^{x}	± 0.79	16.22^{x}	± 0.82	15.10^{x}	± 0.55	14.48^{x}	± 0.34	15.46^{x}	± 0.76
14 days p.m.	16.44^{x}	± 0.40	15.99^{x}	± 0.63	15.91^{x}	± 0.72	15.61^{x}	± 1.03	14.97^{x}	± 0.76
b*										
After boning	$-4.40^{b;y}$	± 0.37	$-3.92^{b;y}$	± 0.56	$-4.96^{b;y}$	± 0.47	$1.38^{a;y}$	± 0.94	$0.06^{a;y}$	± 0.38
7 days p.m.	$6.02^{a;x}$	± 0.86	$5.03^{ab;x}$	± 0.67	$3.37^{b;x}$	± 0.71	$6.93^{a;x}$	± 1.32	$5.92^{a;x}$	± 0.92
14 days p.m.	$6.61^{ab;x}$	± 0.34	$5.97^{abc;x}$	± 0.89	$3.85^{c;x}$	± 0.57	$6.94^{a;x}$	± 1.01	$4.29^{bc;x}$	± 1.01

*SEM=Standard Error of Mean, n=8 replications for treatments; Same superscript letters in the same row or column among means (^{a,b,c}:rows=treatments; ^{x,y,z}:columns=ageing) indicate no significant difference by Duncan test ($p < 0.05$)

In relation to a* values only immediately after boning significant differences were found among the treatments. The LL of ESCC treatment had a significantly higher a* value (11.99) than hot boned treatments except the ones from the ESHB0 (10.69).

In relation to the b* values, immediately after boning they were negative for the hot boning treatments, in the blue region and significantly different than the positive values situated in the yellow region observed for the ESCC and NESCC treatments. When using hot boning, the oxygen consumption rate by mitochondria is very rapid and reduced myoglobin (purple colour) is more intense (Renerre a& Bonhomme, 1991). After 7 days of ageing, the lowest value of b* was observed for muscles from the ESHB0 (3.37) treatment which was not significantly different from the ESHB15 (5.03) treatment. After 14 days of ageing the lowest mean value was still observed for the ESHB0 treatment, significantly lower than the value observed for the muscles of the ESCC treatment.

Immediately after boning, the hot boned cuts presented a darker red-bluish color. Tang & Henrickson (1980) demonstrated that electrically stimulated carcasses presented greater oximioglobina percentage, the reason for having a lighter color. Page et al., (2001) established that parameters L *, a* and the b* are inversely correlated to pH. This explains the darker color of the muscles not electrically stimulated (pH_{24}=5.91) in relation to the stimulated ones (pH_{24}=5.48) (Cardoso et al, 2002). In accordance with Page et al., (2001) a muscle with pH_{24}=5,91 would be inside the category of "dark cutting meat", as these authors established as limit for maximum normal meat pH of 5.87. The observed values of L* in this study were compatible to those measured in *longissimus dorsi* with minimum degree of maturity of the skeleton, in accordance with the classification of the USDA given by Wulf & Wise (1999) that would be of 33.70. The same applies in relation to the values of a* and b* given by the authors of 16.6 and 5.3 respectively. The values of a* in this work were in the range of 14.97-16.44 while those of b* were between 3.85-6.61.

Conclusions

1. Up to the 7th day of storage at 0 ± 2ºC the treatments studied showed no significant effect on drip losses. After 14 days of storage, non-stimulated conventionally chilled LL presented highest drip loss not significantly different ($p>0.05$) from the stimulated control.
2. The treatments did not affect cooking losses after 7 and 14 days of storage.
3. The range of WHC values observed for the different treatments at different storage times was small between 0.40-0.52 in spite of statistical differences being observed between treatments.
4. L*, a* and b* values showed that, immediately after boning, cold-boned cuts were significantly lighter and between cold boned cuts the electrically stimulated ones were lighter. Up to 7 days of storage, storage time also turned the hot boned cuts lighter.

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FACTORS AFFECTING ACCEPTABILITY OF DRY-CURED HAM THROUGHOUT EXTENDED RIPENING UNDER "BODEGA" CONDITIONS

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Background

A great number of studies have been devoted to elucidate the relationship between several chemical parameters and different sensory traits in Iberian and other types of dry-cured hams. All these studies indicate these changes contribute an improvement in the quality of the cured ham. However, when these changes are very intense a decrease of the quality is observed, since they are responsible for the appearance of defective textures (soft and pasty to the touch, and exhibits extraneous aroma and flavour). Several studies have suggested that uncontrolled proteolytic activity results in considerably impaired protein mechanical properties.
Little research has been dedicated to determine the relationship among the instrumental measurements of texture and colour, biochemical processes, sensory parameters and acceptability in quality dry-cured hams of white breeds.

Objectives

The aim of this work was to study the evolution of chemical, color, and texture parameters to investigate their influence on different sensory characteristics, and to establish which of these sensory traits show a greater effect on the overall quality of dry-cured ham from white breeds. These would be useful for ham producers, since they would allow controlling those features directly related to the acceptability of the ham.

Materials and methods

Samples were 28 dry-cured hams (9-10 kg) which were obtained from pig breeds accepted by Denomination of Origin Teruel. All hams, used in this work, had been cured for 1 year and were purchased from various retail markets. The samples were stored at 18ºC and 75% relative humidity conditions from 12 months to 26 months. The central part of the ham was used for the analysis. The *pH* of dry-cured ham samples was measured using a micro pH meter model 2001 (Crison Instruments, Barcelona, Spain). *Lipid oxidation* was measured by the 2-thiobarbituric acid (TBA) method of Pfalzgraf (1995). TBARS values were expressed as mg malonaldehyde/kg sample. *The extraction of lipids* was made by the method of Folch et al. (1957). *Acidity* was made by the determination of percentage of oleic acid which was carried out by the method recommended by International Union of Pure and Applied Chemistry. Standard Methods for the Analysis of Oils, Fats and Soaps (1964). *Non protein nitrogen* was carried out by the method recommended by Norma ISO/R 937. The supernatant were analysed for nitrogen content following the AOAC (1984) Kjeldahl method. Nitrogen content in the supernatant, referred to 100 g meat, yielded total non protein nitrogen (NPN). *TVB content* was determined by steam-distillation according to the reference method (Decision 95/149/CE). Results were expressed as mg TVB-N per 100 g dry-cured ham. *Colour instrumental measurement*: a reflectance spectrophotometer (Minolta CM-2002; Osaka, Japan) was used to measure dry-cured ham colour at the surface of *Biceps femoris* and *Semimembranosus* muscles. The parameters registered were CIE L* (lightness), a* (redness), and b*(yellowness). *Texture analysis* was made in form of texture profile analysis (TPA) (Bourne, 1978; Henry et al., 1971) with a TA-XT2 Texture Analyser (Stable Micro Systems, Godalming, UK). From TPA curves, the following texture parameters were calculated: hardness, springiness, cohesiveness, adhesiveness, chewiness and gumminess. *Sensory Analysis:* **Trained panel:** The ham samples were sliced approximately 1,5 mm thickness. Each sample was presented at room temperature for evaluation. Intensities of aroma, flavour, tastes and texture factors were based on a structured scale (1-9). **Consumers**: Thirty consumers were recruited from students, faculty and staff of Faculty of Veterinary Science. Each Panellist evaluated samples (1.5 mm thickness) at home simulating common serving conditions. The hams were evaluated at 12, 14, 16, 18, 20, 22 and 26 months of storage. The score was based

on an acceptability scale of 9 points, where 1= very disgusting and 9= very pleasant. *Statistical analysis:* the effect of maturation-ripening time was carried out by analysis of variance, using the ANOVA, and Principal component analysis PCA procedure (SPSS, 11.5).

Results and discussion

During extended ripening some changes in *acceptability* were evident (Figure 1). The *consumer panel* did not find significant differences ($p>0.05$) in hams from 12 to 26 months (total manufacturing time), despite the fact that a trend to lower values was apparent from the 18th month. Acceptance scores were above 6. The *trained taste panel* found significant differences ($p<0.05$) after 22 months. A sharp decrease was evident at this point; after which scores were below 5.

All *chemical maturation parameters* increased throughout storage time, except pH (Figure 2). The parameters which showed a major variation were the intramuscular and subcutaneous acidity, NPN and TBA. They indicated an increasing ripeness and oxidation. In the last stage (22-26 months) a larger significant change ($p<0.05$) was observed in NPN and TBA indices, whereas acidity (lipolytic index) changed more rapidly during the first months of storage. These results supported the hypothesis of a large aroma development within the first stage, whereas it became stable at the end of storage.

Figure 3 (a, b) shows the changes in the *instrumental parameters of texture*. There were evident changes in *Semimembranosus* muscle, in particular regarding hardness which reached significant higher values from the 18th month. Adhesiveness decreased, but increased significantly ($p<0.05$) at the end of storage. The same changes were observed in *Biceps femoris* muscle though they were not so remarkable.

Figure 4 shows the results of the *visual and olfactive sensory parameters.* Colour of *Biceps femoris* increased significantly ($p<0.05$). Aroma reached a maximum peak at the 18th month, then decreased probably it due to the evaporation of the volatile aromatic substances. With regard to *texture parameters* (Figure 5) crumbliness showed a significant decrease ($p<0.05$) from the 18 month of storage, whereas pastinesss and adhesiveness strongly increased ($p<0.05$) from the 18-20 months to the end of storage.

The evolution of *flavour* (Figures 6) was very similar to that of aroma; it showed no significant variation ($p>0.05$) after 20 months of storage. *Rancidity* decreased significantly ($p<0.05$) at the end of storage.

Figure 7 is a plot of the principal components analysis loadings for the first two partial least squares of components.The first component was able to predict 54.41 % of the variation of the whole study. Acceptability, crumbliness and cohesiveness reached a high negative loading on component 1, whereas chemical parameters of maturation had a positive loading on component 1. The second component explained only 18.33 % of the variation. Positive loadings had *Biceps femoris* cohesiveness and some of the colour parameters. The negative coefficients on component 2 turned out to be the pastiness, adhesiveness and the rest of the colour parameters.

Following PCA, 12-14-month-ripened hams were in the left quadrant with negative coefficients on the component 1. As storage time and ripeness increased they moved to the left quadrant with positive values in the component 2, and thereafter to the upper-right quadrant. The final trend was towards the lower-right quadrant, with positive a coefficient on the component 1 and a highly negative on the component 2. It must be emphasized that adhesiveness and pastiness were located in this same quadrant.

Conclusions

Results demonstrated that ham acceptability showed no significant differences ($p>0.05$) from 12 to 20 months (total manufacturing time), while it decreased significantly ($p<0.05$) at 22-26 months. Principal component analysis of all data brought about a comprehensive explanation of the biochemical, instrumental and sensory parameters involved in the acceptability decrease. In fact, high pastiness and adhesiveness values, as measured by both sensory and instrumental methods, appeared to be most correlated with decreasing acceptability. Those were the result of an excessive proteolysis, as revealed by biochemical maturation indices.

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Acknowledgements

The authors wish to thank the Fondo Social Europeo, the Consejo Regulador de la Denominación de Origen Jamón de Teruel and the Gobierno de Aragón (grant B063/2002; fellowship of author Cilla, I. grant B072/2001, fellowship of author Martínez, L.) for financial support.

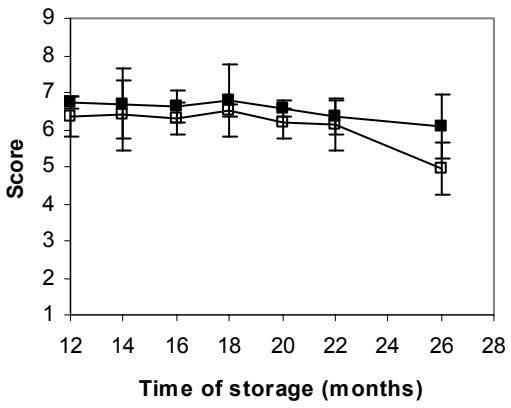

Fig.1. Evolution of the acceptability during time of storage. (■) Consumer's acceptability, (□) Trained Panel's acceptability.

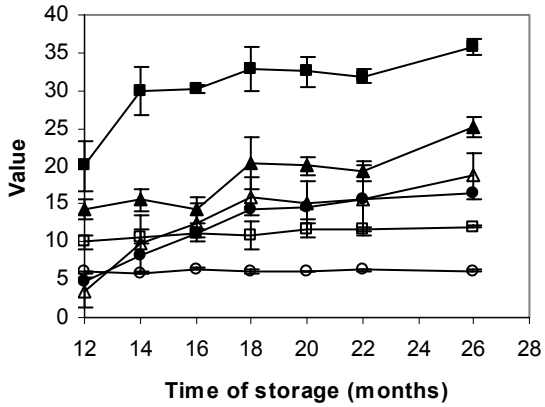

Fig.2. Evolution of chemical parameters during time of storage. (■), TBA x 100 (mg malonaldehído/kg), (□)TVB-N /10 (mg TVB-N/100 g), (▲)NPN/10 (mg NPN/100 g), (Δ) Intramuscular Acidity (% ac. Oleico), (●) Subcutaneous Acidity (% ac. Oleico), (○) pH.

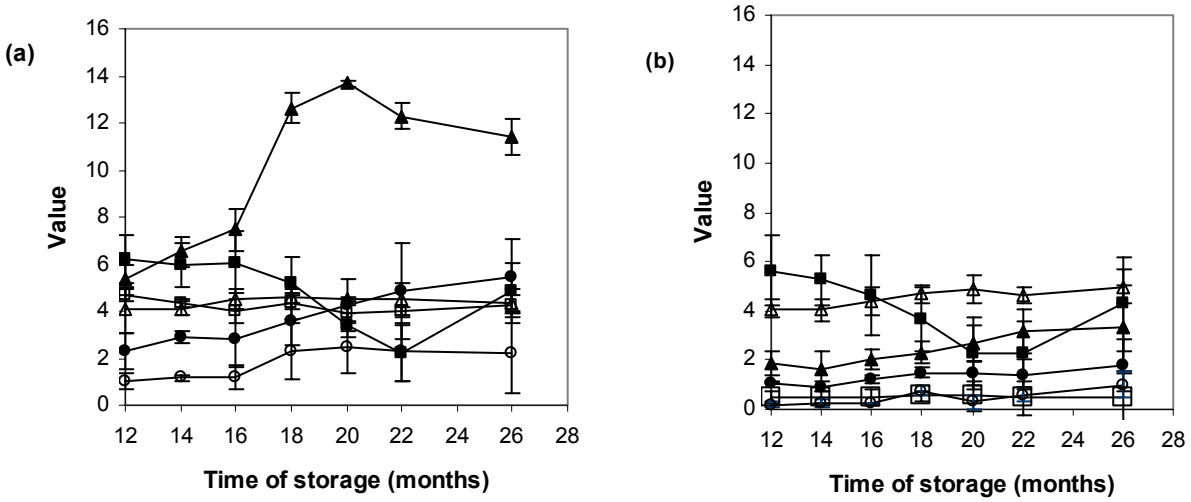

Fig.3. Evolution of instrumental texture parameters during time of storage. (a) *Semimembranosus*, (b) *Biceps femoris*; (■) Adhesiveness/-10 (gs), (□) Cohesiveness x 10 (g), (▲) Hardness/1000 (g), (Δ) Springiness x 10, (●) Gumminess/1000, (○) Chewiness/1000.

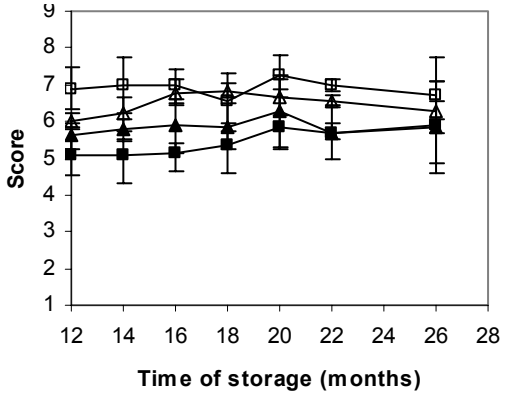

Fig.4. Evolution of sensory analysis parameters of aroma and colour during time of storage. (■)Colour of cured in *Biceps femoris*, (□) Colour of cured in *Semimembranosus*, (▲) Colour homogeneity, (Δ)

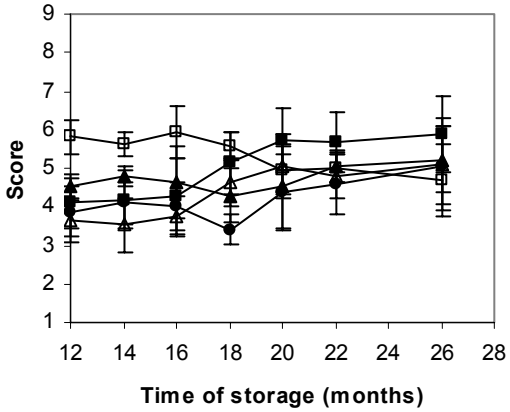

Fig.5. Evolution of sensory analysis parameters of texture during time of storage. (■) Hardeness, (□) Crumbliness, (▲) Pastiness, (Δ) Fibrousness, (●), Adhesiveness.

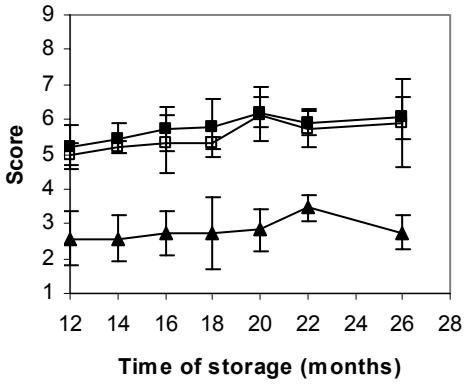

Fig.6. Evolution of sensory analysis parameters of flavour during time of storage. (■) Ham flavour, (□) Saltiness, (▲) Rancidity flavour.

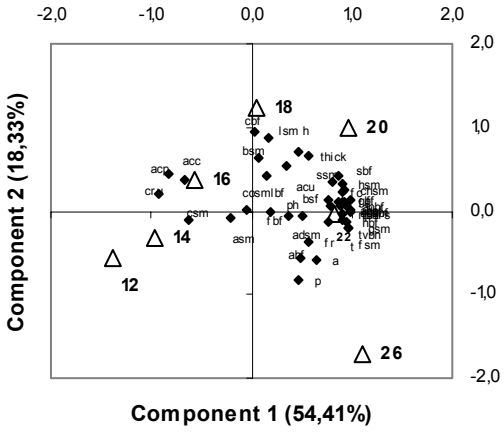

Fig.7. Biplot for the first two principal components. (Δ) centroid for hams at each storage time,(♦)studied parameters

EFFECT OF WALNUT, MICROBIAL TRANSGLUTAMINASE AND STORAGE TIME ON THE WATER AND FAT BINDING CAPACITY OF SALT-FREE BEEF BATTERS

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Background

Consumers are currently more health conscious and as a result demand healthier products. Sodium chloride intake has thus been associated with arterial hypertension and animal fat consumption with cardiovascular disease, one of the main causes of death in industrialised countries. Accordingly, current recommendations aim to reduce daily sodium and saturated fatty acid intake. One way of doing this is to reformulate meat products by reducing the salt content and incorporating fatty acids with proven health benefits.
Different studies have shown that the frequent consumption of walnuts reduces the risk of cardiovascular disease, since it lowers serum cholesterol and favourably modifies the lipoprotein profile (Sabaté, 1993). One way of promoting walnut intake would be to include it as an ingredient in frequently consumed foods, such as meat derivatives, which can thus be made to incorporate health benefits.
In meat products, salt (NaCl) extracts the myofibrillar proteins which are mainly responsible for the heat-induced development of a functional protein matrix by immobilising water (Acton *et al*., 1985). Salt reduction in most products will have an adverse effect on protein extraction, water and fat binding properties and gel strength, and will also alter palatability attributes (Girard *et al*., 1990). In an attempt to reduce the salt level, microbial transglutaminase (MTG) has been increasingly used as a cold meat binder in the absence of salt by combining it with suitable food protein (Kuraishi *et al*., 1997) and crosslinking meat proteins. However, there are hardly any studies on how the storage time in chilling (real commercial conditions) affects the characteristics of raw meat products prepared with MTG as a cold-set binder.

Objectives

The aim of this work, using response surface methodology, was to test how the addition of increasing amounts of walnut (W), microbial transglutaminase/sodium caseinate (MTG/C) and the storage time (ST) (up to 11 days) at 3 °C affect the water and fat binding properties of beef batters formulated in the absence of salt and phosphates.

Materials and methods

Post-rigor beef *semimenbranosus* (top round) was purchased from a local market, ground through a 6 mm plate and divided into 20 (400 g) batches, vacuum packed and stored at -20° C until use. Additives used for preparing meat batters included walnut, supplied by La Morella Nuts, S.A. (Tarragona, Spain), (particle size of 12μ) and microbial transglutaminase/caseinate (ACTIVA EB, Ajinomoto Europe Sales GmbH, Germany) in a formulation containing sodium caseinate 60%, maltodextrin 39.5% and transglutaminase 0.5%. Transglutaminase activity was approximately 34-65 units/g. In all the formulations the meat protein content was adjusted to a constant level (18%) with water, although the total protein content was dependent on the proportion of added walnut.

Beef meat, MTG/C (dissolved in added cold water) and walnut were homogenized in a cutter (Stephan Universal Machine UM5, Stephan U. Stephan U. Söhne GmH & Co., Hameln, Germany) under chilled vacuum conditions. Preparation of the meat batters took 3 min., during which time the final chopping temperature did not exceed 12 °C. Immediately after the homogenate preparation, the batters were stuffed into plastic tubes (diam 3.4 cm) and hermetically sealed. The homogenate samples were then stored in a cold room at 3 °C at various storage times (ST) shown in Table 1.
After the storage time required in a cold room, the meat batters in the plastic tubes were heated (30 min. 70 ° C) in a waterbath. After heating, the containers were opened and left to stand upside down (for 40 min.) to release the exudate. Total loss (TL) was expressed as a % of initial sample weight. Water loss (WL) was

determined as a % weight loss after heating the total loss (TL) for 16 h on a stove at 100 °C. Fat loss (FL) was calculated as the difference between TL and WL. Determinations were carried out in quintuplicate.
Response surface methodology (RSM) was used to study the simultaneous effect of the three experimental variables. Experimental design and statistical analysis were performed using Statgraphics plus 2.1 (STSC Inc., Rockville, MD). Five levels of each variable were chosen following the principles of the central composite design principle (Khuri and Cornell, 1996). The levels of the variables are shown in Table 1.

Results and discussion

Water and fat binding properties
Analysis of variance indicated that among the regression models for the water and fat binding properties of the salt-free beef batters with varying levels of walnut and MTG/C at different storage times, only the model for total loss (TL) was significant (Table 2), while the models for water loss (WL) and fat loss (FL) were not significant. Furthermore, the absence of any interaction effects on total loss indicates that the effect exerted by each variable was not affected by the others. The effect of the variables studied is shown in Fig. 1.
The level of the added walnut has a negative linear effect, the greater the amount of walnut in the meat batters the lower the total loss (TL) values (Fig. 1A, C). This behaviour could be due to the lower water content present in the samples with greater walnut content, since the incorporation of the walnut is at the expense of water, with the concentration of the meat protein remaining constant. Therefore, for the same concentration of meat protein, the samples with greater water content will lose more than the samples with a lower water content and a greater amount of walnut, since the water content of the walnut is very low.
The incorporation of increasing amounts of MTG/C does not seem to affect the amount of TL during heating (Fig. 1A, B). The apparently high results of total loss in this experiment in salt-free beef batters without walnut are agreed with the low ionic strength in the formulation, which reduced myofibrillar protein extraction and therefore increased cooking losses. These results are the same as those obtained by other authors in restructured meat prepared with MTG and without salt (Kerry, 1999). Although some authors suggest that non-meat proteins like caseinate with MTG are necessary to ensure proper binding properties, in the conditions assayed in this study, meat batters were obtained with poor water and fat binding properties. There are apparently contradictory results about the effect of MTG on water and fat binding properties and these differences could be due to the level and different type of MTG added and the conditions in which they are used (time and reaction temperature; presence of other ingredients such a salt, phosphates, and non-meat proteins, species of origin, etc.) (Pietrasik and Li-Chan, 2002). The storage time in chilling did not have any significant effect ($P > 0.05$) on total loss.

Conclusions

The results obtained in this study suggest that the gel/emulsion systems induced during the heating process prepared with MTG/C in the absence of salt and phosphates form structures with poor binding properties. The detrimental effects caused by the absence of salt in beef batters were not overcome by the addition of MTG/C used in this study. However, the presence of walnut in salt-free beef batters made with MTG/C reduces cooking losses, thereby improving water and fat binding properties.

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Acknowledgments
This research was supported under the project AGL2001-2398-CO3-01. Plan Nacional de Investigación Científica, Desarrollo e Innovación Tecnológica (I+D+I) Ministerio de Ciencia y Tecnología. Thanks are due to Morella Nuts, S.A. (Tarragona, Spain) for supplying the walnuts.

Table 1. Levels of variables established according to the central composite rotatable design.

Treatment	Walnut %	MTG %	Storage time (days)
1	12.50	1.00	3
2	3.20	1.00	9
3	15.67	0.65	6
4	12.50	0.30	9
5	12.55	0.30	3
6	7.85	0.65	6
7	0	0.65	6
8	7.85	0.65	6
9	7.85	0	6
10	3.20	0.30	3
11	3.20	1.00	3
12	7.85	0.65	1
13	3.20	0.30	9
14	7.85	1.24	6
15	12.50	1.00	9
16	7.85	0.65	11
17	7.85	0.65	6
18	7.85	0.65	6
19	7.85	0.65	6
20	7.85	0.65	6

Table 2. Analysis of variance of the regression models for water and fat binding properties of salt-free beef batters.

	MODEL[a]	R^{2b}
Water and fat binding properties		
TL	0.023 (*)	77.5 – 57.39
WL	NS	
FL	NS	

[a] * = significant at P<0.05; NS = not significant.
[b] Fitted for degrees of freedom.

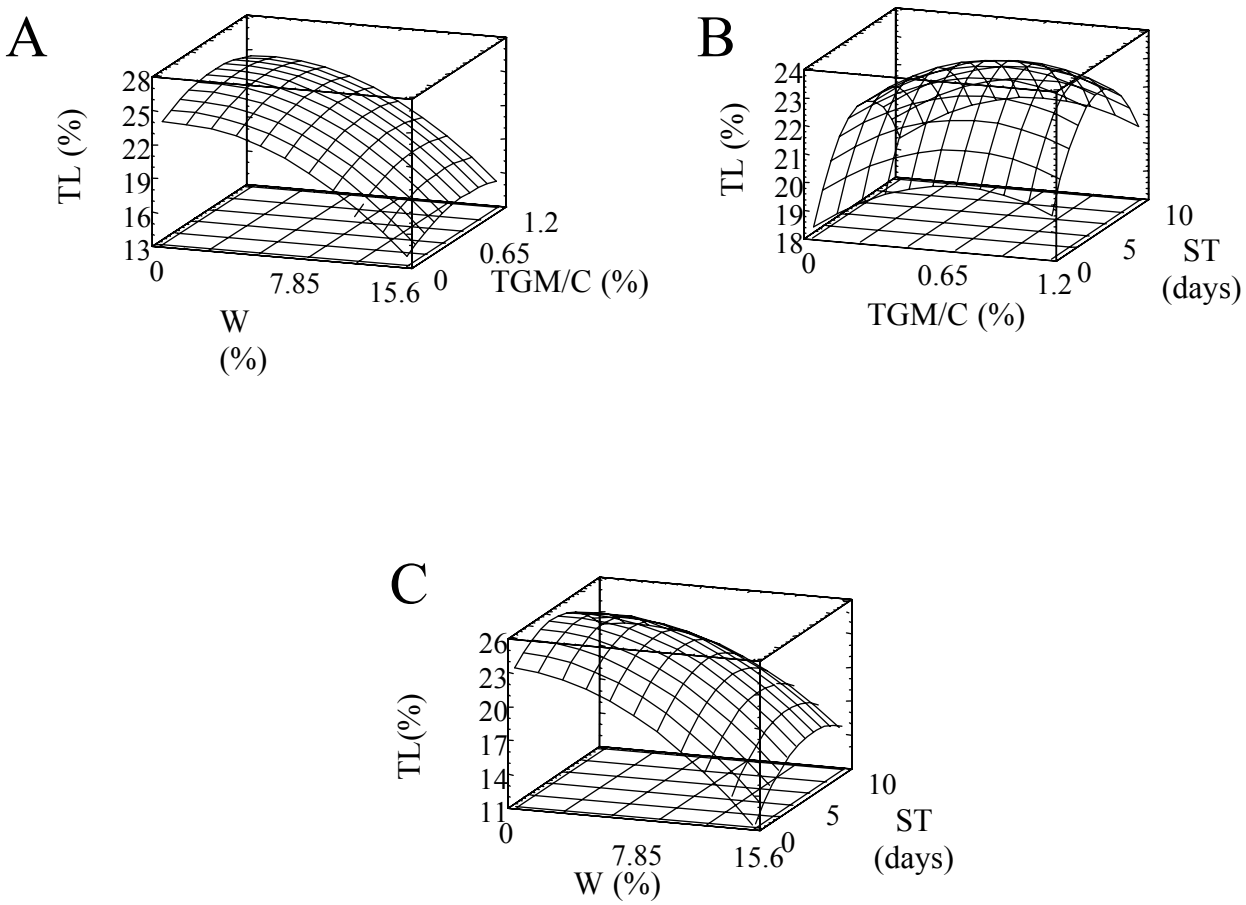

Figure 1. Effect of walnut (W), microbial transglutaminase/sodium caseinate (MTG/C) and storage time (ST) on total loss (TL) of salt-free beef meat batters. (A) W and MTG/C at 6 days (ST); (B) MTG/C and ST at 7.85% W; (C) W and ST at 0.65% MTG/C.

ADDITION OF WHEY PROTEIN CONCENTRATE AND SODIUM CHLORIDE TO BEEF MUSCLES: EFFECTS OF TUMBLING PROCEDURES AND *SOUS VIDE* COOKING TREATMENT ON TECHNOLOGICAL PARAMETERS, PHYSICAL PROPERTIES AND SENSORY QUALITY

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Background

Sous vide processing is the application of a cooking-pasteurization thermal process to food packaged in a hermetically sealed vacuum pouch or tray (Church & Parsons, 1993). Advantages associated with *sous vide* processing include: a superior flavour profile compared to conventionally prepared food, increased tenderness and moisture, improved colour retention and reduced nutritional loss. Furthermore, processing and storage in evacuated pouch increase product shelf life by inhibiting microbiological and chemical spoilage (Vaudagna *et al*., 2002). The *sous vide* cooked beef muscle is an interesting alternative to diversify the marketing of meat, however, this type of products present technological difficulties such as the retention -inside the packaging- of the juice lost during processing (Church & Parsons, 2000). This problem is relevant, particularly regarding commercial profit and preservation of the sensorial and nutritional characteristics of the product. In order to increase water holding capacity (WHC) of meats and consequently reduce cooking weight loss, it has been suggested the use of mild thermal treatments (Vaudagna *et al.*, 2002), the addition of salts, such as sodium chloride (SC) and/or alkaline phosphates (Sofos, 1989), and more recently, the use of natural functional ingredients, i.e. whey protein isolate (WPI) and concentrate (WPC), soy protein isolate, polysaccharide gums, starches, blood plasma, etc. (Chen & Trout, 1991). The higher viscosity of the brines containing those natural ingredients resulted in a non homogenous brine distribution with detrimental consequences for WHC, cooking weight loss and sensory quality. This is particularly relevant in high quality products processed with low injection rates (10 % - 20%) where concentrated brines are used. Consequently, to improve brine distribution, the injection and tumbling procedures become critical. Mechanical treatment of meat tissue by tumbling (massaging) is well recognized and accepted techniques in the meat industry (Xargayo & Lagares, 1992). However, little information is available regarding the effect of extended tumbling procedures on binding and sensory quality of cooked beef pieces (Pietrasik & Shand, 2004), particularly when natural functional ingredients are added to them. The combination of pre- and post- injection tumbling procedures may provide a useful means to improve brine distribution, water binding properties and sensory quality.

Objectives

To evaluate the combined effect of two tumbling procedures (pre- and post- injection step) on technological parameters, physical properties and sensory quality of WPC + SC added beef muscles cooked using the *sous vide* system.

Materials and methods

A 3 x 3 factorial design with three replicates was applied in this experiment. The major variables investigated were tumbling treatments prior to injection step (pre-injection tumbling, PreIT) and after injection step (post-injection tumbling, PostIT), see Table 1. For the experimental design, fifty four *Semitendinosus* muscles were dissected from British breed steer carcasses 48 h post slaughter, trimmed free of fat, vacuum packaged (Cryovac BB4L, Sealed Air Co., Buenos Aires, Argentina), stored for 72 h at 1.5 ± 0.5°C and then processed. The trimmed raw muscles had an average weight of 1579.8 ± 166.1 g, and an average pH of 5.62 ± 0.08. Then, two procedures were carried out, by one hand, the muscles selected for PreIT were weighed, vacuum packaged in proper bags (Cryovac CN510, Sealed Air Co., Buenos Aires,

Argentina) and continually tumbled (2.5 rpm) for 1.5 or 3 h at 1.5 ± 0.5 °C in a Lance Industries tumbler (model LT-15, Allenton, USA) using a drum load of 45 kg (half of working capacity). On the other hand, the muscles non tumbled (NPreIT) were weighed, vacuum packaged in the bags previously described and stored at 1.5 ± 0.5 °C until further injection. Then, the PreIT and NPreIT muscles were weighed and injected using a hand operated brine pump (Dick Lokespritze Esslingen A.NX., Germany). Brine was formulated to give a concentration of 3.5 %WPC (Lacprodan 80, Arla Food Ingredients S.A., Buenos Aires, Argentina) and 0.7% SC (Dos Anclas, Buenos Aires, Argentina) in the injected product, and it was added at a rate of 20% (w/w). After injection, two procedures were applied, by one hand, non tumbled muscles (NPostIT) were vacuum packaged in proper bags (Cryovac CN510, Sealed Air Co., Buenos Aires-Argentina) and then refrigerated overnight at 1.5 ± 0.5°C until *sous vide* cooking was performed. On the other hand, the muscles selected for PostIT were vacuum packaged in the Cryovac CN510 bags, continually tumbled (5 rpm) for 2 or 10 h at 1.5 ±0.5°C in the equipment previously described and stored overnight at 1.5 ± 0.5 °C until *sous vide* cooking was carried out. Then, all muscles were weighed, vacuum packaged into cook-in bags (Cryovac CN510, Sealed Air Co., Buenos Aires, Argentina) and cooked in a water cascading retort (Microflow Barriquand, Roanne, France) in batches of twenty four muscles (the device was operated in static basket mode). Time-temperature evolutions in the slowest heating point (SHP) of three muscles and in the retort chamber were measured using a T type thermocouple and recorded with a digital multimeter Hydra 2625A date logger (John Fluke Mfg. Co., Inc., Everett, USA). In the present study, a processing temperature and time combination of 70°C – 2 min were applied at the muscle SHP. This treatment has been suggested in order to achieve a 6D reduction of *Listeria monocytogenes* (FAIR CT96-1020, 1999). Immediately after the heat treatment, samples were immersed in an ice-water bath until the temperature at SHP reached 10 °C and then were stored at 1.5±0.5 °C for 18 h until further testing.

The technological parameters measured on each muscle were PreIT weight loss percentage (P_1), PostIT weight loss percentage (P_2), cooking-pasteurisation weight loss percentage (P_3) and total weight loss percentage (P_T). Each weigh loss percentage was determined using the relationship $P_i = 100 \times (m_i - m_f)/m_{trm}$, where, for P_1: m_i is the mass of the trimmed raw muscle (non injected) and m_f is the mass of the muscle after PreIT treatment. For P_2, m_i is the mass of the injected muscle and m_f is the mass of the muscle after PostIT treatment, while for P_3, m_i is the mass of muscle after PostIT treatment and m_f is the mass of the muscle after thermal process. Finally, for P_T, m_i is the mass of the trimmed raw muscle (non injected) and m_f is the mass of the muscle after thermal process. All percentages were based on the weight of the trimmed raw muscle (non injected), m_{trm}. The pH values of raw and cooked muscles were measured in duplicate on an homogenate (5 g of sample: 25 ml of distilled water buffered at pH 7) with a pH-meter (Thermo Orion 710A+, Beverly MA, USA) equipped with a combination pH electrode (Thermo Orion Model 8102BN ROSS Electrode). Warner Bratzler shear (WBS) was determined on ten cylinders (3 cm height; 1.27 cm in diameter) obtained from 2.0 cm slice located in the muscle medial portion. For this purpose, Warner–Bratzler meat shear device (Chatllon, New York, USA) with a triangular shear was used. Also, visual appearance of 1.5 cm slice also separated from the muscle medial portion was judged by ten trained panellists using a Veri-Vide CAC120 box (illuminant D_{65}). A seven-point scale was used for cooked beef colour, and a five-point scale for colour uniformity, following the specification of AMSA (1991). The level of defects in the slices was evaluated by a five-point scale of 1=*None*, 5= *Extreme*. The amount of defects (percentage by slice area) was also evaluated using a seven-point scales of 1= *No defect* (0%), 7= *Total* (100%). For statistical analysis, data were analysed as a 3 x 3 factorial design with PreIT and PostIT treatments as main effects (A and B, respectively). All data were analysed by SPSS (Statistical Package for the Social Sciences) 12.0 for Windows (SPSS Inc., Chicago, IL, USA). The Tukey's multiple range test at P=0.05 was used to determine differences between means. Principal component analysis (PCA) was also performed with SPSS software.

Results and discussion

Neither PreIT nor PostIT treatments had significant effect ($P > 0.05$) on the pH of cooked muscles (injected or non injected). However, a significant pH increase was observed ($P<0.05$) for the injected + cooked muscles (pH = 6.11 ± 0.09) in comparison to, raw muscles (pH = 5.61 ± 0.08) and non injected + cooked (pH= 5.87 ± 0.07) ones. An overview of Figure 1 and Table 2, showed that the highest weight losses correspond to cooking-pasteurisation step (P_3). In addition, PostIT weight losses (P_2-Table 2) were higher than the PreIT ones (P_1-figure 1), probably as consequence of the more severe PostIT procedure. In Fig. 1 it is also observed that increasing PreIT times up to 3 h had non significant effect on P_1 parameter ($P > 0.05$),

which values were in the range of 1.0-1.4%. In the present study, PreIT treatments were applied in order to provide more suitable physical conditions for the injection procedure. These treatments were suggested considering both, the high viscosity of the brines used and the higher resistance of beef to brine addition compared to pork and poultry (Pietrasik & Shand, 2004). According to Table 2, P_2, P_3 and P_T parameters were not significantly affected by PreIT times ($P > 0.05$) but they were markedly increased ($P < 0.05$) by PostIT times (Table 2). Then, PostIT time up to 2 h had not effect on them, but the extending of PostIT time to 10 h increased significantly these variables (Table 2). None of the evaluated weight losses was affected by interactive effects between PreIT and PostIT treatments (P=0.1341, P=0.3068 and P=0.4642, for P_2, P_3 and P_T respectively). As it was expected, PostIT weight loss (P_2) corresponding to a tumbling time of 10 h was higher than those one from muscles, either tumbled by 2 h or non tumbled (NPostIT). This result could be due to a larger tissue disruption induced by the extended tumbling time. Also, muscles treated by PostIT of 10 h presented a cooking-pasteurisation weigh loss (P_3) higher than those muscles processed for 2 h or NPostIT. This effect is different from the one observed in muscles added of conventional functional ingredients, in which the extended tumbling has been described as a factor that improves the water binding characteristics and reduces purge and cooking losses. Generally, extended tumbling is required to incorporate brine into the muscle cells and to provide more suitable conditions for protein solubilization and extraction (Xargayo & Lagares, 1992; Pietrasik & Shand, 2004). The described discrepancy can be attributed to differences in formulation (WPC and SC ingredients), in injection levels, in meat composition and structure, and in cooking procedure (heating rate, temperature and time).Even though the injected muscles processed by PreIT and PostIT treatments have P_T in the range of 5 – 10 % (Table 2), these are significantly lower ($P < 0.05$) than those of non injected muscles similarly processed (25-30%, data not shown). It proved that WPC and SC addition successfully reduced weight losses between 2-3 times. As can also be seen in Table 2, neither PreIT nor PostIT treatments had significant effect ($P > 0.05$) on the instrumental tenderness (WBS) of injected + cooked muscles. However, it was detected a trend to increase tenderness in muscles treated by PostIT for 10 h. A correlation between WBS values and a hedonic scale, let us classified injected + cooked muscles as "very tender".

In Figure 2, P_2, P_3 and P_T weight losses, sensory ("cooked beef colour", "colour uniformity", "level of defects") and instrumental (WBS) parameters, and NPostIT and PostIT treatments have been plotted as it was obtained by PCA. The first and second axes (PC1 and PC2) describe 62.3 and 19.8 % of overall variation, respectively. The horizontal axis (PC1) discriminates treatments by the three weight losses (left side) and sensory and instrumental parameters (right side). Even though muscles processed by the PostIT time of 10 h had higher weight losses than those of the muscles submitted to other treatments (NPostIT and PostIT of 2 h), they showed a more uniform *pinkish-gray* colour (3= s*mall amount of variation*) and improved tenderness (lower WBS values). Since the descriptor "level of defects" presented similar loadings for both components (0.65 and 0.66 for PC1 and PC2, respectively), then this sensory parameter would also be related to PC2 axis. Thus, Fig. 2 shows that muscles without PostIT (NPostIT) or processed by a PostIT time of 2 h exhibited a higher "level of defects" than those treated by a PostIT time of 10 h (this descriptor was evaluated as s*mall* and involved 20 to 40% of slices area). Taking into account present results, the PostIT time of 10 h seemed to be the most appropriate treatment to improve quality characteristics.

Conclusions

Contrary to our expectations, tumbling procedure applied prior to the injection one did not affect either technological parameters (tumbling and cooking weight losses) or sensory quality (tenderness and visual appearance) of cooked beef muscles. As well, a post injection tumbling time of 2 h had not influence on these parameters. However, extending post injection tumbling times to 10 h, improved brine distribution and sensory quality (cooked beef colour, colour uniformity, level and amount of defects and tenderness). The drawback was that tumbling and cooking weight losses were increased by this treatment.

Acknowledgements

This work has been funded by Instituto Nacional de Tecnología Agropecuaria (INTA) & Agencia Nacional de Promoción Científica y Tecnológica de Argentina (Subsidio BID 1201/OC-AR PICT 09-08831), and Facultad de Agronomía y Ciencias Agroalimentarias - Universidad de Morón (Subsidio 06A001/02).

TABLE 1: Description of tumbling procedures applied.

PRE-INJECTION TUMBLING (PreIT)	POST-INJECTION TUMBLING (PostIT)
NPreIT (*)	NPostIT (**)
NPreIT (*)	5 rpm – 2 h
NPreIT (*)	5 rpm – 10 h
2.5 rpm – 1.5 h	NPostIT (**)
2.5 rpm – 1.5 h	5 rpm – 2 h
2.5 rpm – 1.5 h	5 rpm – 10 h
2.5 rpm – 3 h	NPostIT (**)
2.5 rpm – 3 h	5 rpm – 2 h
2.5 rpm – 3 h	5 rpm – 10 h

(*) PreIT non carried out. (**) PostIT non carried out.

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Figure 1: Percentage of pre-injection tumbling (PreIT) weight loss at the different PreIT times

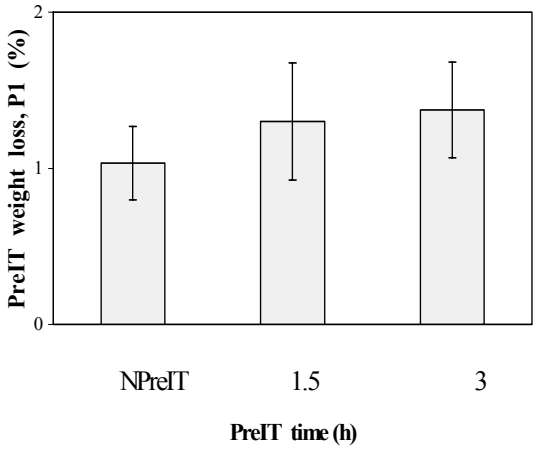
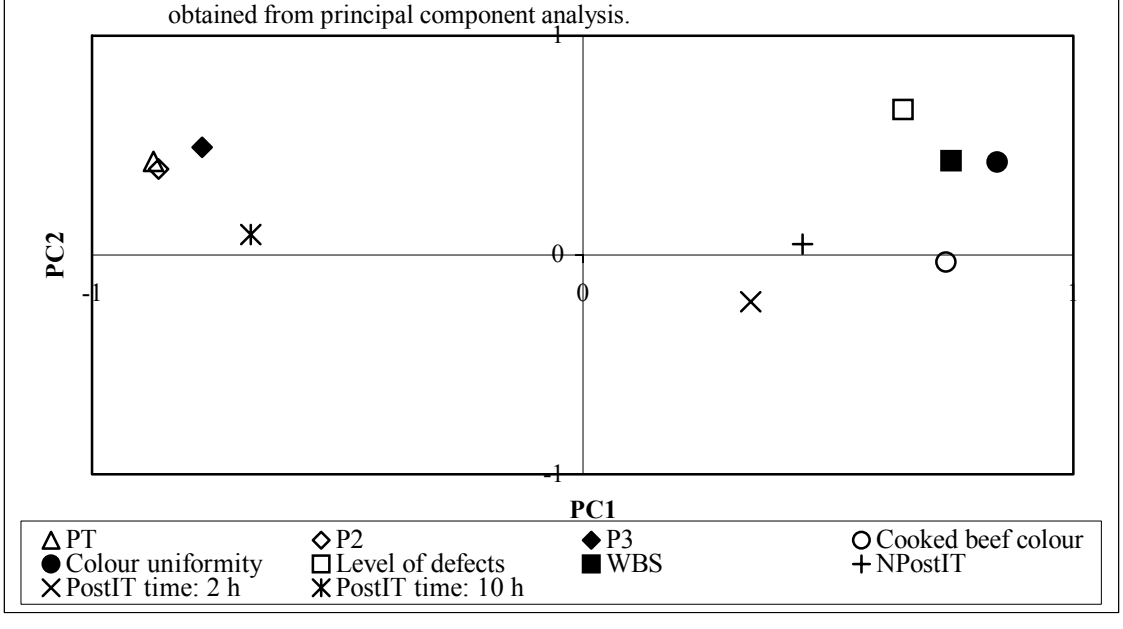


TABLE 2: Technological parameters and Warner Bratzler Shear (WBS) values of muscles processed by the different pre- and post tumbling procedures.

FACTORIAL MAIN EFFECT AND INTERACTION		TECHNOLOGICAL PARAMETERS			WBS
		P_2 (%)	P_3 (%)	P_T (%)	(lb in^{-2})
A: PreIT	NPreIT	4.2 ± 1.0	21.8 ± 1.6	7.3 ± 2.4	5.0 ± 0.2
	2.5 rpm – 1.5 h	3.9 ± 1.0	21.4 ± 1.1	6.7 ± 2.2	4.8 ± 0.1
	2.5 rpm – 3 h	4.2 ± 1.1	21.8 ± 1.8	7.6 ± 2.7	4.8 ± 0.1
	P-value	0.6800	0.6900	0.6609	> 0.05
B: PostIT	NPostIT	3.8 ± 0.8 **b**	21.0 ± 1.0 **b**	6.3 ± 1.3 **b**	4.9 ± 0.1
	5 rpm – 2 h	3.5 ± 0.8 **b**	21.1 ± 1.1 **b**	5.9 ± 1.7 **b**	5.1 ± 0.1
	5 rpm – 10 h	4.9 ± 0.8 **a**	22.9 ± 1.5 **a**	9.4 ± 2.3 **a**	4.7 ± 0.2
	P-value	0.0021	0.0066	0.0020	> 0.05
Interaction A x B	*P-value*	0.1341	0.3068	0.4642	> 0.05

Means with different letters in the same column (within each main effect, A or B) are significantly different ($P < 0.05$)

Figure 2: Loadings of technological, instrumental and sensory parameters and post-injection tumbling (PostIT) treatments on the first two components (PC1 vs PC2) obtained from principal component analysis.



OPTIMISING THE MEASURING OF MEAT FLAVOURS BY MEANS OF AN ELECTRONIC NOSE VIA SENSOR SELECTION

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Background

A reproducible method for detecting flavours is an important precondition for the successful employment of the electronic nose when evaluating the quality of foodstuffs. The results of the executed measuring of meat flavours showed a number of factors which are to be optimised to obtain reliable results when using gas sensor arrays (Rosenbauer *et al*. 1998). The precise knowledge of the sensor selectivity, i.e. what the signals of individual sensors contribute to the differentiation, apart from the factors examined by us, like the reference air temperature and humidity, the mass flow of the carrier gas, the sample volume, the surface, the incubation period and temperature of the sample is essential for the distinctiveness of a sensor system (Dederer and Troeger 2000).

Objectives

Subsequently, the possibility to improve the efficiency of the sensor system VOCmeter (Messrs. Motech in Reutlingen) was demonstrated by selecting the appropriate sensors and by optimising the evaluation of the sensor signals by means of a practical example (comparison of beef samples with different pH values during ageing).

Materials and methods

The employed system works on the principle of mixed sensors: It contains 4 metal oxide (MOX) and 8 quartz microbalance sensors (QMB).
The sample material was beef meat (*M. longissimus dorsi*) with a pH value of 5.5 and 5.9. The samples were stored in multilayer plastic bags at +2 °C for a total of 5 weeks. In each storage week, the samples were measured in a dynamic measuring modus under the following experimental conditions: Sample volume 10 g; thermostatisation of the samples by 35 °C for 30 minutes; carrier gas – purified air with 55 % humidity (related to 30°C); flow rate of the reference air 25 ml/sec; equilibration time of the sensors 120 sec; measuring period 5 min. Parallel to their measuring by means of the electronic nose, the meat samples were evaluated sensorial according to the criteria of flavour and tenderness on the basis of a 6-point scale.

Results and discussion

To establish the sensor rate relevant for differentiating the ageing condition of the beef samples, the sensor signals of the 4 MOX sensors and the 8 QMB sensors were evaluated separately. Particularly those sensors mainly reacting on polar substances and on common hydrocarbons, showed a significant increase in the signal intensity during the meat ageing.

The Figure 1 and 2 show the mean value of the maximum of the sensor reactions for beef samples with different pH-value and storage period. In case of MOX sensors, slight differences were established in the signal intensity between all samples stored for one week and an increasing signal intensity of all sensors after the second storage week. The highest signals were established for the samples with the pH-value 5.9. In this connection, sensor MT-J20 particularly reacting on polar substances and sensor MT-J0 which reacts on common hydrocarbons showed a significant increase of the signal intensity during meat ageing. The sensor indicated by the manufacture selectively for methane and permanent gases, reacted the least on the flavour differences. Only two of the eight QMB sensors reacted on the ageing-related differences of the meat flavour.

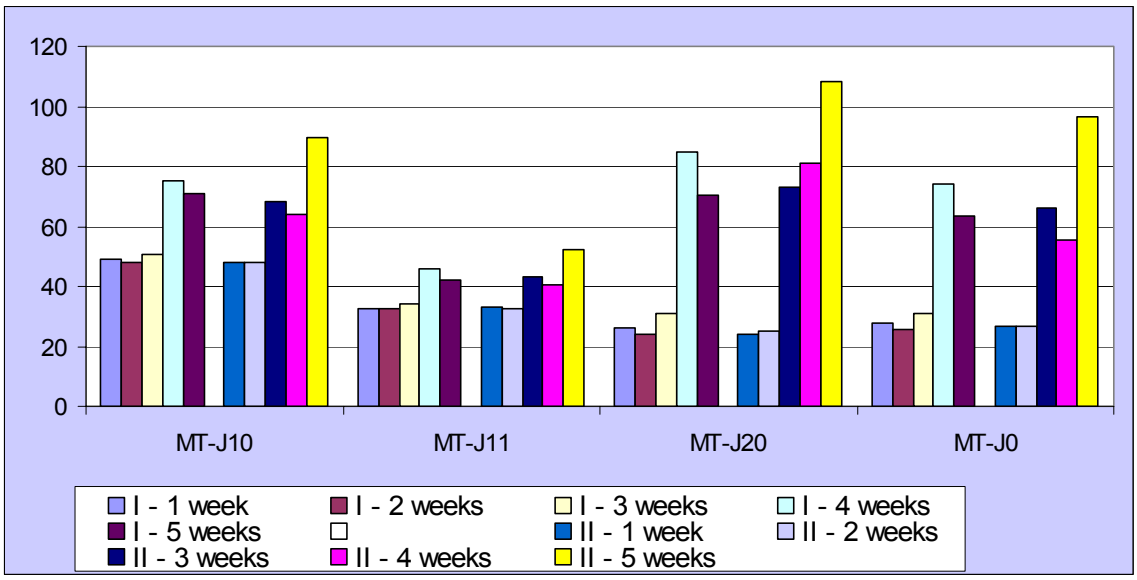

Fig. 1. Mean value of the signal maxima of MOX sensors of the beef samples with the pH-value of 5.5 (I) and of 5.9 (II) during ageing.

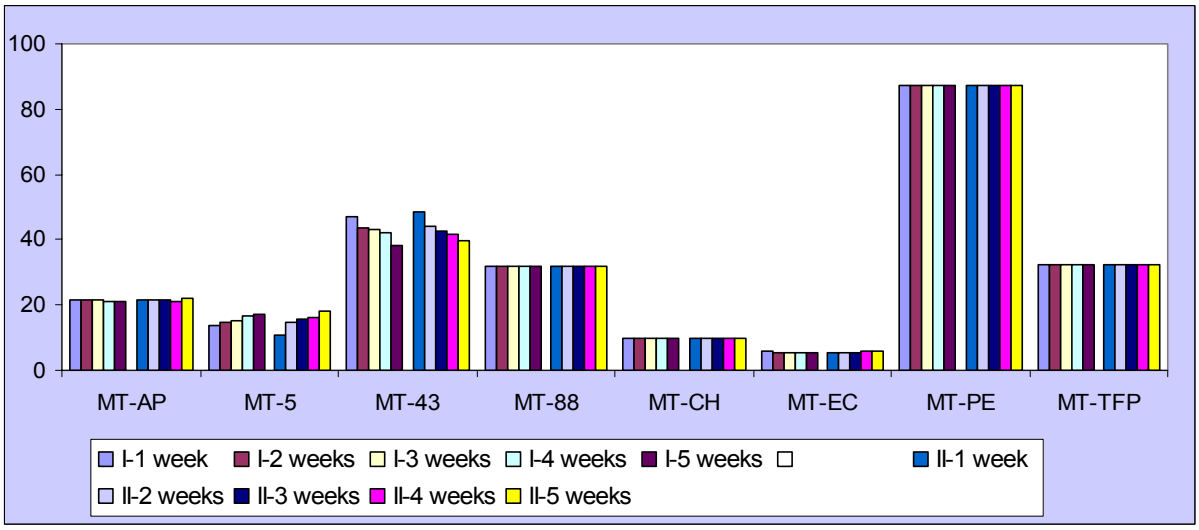

Fig. 2. Mean value of the signal maxima of QMB sensors of the beef samples with the pH-value of 5.5 (I) and of 5.9 (II) during ageing.

A smaller decrease in the signal intensity of storage of 1 to 5 weeks was determined for the sensor MT-43 specified for medium polar hydrocarbons and an increase in the signal intensity was determined for the sensor MT-5 reacting on nonpolar hydrocarbons. On the basis of the signal intensity, those sensors reacting on the differences in the meat flavour were selected and taken up for further evaluation.

In Figure 3 you can see the principal component analysis of the beef samples with different initial pH-values for the ageing process. The evaluation only contains the sensor signals reacting on differences in the meat flavour. Here it proved to show that the meat samples with a different storage period and a different pH-value can be separated by means of their sensor signals.
The differently evaluated samples in the multidimensional space are separated in a way that the differences of the sensorial testing can be found in the distances. You can see smaller distances between the clusters of the samples whose flavour has become a similar evaluation. The “good” and “bad” samples with respect to tenderness and flavour are placed in one area of the illustration. Larger differences of the gas composition

were caused by the ageing of the beef samples with the pH-value of 5.9 compared to the samples with the pH-value of 5.5.

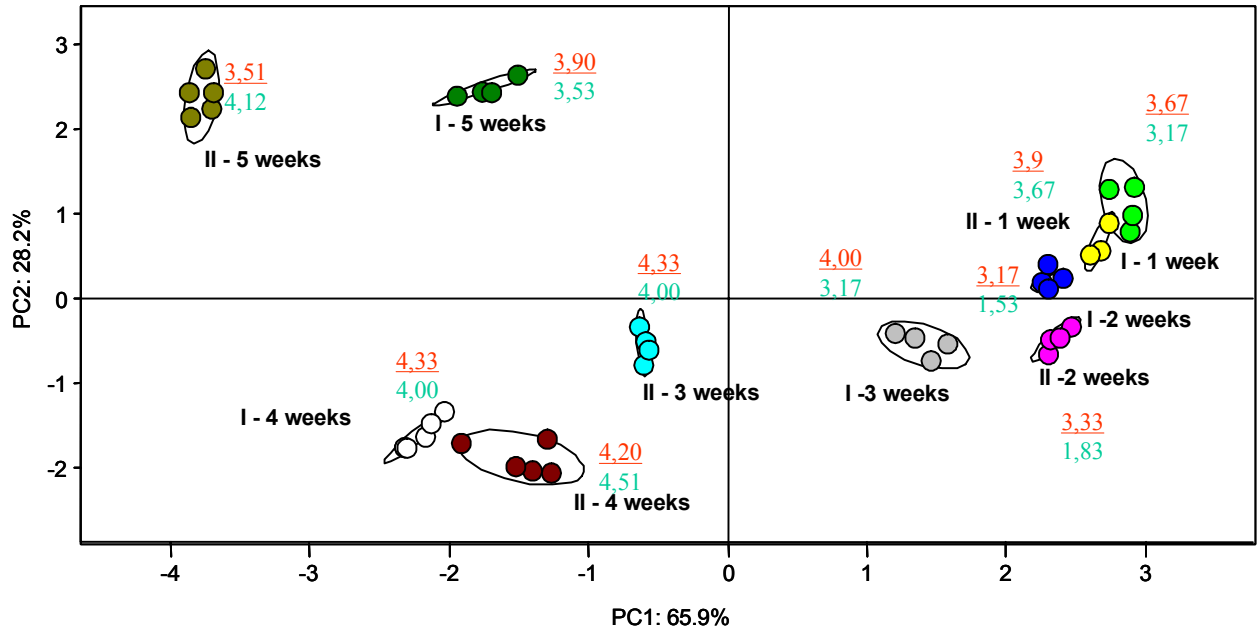

Fig. 3. Principal component analysis of the beef samples with a different pH-value during ageing (I –pH 5.5; II – pH 5.9). 3.67/3.17 – sensorial established values: Flavour/tenderness on the basis of a 6-point scheme.

The principal components analysis showed that the meat samples with a different storage period and a different pH value can be separated by means of their Headspace gas composition.

Conclusions

Due to this information, it would be possible to replace sensors with low distinctiveness by sensors of another selectivity being more relevant for the problem of separation (meat ageing), thus increasing the distinctiveness of the Cassensor-Arrays.

The available results show that the chosen sensor arrangement of 4 MOX sensors and 2 QMB sensors has been able to recognize the ageing condition of beef samples with a different pH-value corresponding to the sensorial evaluation.

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SENSORIAL AND RHEOLOGICAL PROPERTIES OF "PAINHO DE PORTALEGRE". THE INFLUENCE OF MEAT AND FAT SIZE PORTIONS AND NACL CONCENTRATION.

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Background

Traditional products of processed meat products have a positive impact on the sustainability of regional economy. Their consumption, mainly in those using raw materials from Alentejano pig breed, present some variability due to changes occurred on processing practices. Among these, meat and fat mincing size and the level of salt (NaCl) addition could fulfil a great influence on sensory and textural properties of the final product.

Objectives

The purpose of this work is to evaluate the rheological and sensorial properties occurred in "Painho de Portalegre" using low levels of salt in the formulation (0,5% and 1% NaCl) and two different minced meat called small portions and large portions.

Material and methods

"Painho de Portalegre" is a regional and traditional portuguese smoked sausage (cylindrical shape; length 30cm; diameter 25 to 40 mm) made from meat and fat obtained from Alentejano pig, a Portuguese breed.
The small portions refers to parallelepiped portions 1,7 X 1,7cm and large portions to 5,5 X 2,5cm. Two different concentrations of salt, 0,5% and 1% NaCl, were used in the formulation. Three samples of final product were used for each one of the 4 modalities (small portions with 0,5% NaCl, in the formulation; small portions with 1% NaCl, in the formulation; large portions with 0,5% NaCl; large portions with 1% NaCl).
The sensorial evaluation was a descriptive and quantitative analysis, considering a scale from 1 to 100, and the number of panellists was 12. The attributes considered were Colour Intensity, Aroma Intensity, Taste Intensity, Tenderness, Fibrouseness, Juiciness, Undesired Taste, Salt and General Appreciation.
A Texture Profile Analyze (TPA) with a compress platen and a cutting test with a blade knife was performed using a Stable Micro System TA-Hdi.in order to define the texture and compare it with the sensorial evaluation. The samples for the first test were cylindrical with 3,5cm of diameter and 3,5cm of height and were compressed twice to 10% of the initial height. For the cutting test the samples were slices with 4 mm of height and the cut was until reach 87,5% of the sample and the maximum force was measured.
Results were analysed trough an ANOVA-MANOVA considering 2 factors (salt concentration and minced) and their interactions, using the STATISTICA software. A Tukey test for comparation of means was done too.

Results and Discussion

None significant differences were found in the sensorial evaluation results (Table 1). However can be noticed that samples obtained from "Small Portions" exhibit higher values of aroma intensity and fibrouseness lower than those of "Large Portions" (Table 2).
Samples obtained with 1% NaCl exhibit higher values of tenderness (confirmed by rheological results), juiciness and taste intensity. This high classification of the two last attributes is possibly due to the salt effect on the salivation and increasing the flavour of food. Samples with 0,5% NaCl showed higher intensity of colour than those with 1% NaCl
Panellists only distinguish salt intensity for the samples produced with "Small Portions" (56 points for sausages with 0,5% NaCl and 64 points for those with 1% NaCl).
The general appreciation revealed a clear preference by the sausages produced with "Small Portions" and 0,5% NaCl.
Coutron-Gambotti *et al*. (1999) studied the effect of low concentration of salt under lipid composition and the sensorial attributes in dry ham and they observed decreasing of auto oxidation processes and a better aroma and flavour.
The analysis of the rhelogical results revealed that cohesiveness showed significant differences ($p<0,01$) for factor Minced (average value 0,74 for "Small Portions" and 0,68 for "Large Portions"). On the other hand all the other parameters didn't exhibit significant differences. Results of springiness, chewiness and cohesiveness were superior for

"Small Portions" than those of "Large Portions" due to a better binding of the meat and fat portions because after prepared they had higher contact area among them, so the extraction of soluble protein is higher too. This is corroborated by high values of cutting test found in the sausages obtained with "Small Portions" and this parameter was significant for the factor Minced ($p<0,05$).

Conclusions

A general approach of the results of Sensorial Evaluation conclude that sausages obtained through "Small Portions" exhibit a better classification than those obtained from "Large Portions". Panellists also noticed a high concentration of salt in the products from "Small Portions" and more intensity of aroma. Although none of the sensorial parameters showed significant results from Anova.
Products with 1% of NaCl (in the formulation) were classified with higher values of tenderness, juiciness and taste but lower colour intensity when compared with those with 0,5% of salt (in the formulation).
It is possible to produce sausages of high level of quality using the usual technology but with lower concentration of salt.

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Table 1 – Anova analysis for sensorial evaluation considering two factors Minced **(M)** and Na Cl concentration **(S)** and their interaction

	Minced (M)			**NaCl concentration (S)**			**Interaction (M x S)**		
	F	**p**	**Significant level**	**F**	**p**	**Significant level**	**F**	**p**	**Significant level**
Colour Intensity	0,886	0,3605	NS	3,950	0,0643	NS	0,175	0,6812	NS
Aroma Intensity	0,341	0,5675	NS	0,123	0,7307	NS	0,085	0,7741	NS
Tenderness	0,287	0,5996	NS	1,814	0,1968	NS	0,045	0,8356	NS
Fibrouseness	0,675	0,4233	NS	0,102	0,7538	NS	0,224	0,6421	NS
Juiciness	0,548	0,4704	NS	0,985	0,3367	NS	0,096	0,7609	NS
Taste Intensity	0,076	0,7863	NS	1,217	0,2863	NS	0,149	0,7045	NS
Undesired Taste	0,051	0,8243	NS	0,354	0,5602	NS	0,0004	0,9839	NS
Salt Intensity	1,043	0,3223	NS	0,718	0,4092	NS	0,794	0,3861	NS
General Appreciation	0,898	0,3573	NS	0,003	0,9544	NS	0,135	0,7180	NS

Legend: NS – no significant, $p \geq 0,05$

Table 2 – Means and Standard deviation of Sensorial Evaluation considering two factors Minced **(M)**, NaCl concentration **(S)**

Minced	NaCl (%)	Colour Intensity	Aroma Intensity	Tenderness	Fibrouseness	Juiciness	Taste Intensity	Undesired Taste	Salt Intensity	General Appreciation
Small Portions	0,5	75,6 ± 9,317	69,4 ± 12,260	65,0 ± 16,583	31,4 ± 22,109	66,75 ± 9,708	67,6 ± 10,550	5,2 ± 8,438	56,0 ± 8,944	71,2 ± 11,189
	1	61,8 ± 18,199	65,0 ± 8,775	74,6 ± 14,519	29,8 ± 19,880	70,0 ± 10,000	72,2 ± 5,310	8,0 ± 13,038	64,0 ± 11,402	69,0 ± 6,519
Large Portions	0,5	78,6 ± 11,824	63,4 ± 15,550	69,6 ± 12,280	35,0 ± 19,365	68,8 ± 8,786	65,2 ± 5,805	4,0 ± 8,944	55,4 ± 9,788	64,4 ± 9,317
	1	69,6 ± 10,015	63,0 ± 21,679	76,6 ± 11,082	43,2 ± 29,685	75,0 ± 12,329	70,6 ± 9,476	7,0 ± 12,410	55,2 ± 10,849	66,0 ± 16,733

Table 3 – Anova analysis for rheological evaluation considering two factors Minced **(M)** and NaCl concentration **(S)** and their interaction

	Minced (M)			NaCl concentration (S)			Interaction (MxS)		
	F	p	Significant level	F	p	Significant level	F	p	Significant level
Hardness (N)	0,075	0,7867	NS	0,112	0,7413	NS	0,035	0,8541	NS
Cohesiveness	9,368	0,0062	**	0,002	0,9633	NS	0,031	0,8616	NS
Springiness	2,487	0,1305	NS	0,159	0,6941	NS	0,211	0,6512	NS
Gumminess (N)	0,008	0,9276	NS	0,084	0,7744	NS	0,015	0,9023	NS
Chewinness (N)	0,178	0,6776	NS	0,015	0,9048	NS	0,0001	0,9932	NS
Cutting Test (N)	7,131	0,0147	*	0,023	0,8801	NS	0,042	0,8392	NS

Legend: NS – no significant, p≥0,05; * - significant, p<0,05; ** - significant, p<0,01

Table 4–Means and Standard deviation for rheological evaluation considering two factors Minced **(M)**, NaCl concentration **(S)**

Minced	NaCl (%)	Hardness (N)	Cohesiveness	Springiness	Gumminess (N)	Chewinness (N)	Cutting Test (N)
Small Portions	0,5	1005,997 ± 825,559	0,744 ± 0,037 a	0,842 ± 0,068	761,274 ± 650,757	648,664 ± 571,598	2321,676 ± 524,609 a
	1	969,968 ± 345,202	0,740 ± 0,049 a	0,840 ± 0,091	730,524 ± 294,025	628,042 ± 282,617	2375,954 ± 679,956[a]
Large Portions	0,5	1117,761 ± 624,355	0,682 ± 0,040b	0,761 ± 0,096	767,245 ± 420,917	580,193 ± 293,899	3608,670 ± 1729,545b
	1	991,331 ± 471,762	0,684 ± 0,060b	0,795 ± 0,127	690,489 ± 367,084	562,301 ± 337,990	3532,009 ± 1271,392b

Acknowledgements
This research work was supported by the IFADAP under Project AGRO 218.

LIPID OXIDATION DURING REFRIGERATED STORAGE OF LIVER PÂTÉS FROM EXTENSIVELY REARED IBERIAN PIGS AND INTENSIVELY REARED WHITE PIGS

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Background

Iberian pigs have been traditionally reared extensively in evergreen-oak forests named 'dehesas' in which animals are fed on natural resources (grass and acorns). On the other hand, pigs from industrial genotypes are selected for high growth rates and traditionally reared intensively, under controlled conditions and fed with concentrated diets, in order to maximize benefits in the shortest period of time (Sundrum, 2001).
Oxidation of lipids is the major factor reducing quality and acceptability of meat and fat products (Morrissey et al., 1998). Lipid oxidation is a complex process whereby polyunsaturated fatty acids (PUFA) are degraded via formation of free radicals, causing flavour, texture, colour and nutritional deterioration of foodstuffs (Morrissey et al., 1998).
Liver pâté is a traditional product with excellent nutritional and sensory properties (Echarte et al., 2004). Ingredients (mainly liver, fat and meat) are finely minced and cooked during pâté manufacture that could favour the development of lipid oxidation. Thus, liver pâtés exhibit high amounts of fat and iron, and therefore, oxidative deterioration of liver pâtés during refrigeration is expected. The differences between pâtés from Iberian and white pigs in terms of their fatty acid composition and antioxidative status (Estévez et al., 2004) are expected to influence on their oxidative deterioration during refrigerated storage.

Objectives

The aim of the present work was to study the lipid oxidative changes of liver pâtés from Iberian and white pigs during refrigerated storage as assessed by PUFA degradation and generation of thiobarbituric acid reactive substances (TBA-RS) and hexanal.

Material and methods

Animals: Iberian pigs were free-range reared and fed on natural resources (grass and acorns) following the traditional livestock farming for Iberian pigs. The animals were slaughtered at ~150 kg live weight and at the age of 12 months. White pigs (Large White x Landrace) were intensively reared under controlled conditions in a typical industrial livestock farm. The animals were fed on a concentrate feed and slaughtered at 85 kg live weight and at the age of 7 months.
Samples, manufacture of pâté and refrigerated storage: After slaughter, back fat, muscle *Quadriceps femoris*, and liver were removed from the carcasses, vacuum packaged and stored at -80ºC until the day of the manufacture of the pâté. For this, ingredients were as follows per 100g of elaborated product: 28g liver, 40g adipose tissue, 5g muscle, 23g distilled water, 2g sodium caseinate, 2g sodium chloride. Sodium di- and tri-phosphates (0.3%) sodium ascorbate (0.05%) and sodium nitrite (0.03%) (ANVISA, Madrid, Spain) were also added. The protocol followed for the manufacture of liver pâtés was described elsewhere (Estévez et al., 2004). Liver pâtés were packed in glass containers prior to thermal treatment (80ºC/30min.). Liver pâtés were stored in the darkness at 4ºC during 90 days since the day of the manufacture (day 0). Liver pâtés were analysed at days 0, 30, 60 and 90 for PUFA content, TBA-RS and hexanal. After being accomplished each of the refrigeration stages, liver pâtés were stored at -80ºC until analytical experiments.
Chemical Analysis: Moisture, total protein, total fat, and ash were determined using official methods (AOAC, 2000). Fatty acid methyl esters (FAME) were prepared following the method of López-Bote et al. (1997). FAME were analysed using a Hewlett Packard, gas chromatograph, equipped with a flame ionisation detector (FID) as described Estévez et al. (2004). Identification of FAME was based on retention times of reference compounds (Sigma). Proportions of saturated, monounsaturated and polyunsaturated fatty acids (SFA, MUFA and PUFA, respectively) were calculated as percentages of total fatty acids analysed. The quantification of PUFA (sum of C18:2, C18:3 and C20:4) was carried out by using C13 as internal standard. Results are expressed as g PUFA $100g^{-1}$ pâté. TBARS were determined using the method of Rosmini et al.,

(1996). Hexanal was determined in the headspace of liver pâtés using the solid-phase microextraction (SPME) sampling coupled to gas chromatography and mass spectrometry (GC-MS) (Estévez et al., 2003).
Data Analysis: Results of the experiments were used as variables and analysed by using a Student's t-test for independent variables (SPSS, 1997) in order to compare pâtés from Iberian and white pigs. The effect of refrigerated storage on liver pâtés was assessed by using an Analysis of Variance (ANOVA) from SPSS software. Statistical significance was considered as follows: $p>0.05$ (ns), $p<0.05$ (*), $p<0.01$ (**), $p<0.001$ (***).

Results and Discussion

Liver pâtés from Iberian and white pigs showed no differences for moisture, fat, protein or ash contents (Table 1). However, compared to pâtés from white pigs, pâtés from Iberian pigs presented a larger proportion of MUFA and smaller of SFA and PUFA. Liver pâtés reflected the fatty acid composition of the raw material (fat, liver and meat) used for their manufacture (Estévez et al., 2004). Muscles, back-fat and liver tissues from Iberian pigs present large amounts of oleic acid and MUFA as a result of the intake of acorns during the last stage of their fattening period that has been linked to several quality traits (López-Bote et al., 1997).
The amount of PUFA gradually decreased in both types of pâté during refrigerated storage as a likely consequence of the development of oxidative reactions. The decrease rate of PUFA content as measured by the slopes of the calculated trend lines (Figure 1) revealed that the loss of PUFA was more intense in pâtés from white pigs than in the Iberian counterparts. After 90 days of refrigerated storage, pâtés from white pigs lose 2.3 g PUFA/100g pâté as an average while those from Iberian pigs lose a significantly ($p<0.05$) smaller amount (1.5 g PUFA/100g pâté). The oxidative degradation of PUFA led to a gradually increase in the amount of TBA-RS and hexanal between day 0 and day 90 for liver pâtés from Iberian and white pigs (Figure 2). Pâtés from white pigs presented significantly ($p<0.05$) higher TBA-RS numbers and hexanal contents than pâtés from Iberian pigs at day 0 and day 90. These results are consistent with those obtained from the PUFA degradation and agree with those obtained in previous works devoted to the study of the oxidation stability of raw and cooked meats from Iberian and white pigs (Estévez et al., 2003; Estévez et al., 2004) and could be partly explained by the equilibrium between prooxidant and antioxidant factors in the pâtés. Pâtés from white pigs presented a higher proportion of PUFA and lower of MUFA than pâtés from Iberian pigs that makes the former more prone to oxidation than the latter. Moreover, in a previous work (Estévez et al., 2004) we reported a significantly higher amount of vitamin E in the raw material used for the manufacture of pâtés (fat, liver and muscles) from Iberian pigs when compared to those from white pigs. The relationship between the nutritional background (pasture- and mixed diet finishing) and the fatty acid profile and oxidation stability of liver, pork and on them based products is profusely documented (Cava et al., 2000; Nilzén et al., 2001). The intake of pasture by animals increases in their tissues the level of vitamin E, enhancing their oxidation stability (Cava et al., 2000; Nilzén et al., 2001).

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Table 1. Chemical composition and fatty acid profile of liver pâtés from extensively reared Iberian pigs and intensively reared white pigs.

	Iberian	white	P[2]
Moisture[1]	48.42±1.37	50.51±0.62	ns
Protein[1]	10.34±0.24	10.04±0.70	ns
Ash[1]	2.69±0.09	2.78±0.21	ns
Fat[1]	33.37±1.81	31.82±0.57	ns
Fatty acids[3]			
SFA	32.87±0.09	37.98±0.12	***
MUFA	57.52±0.06	47.58±0.10	***
PUFA	9.63±0.11	14.40±0.25	***

[1]g/100g pâté; [2]Statistical significance; [3]Percentage of total methyl esters analysed
SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids
ns: non significant; *: p<0.05; **: p<0.01; ***: p<0.001

Figure 1. Trend lines of the evolution of PUFA amounts (g/100g pâté) during refrigerated storage of liver pâtés from extensively reared Iberian pigs and intensively reared white pigs.

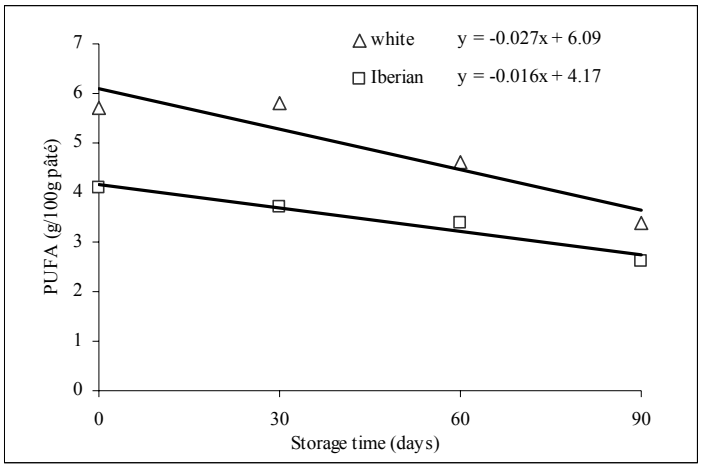
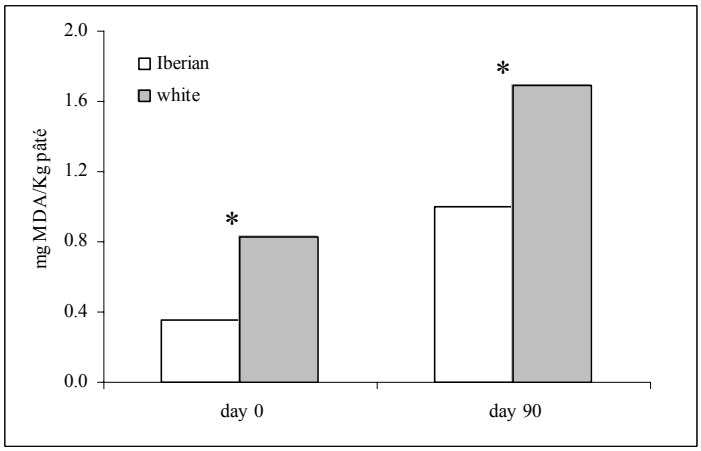
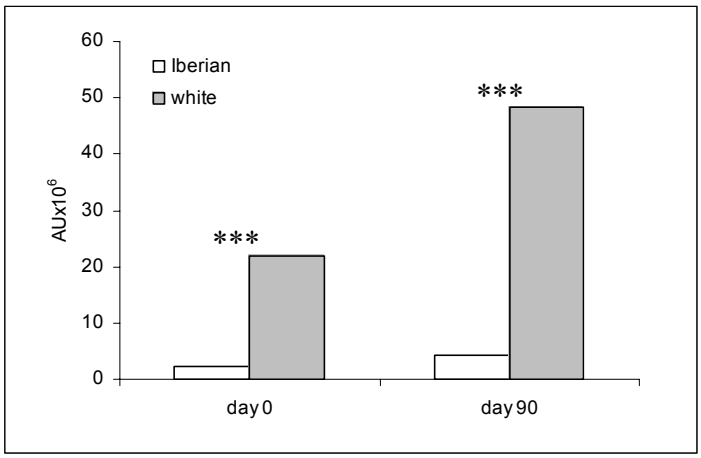


Figure 2. TBA-RS in liver pâtés from extensively reared Iberian pigs and intensively reared white pigs before (day 0) and after 90 days of refrigerated storage (day 90).



ns: non significant; *: p<0.05; **: p<0.01; ***: p<0.001

Figure 3. Hexanal content in liver pâtés from extensively reared Iberian pigs and intensively reared white pigs before (day 0) and after 90 days of refrigerated storage (day 90).



ns: non significant; *: p<0.05; **: p<0.01; ***: p<0.001

EFFECT OF THE ADDITION OF SAGE AND ROSEMARY EXTRACTS ON THE OXIDATIVE STABILITY OF DIFFERENT TYPES OF LIVER PÂTÉS

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Background

Liver pâté is an emulsion-like product that exhibits high contents of fat and iron that would make of it a product with high oxidative instability (Russell et al., 2003; Estévez et al., 2004). The pâté matrix is relatively poor in natural antioxidants, which justifies the addition of exogenous antioxidants (Madhavi et al., 1996) in order to inhibit the development of the aforementioned oxidative reactions. Synthetic phenolic antioxidants, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl, octyl and dodecyl gallates (PG, OG, DG), are easily available and largely used in different food products (Pinho et al., 2000). Using such synthetic compounds has been linked to health risks generally referred to carcinogenic potential (Clayson et al., 1986). Consequently, many scientific efforts have been led to select different natural antioxidant extracts and prove their antioxidant activities as alternatives to synthetic antioxidants. Sage (*Salvia officinalis*) and rosemary (*Rosmarinus officianalis*) are popular *Labiatae* herbs with a verified potent antioxidant activity in meat and fat products (McCarthy et al., 2001; Yu et al., 2002). On the other hand, the compositional characteristics of the raw material influence on the quality characteristics and oxidative stability of the manufacture product (Estévez et al., 2004). Liver pâtés from extensively reared Iberian pigs demonstrated to have a higher oxidative stability than pâtés from intensively reared white pigs as a result of the fatty acid composition and the presence of higher amounts of endogenous antioxidants such as vitamin E (Estévez et al., 2004).

Objectives

The aim of the present work were to investigate the effect of the addition of natural antioxidants (extracts of sage and rosemary) on the oxidative stability of refrigerated stored pâtés from extensively reared Iberian pigs and intensively reared white pigs.

Material and methods

Animals: Iberian pigs were free-range reared and fed on natural resources (grass and acorns) following the traditional livestock farming for Iberian pigs. White pigs (Large White x Landrace) were intensively reared under controlled conditions in a typical industrial livestock farm a fed on a concentrate feed.
Samples, manufacture of pâté and refrigeration: After slaughter, back fat, muscle *quadriceps femoris*, and liver were removed from the carcasses, vacuum packaged and stored at -80ºC until the day of the manufacture of the pâté. For this, ingredients were as follows per 100g of elaborated product: 28g liver, 40g adipose tissue, 5g muscle, 23g distilled water, 2g sodium caseinate, 2g sodium chloride. Sodium di- and tri-phosphates (0.3%) sodium ascorbate (0.05%) and sodium nitrite (0.03%) (ANVISA, Madrid, Spain) were also added. Depending on the origin of the raw material (from 'Iberian' or 'white' pigs) and the addition of different antioxidants ('rosemary' and 'sage' extracts, 0.1%) different types of pâtés were elaborated. A 'control' batch without added extract was also considered. The protocol followed for the manuacture of liver pâtés was described elsewhere (Estévez et al., 2004). Liver pâtés were packed in glass containers prior to thermal treatment (80ºC/30min.). After manufacture, liver pâtés were stored in the darkness at 4ºC during 90 days in order to allow the development of oxidative reactions. Liver pâtés were analysed at days 0 and 90 for the amount of reactive substances to the tiobarbituric acid (TBA-RS), increase of carbonyls from protein oxidation and hexanal.
ChemicalAnalysis: TBA-RS were determined using the method developed by Rosmini et al., (1996) for liver pâtés. Protein oxidation as measured by the total carbonyl content was assessed following the method described by Oliver et al., (1987). Hexanal was determined in the headspace (HS) of liver pâtés using the solid-phase microextraction (SPME) sampling coupled to gas chromatography and mass spectrometry (GC-MS) (Estévez et al., 2003).

Data Analysis: Results from the experiments were used as variables and analysed by using a Student's t-test for dependent variables (SPSS, 1997) in order evaluate significant changes between days 0 and 90. The effect of the addition of the antioxidants was assessed by using an one-way Analysis of Variance (ANOVA) from SPSS software. When statistical differences were detected, data were analysed using Tukey's tests. Statistical significance was set at $p<0.05$.

Results and Discussion

The development of oxidative reactions during refrigerated storage led to a gradual increase in the amount of residual components in liver pâtés such as those generated from lipids and proteins. TBA-RS significantly increased ($p<0.05$) in all groups during refrigerated storage (Figures 1A, 2A). Similarly, the amount of carbonyls from protein oxidation had significantly increased ($p<0.05$) after 90 days of refrigerated storage in control and treated pâtés (Figures 1B, 2B). Due to significant correlations found between some particular lipid-derived volatiles and other lipid oxidation products such as TBA-RS (Shahidi & Pegg, 1994), hexanal has been considered as a fairly good indicator of lipid oxidation. During refrigerated storage, the hexanal significantly ($p<0.05$) increased in the pâtés HS (Figures 3A, 3B), agreeing with the results obtained from the oxidation of lipids and proteins. In general, the addition of sage and rosemary essential oils had a significant effect on the oxidative stability of liver pâtés but this effect was different depending on whether they were added on 'Iberian' or 'white' pâtés. In agreement with previous research on several meats and meat products (McArthy et al., 2001; Yu et al., 2002), the addition of sage and rosemary essential oils had an antioxidant effect on pâtés from Iberian pigs as long as smaller amounts TBA-RS, carbonyls from protein oxidation and hexanal were detected in treated pâtés when compared to the 'control' counterparts. In contrast, the addition of sage and rosemary in pâtés from white pigs had an opposite behaviour, significantly increasing ($p<0.05$) the generation of lipid and protein oxidation products, while no effect was detected for the generation of hexanal. Results from the present work suggest that the activity of sage and rosemary essential oils is dependent on the compositional characteristics of the food matrix. Food systems, and specifically liver pâtés, are very complex in the number and the type of chemicals in the mixture, and a combination of these compounds may behave differently from the individual components. In this sense, Wong et al. (1995) and Fang & Wada (1993) reported possible interactions between phenolic compounds from sage and rosemary essential oils and vitamin E, resulting in different activities depending on the individual amounts of these substances in the food system. Significant differences ($p<0.05$) were found between Iberian and white pigs regarding vitamin E content in muscles (6.18 vs 1.94 mg/kg muscle), livers (7.93 vs 3.49 mg/kg liver) and adipose tissues (19.67 vs 1.21 mg/kg adipose tissue) used for the manufacture of liver pâtés (Estévez et al., 2004) as a likely consequence of the intake of pasture during fattening. The presence of a certain amount of an endogenous antioxidant (vitamin E) in the raw material and manufacture product might influence on the activity of exogenous active extracts, leading to antioxidant or pro-oxidant effects.

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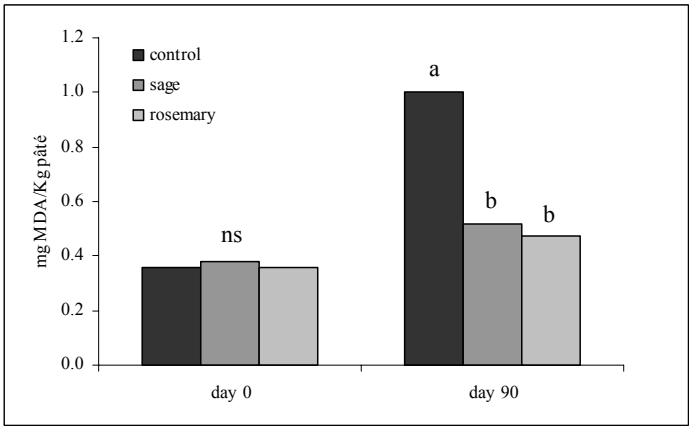
Figure 1. TBA-RS numbers (A), carbonyls from protein oxidation (B) and hexanal content (C) in pâtés from **IBERIAN PIGS** as affected by the addition of sage and rosemary essential oils

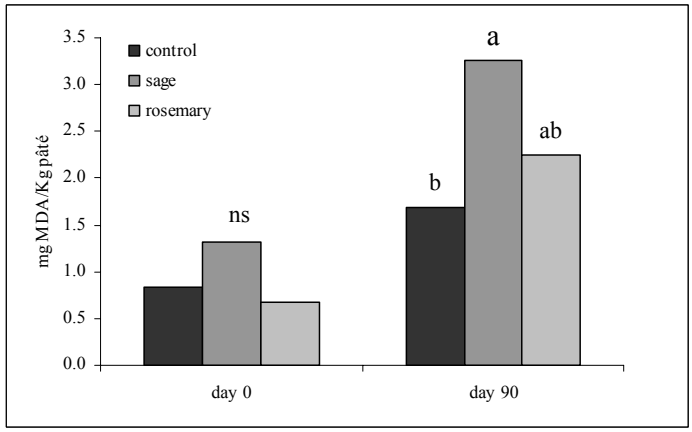
ns: non significant; a,b: different letters denote differences betweeen groups withinh a day

Figure 2. TBA-RS numbers (A), carbonyls from protein oxidation (B) and hexanal content (C) in pâtés from **WHITE PIGS** as affected by the addition of sage and rosemary essential oils

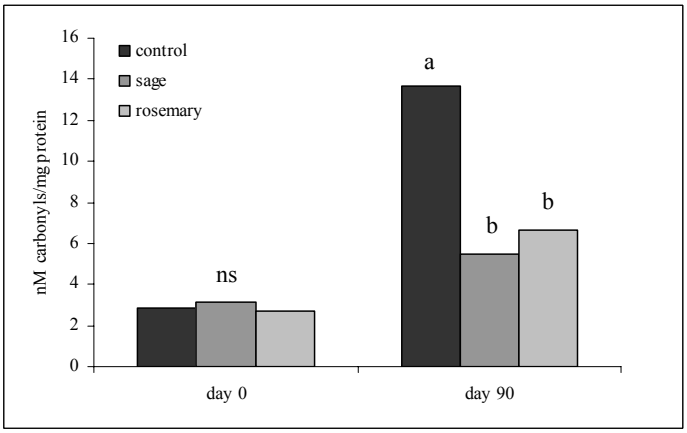
ns: non significant; a,b: different letters denote differences betweeen groups withinh a day

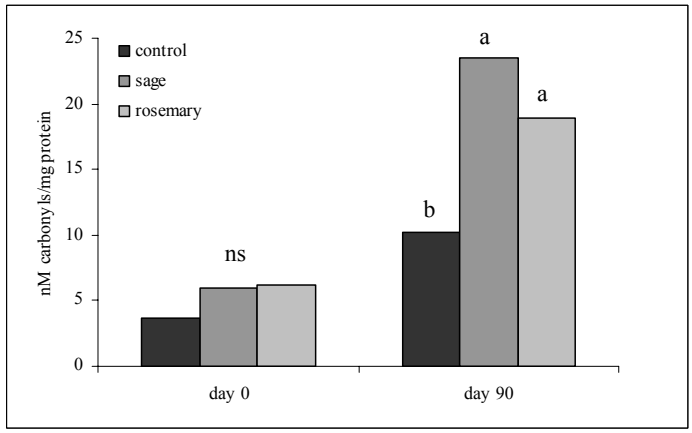
(A) TBA-RS NUMBERS



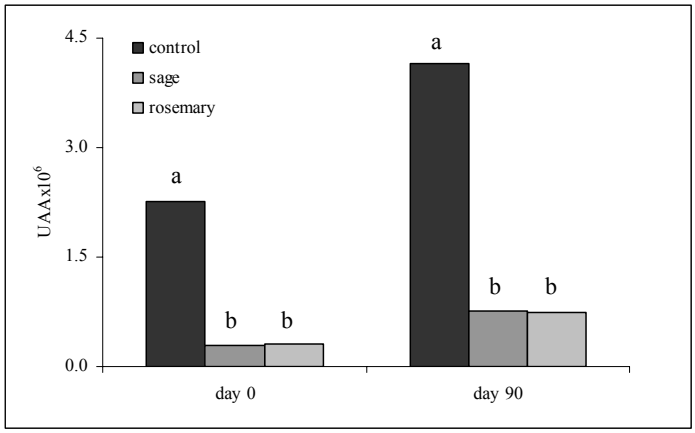


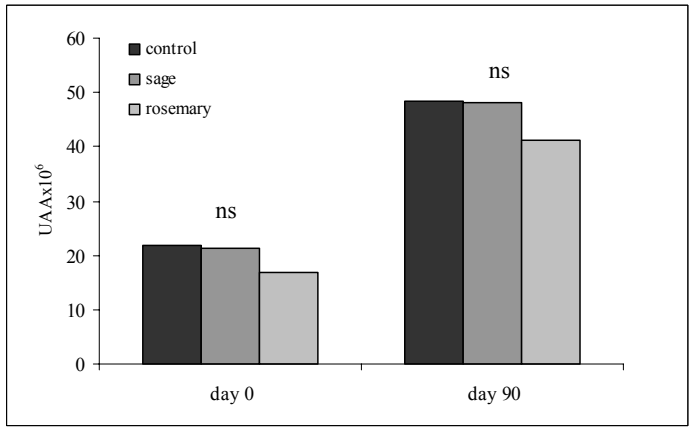
(B) CARBONYLS





(C) HEXANAL





ANALYSIS OF UNIQUE FLAVOR OF CHINESE TRADITIONAL XUANWEI HAM

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Background

The cured ham was initially intended to solve the problem of storing fresh meat. But now with the wide use of the refrigeration, the major attention has been shifted to its unique flavor. A typical flavor can provide a pleasant appreciation and wide acceptance for customers. For example, Iberian Ham has become one of the most famous meat products of high quality in Europe. Xuanwei Ham is a famous special product in Yunnan Province of China. At present, studies on the characteristics of Xuanwei Ham are not enough.

Objectives

This research was conducted to analyze the impact of the climate character in Xuanwei area, raw materials and traditional techniques on the quality of traditional Xuanwei ham.

Materials and Methods

Ham Process: Wujin Pigs were slaughtered when they grew to 8~12 month old, 90~100kg. Hams were made in traditional procedures. Green hams were held for 12~24h at 5~10℃ after slaughter. The hams were thoroughly rubbed with local salt and placed in piles with alternate beds of hams and salted for 30 days at 5~10 ℃ and 65%~85% relative humidity. The salt was added on the proportion of 7% of green legs and was rubbed two or three times at a 7 day interval. The salting started from late October and ended early February in the following year. After washing to remove the excess salt and dirt on the surface of the ham, they were hung with straw strings and dried in the sunlight for 3~4 days. The hams were hung on the strings to ripen for 8~12 months in a ventilated chamber (temperature ranging from 10~20℃ and relative humidity 60%~80%).

Sampling. The *Biceps femoris* were taken from 10 hams of 12-month maturation and the physicochemical and the volatile compounds were analyzed and identified immediately.

Chemical Analyses. Sodium chloride was determined by potentiometric titration with $AgNo_3$ in an autotitrator, and results are given as a percentage of NaCl (w/w). *Water content* was determined after drying of a 3.00 g homogenized sample at 105℃ for 18 h and subsequent cooling in a desiccator. Results are given as a percentage of water (w/w). *Water activity* (a_w) was determined at 25℃ by the graphic interpolation method using saturated salt solutions as standards. *Free amino acids*: Samples of 1 g of ham was homogenized with 25 ml of sulphosalicylic acid (8%) in a 50ml centrifugal tube and the tube was set in ice. The homogenate was centrifuged in a refrigerated centrifuge at 8000 rpm for 13 min. The supernatant was filtered through Whatman No. 54 filter paper. Some amino acids were identified and quantified were measured by L-8500 Amino Acid Analytical Apparatus. *Volatile compounds analysis*: The volatile compounds were extracted by employing a simultaneous distillation-extraction method (SDE), and were separated and identified by Agilent 6890 Series gas chromatographer (GC) and Agilent 5973 Network Mass Selective Detector (MS).

Result and Discussion

1. Physicochemical analysis of traditional Xuanwei ham

The production yield of traditional Xuanwei ham maintained about 69.30%~73.60% (Table 1). The proportion of muscle to fat varied according to the composition of raw hams. The raw hams usually come from pigs with more fat and thus, the finished ham is rich in fat.

The traditional Xuanwei ham is rich in salt, which has an influence on consumers' appreciation and acceptance. Compared with other dry cured ham products, there were almost no differences concerning water content and a_w in the *M. biceps femoris* and subcutaneous fat (Table 2).

The free amino acids in Xuanwei ham are rich (Table 3). The most abundant is Glutamic acid，followed by Alanine and Leucine. The total free amino acids are 9040.45mg·100g^{-1} *M. biceps femoris* dry matter, similar to the results obtained from Iberian ham[1, 2]. Iberian ham was processed with long ripening and drying periods (18~24 months) and Xuanwei ham only had shorter production periods (8 ~ 12 months). This illustrated that Xuanwei ham has characteristics of quicker ripening process, higher activity of proteolytic enzyme, more free amino acids formed than those of Iberian Ham. These characteristics may well be related to the climate character, under which Xuanwei hams were dried and ripened.

2. Separation, identification and content evaluation of volatile compounds in Xuanwei ham

Separating and identifying results of the volatile compounds in the *Biceps femoris* of traditional Xuanwei ham were given in table 4.

Results showed that 75 volatile components were tentatively identified in the volatile fraction, including 15 hydrocarbons, 9 alcohols, 22 aldehydes, 6 ketones, 3 acids, 7 esters and 13 others. In the recent years, studies on volatile aroma compounds about Iberian ham, Serrano ham, Parma ham and Jinhua ham have been carried out by some experts[3, 4, 5, 6]. Some of the volatile compounds separated and identified from traditional Xuanwei ham had some similarities to those results reported of those dry cured ham products, but there were some differences. For example, in our samples, less species of ketones, esters and low molecular weight compounds were identified, but more branched compounds were obtained. Short chain hydrocarbon compounds less than C_{12} were not found. Such differences were correlated with experimental methods, sampling time and materials. Therefore, as regards the typical flavor properties and the exact quantity of the volatile compounds, it is hard to identify them and needs more careful future study.

3. The traditional Xuanwei ham is the work of nature and the special terrain

Xuanwei City is located in the north-east of Yunnan Province in China, east longitude 103º35′ 30″ ～ 104º 40′ 50″, north latitude 25º53′ 30″ ～ 26º44′ 50″. It is located in the transitional draping belt of Yuannan Plateau and Guizhou Plateau, its elevation is 2,868 to 920 meters. The winter begins from November 6th and ends on March 5th of the next year, and the spring begins from March 6th and the autumn ends on November 15th. There is no summer. The main weather reports of Xuanwei town are seen in the table 5. The unique weather creates an appropriate natural climate environment of curing, drying and ripening Xuanwei ham. The hams cured in winter were dried and ripened in spring and autumn. Therefore, Xuanwei City is a natural workshop of dry-cured hams. The natural climate character is the key factor of forming high quality.

4. The special pig specie and feeding way are the quality guarantee of the traditional Xuanwei ham

Wujin pig is a typical local specie. It has higher ability of digesting crude feedstuff. It not only adapts to the climate of high and cold mountain area, but also the warm and hot river valley area. Its body fat is high, it grows slowly, and the quality of muscle is good. In the early growth stage, the greens, straw chaff and rice bran were primary feedstuff. When the weight reaches to 40～50kg, proportion of corn flour and potato were increased in primary feed. The common green stuff are the straw chaff of horsebean, pease, buckwheat and sweet potato, the wild feedstuff are sweet potato vine, potato, carrot, pumpkin, chayote, cress, and so on. According to the feeding way of the local farmers, at the beginning, the piglet is 6.5kg, after feeding for 323 days, its weight can reach to 83.5kg. The daily average weight increase is 239g. It needs about 2.34kg foodstuff and 24.25kg green stuff to increase 1kg.

Wujin pigs' intramuscular fat content is abundant(14.37±3.82%), the flesh is tender, and cooking yield is high (71.64±5.32%). The water content in buttocks muscle is 67.3%. It is an ideal material for producing the high qualtity Xuanwei ham. The aroma of ham made of Wujin pig is good than that of others. The reasons require further investigation.

Conclusion

The ratio of finished product of traditional Xuanwei ham is 69.30%～73.60%, and that the water content, salt content, a_w of *M. Biceps femoris* were 47.30%~50.50%, 9.10%～11.20%, 0.83～0.85 respectively, it has a good storage stability. The ripening ham muscle was rich in free amino acid, the free amino acid content of *M. Biceps femoris* dry matter amounted to 9040.45mg/100g, similar to that of Iberian ham. Because of the different ripening time of two finished products, the ripening process of Xuanwei ham is quicker than that of Iberia. 75 volatile compounds are tentatively identified from the extracts in the *Biceps femoris* muscle. There are 15 hydrocarbons, 9 alcohols, 22 aldehydes, 6 ketones, 3 acids, 7 esters and 13 others. The technology of Xuanwei ham showed that the unique trait of Xuanwei ham resulted from the typical climate character in Xuanwei area, the Wujin pig, and the refined traditional processing technology.

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Table 1 The ratio and composition of end products of traditional Xuanwei ham

Index	End product ratio %	Skin %	Bone %	Lean %	Fat %
Scope	69.30~73.60	7.19~7.61	13.53~13.79	52.59~56.41	22.62~26.50

Table 2. Physicochemical index of traditional Xuanwei ham

Samples	Moisture /%	a_w/ %	NaCl /%
M. Biceps femoris	47.30~50.50	0.83~0.85	9.10~11.20
M. Subcutaneous fat	2.90~3.30	0.75~0.80	0.70~0.85

Table 3. Free amino acid content of the *M. Biceps femoris* dry matter in Xuanwei ham

Amino acids	Thr	Ser	Glu	Gly	Ala	Cys	Val	Met
$Mg \cdot 100g^{-1}$	504. 81	487.13	1274.10	390.84	882.28	0.00	565.98	279.11
Amino acids	Ile	Leu	Try	Phe	Lys	His	Arg	Pro
$Mg \cdot 100g^{-1}$	456.90	869.28	316.56	469.82	1130.67	271.40	630.11	511.51

Table 5. Xuanwei City 2002 monthly average Temperature, Relative Humidity, Rainfall and the Hours of sunlight

Month	1	2	3	4	5	6	7	8	9	10	11	12
Temperature/℃	5.5	9.9	13.0	17.3	17.2	20.2	19.2	17.8	16.4	14.0	10.6	8.0
RH /%	73	65	58	49	71	74	79	80	72	76	76	68
Rainfall /mm	21	10	19	19	141	160	111	224	106	61	9	11
sunlight /h	124	194	228	253	168	177	138	153	173	159	123	177

Table 4. The volatile components of the traditional Xuanwei ham *M. Biceps femoris* (DM)

No.	Volatile components	μg/100g	No.	Volatile components	μg/100g
	Hydrocarbons		39	2-Octenal, (E)-	62.12
1	1,3-Hexadiene, 3-ethyl-2-methyl-	14.17	40	Tetradecanal	40.33
2	(3Z,5E)-1,3,5-Undecatriene	5.45	41	Pentadecanal-	85.01
3	Tetradecane	7.63	42	Hexadecanal	5784.10
4	1-Pentadecene	18.53	43	9,17-Octadecadienal, (Z)-	13.08
5	Pentadecane	51.23	44	13-Tetradecenal	456.67
6	Pentacosane	3.27	45	9-Octadecenal, (Z)-	175.47
7	Pentadecane, 4-methyl-	4.36	46	Octadecanal	567.84
8	Pentadecane, 3-methyl-	5.45		**Ketones**	
9	Hexadecane	11.99	47	Cyclohexanone	7.63
10	Cyclopentadecane	14.17	48	2-Heptanone	35.97
11	1-Octadecene	6.54	49	Ethanone, 1-(1H-pyrrol-2-yl)-	7.63
12	Triacontane	10.90	50	2-Nonanone	18.53
13	1, 13-Tetradecadiene	10.90	51	5-Nonanone, 2,8-dimethyl-	9.81
14	Oxirane, tetradecyl-	10.90	52	2-Pentadecanone	55.58
15	Oxirane, hexadecyl-	152.59		**Acids**	
	Alcohols		53	n-Decanoic acid	9.81
16	1-Pentanol	41.42	54	n-Hexadecanoic acid	47.74
17	2-Furanmethanol	16.35	55	9,12-Octadecadienoic acid (Z,Z)	21.80
18	1-Hexanol	64.30		**Esters**	
19	Cyclohexanol	41.42	56	Formic acid, octyl ester	29.43
20	1-Heptanol	32.70	57	Decanoic acid, methyl ester	5.45
21	Phenylethyl Alcohol	7.63	58	Methyl tetradecanoate	10.90
22	3-Cyclohexene-1-methanol, .alph., .alph.	5.45	59	9-Hexadecenoic acid, methyl ester	10.90
23	E, E-2,13-Octadecadien-1-ol	68.66	60	Hexadecanoic acid, methyl ester	69.75
24	Z, E-3,13-Octadecadien-1-ol	27.25	61	8-Octadecenoic acid, methyl ester	28.34
	Aldehydes		62	9-Octadecenoic acid (Z)-, methyl ester	19.62
25	Hexanal	308.44		**Others**	
26	Furfural	13.08	63	Furan, 2-ethyl-	25.07
27	Propanal, 3-(methylthio)-	56.67	64	Pyrazine, methyl-	3.27
28	Benzaldehyde	41.42	65	Pyrazine, 2,6-dimethyl-	23.98
29	2-Thiophenecarboxaldehyde	19.62	66	Phenol, 4-ethyl-	15.26
30	Octanal	110.08	67	Furan, 2-pentyl-	37.06
31	Benzeneacetaldehyde	304.08	68	Hexanoic acid, anhydride	17.44
32	2-Octenal, (E)-	25.07	69	Phenol, 4-methyl-	17.44
33	Nonanal	152.37	70	Benzene, 1-ethenyl-4-methoxy-	3.27
34	2-Nonenal, (E)-	27.25	71	Naphthalene	5.45
35	2-Decenal, (Z)-	51.23	72	Benzothiazole	2.18
36	Decanal	8.16	73	2(3H)-Furanone, 5-butyldihydro-	8.72
37	Undecanal	5.45	74	Naphthalene, 2-methyl-	2.18
38	2,4-Decadienal	30.52	75	Naphthalene, 2,7-dimethyl-	2.18

CALCIUM CHLORIDE MARINATION OF BOVINE BRACHIOCEPHALICUS MUSCLE: EFFECT OF TUMBLING ON TEXTURAL PROPERTIES

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Background

Meat tenderness is one of the most important attributes for consumers and depends on a great number of intrinsic biological factors, as breed, age, sex, feeding, and kind of muscle, besides the ante- and postmortem conditions (Thompson, 2002). Meat ageing is an alternative to achieve more tenderness and improve the sensory and nutritional quality. Nonetheless, in Mexico and many countries in the world ageing or maturation process is not a common practice, due to higher storage cost and energy required. The tenderness obtained during meat ageing is attributed to the action of two endogenous enzymatic systems, calpains and cathepsins (Jaarseveld et al., 1997). Cathepsins are liberated in lysosomes due to the lipoprotein membrane breakdown caused by the low pH values achieved in the post-mortem muscle. Calpains are located in cells cytoplasm, and they can be activated by the calcium stored in the sarcoplasmic reticule. Many researches have been done to improve the tenderness of meat samples (Morgan et al., 1991; Wheeler et al., 1992; Steen et al., 1997; Pérez-Chabela et al. 1998; Aktas and Kaya, 2001; Berge et al., 2001), but results are limited to certain kind of muscles. On the other hand, marination could be employed to extend the shelf life of meat and increase the value of hard cuts with low commercial value, besides the activation of endogenous proteolytic enzymes, calpains. The most employed marinating method is immersion, with the concomitant impact of the time required (24-48 h) at low temperatures. Tumbling or massaging systems could be employed to reduce marination time since the massaging promotes a faster migration of solution into meat tissues.

Objectives

The objective of this work was to study the effect of calcium chloride marination of *brachiocephalicus* bovine muscle, a relatively tough muscle, in a tumbling system at different rpm, in order to reduce immersion time during meat marination.

Materials and methods

Bovine *brachiocephalicus* muscle (48 h post mortem) was obtained from a local abattoir. Meat was cleaned from visible connective tissue and fat, and cut perpendicularly to muscle fibers and randomly distributed in the different treatments, displayed in Table 1. In order to reduce marination times reported (Koohmaraie et al., 1990; Wheeler et al., 1997; Pérez-Chabela et al., 1998), four different tumbling levels were employed (i.e., 1000, 2000, 3000 or 4000 tumbling/min, obtained in 2, 4, 6 or 8 h, respectively), together with an immersion sample (48 h at 4-6°C), in a 150 mM $CaCl_2$ solution. An untreated sample was used as a control. Vacuum massaging was made employing a SVM-30C machine tumbling (Edel Ingenieros, Monterrey, Mexico), at –25 Hg in. Samples were vacuum packed and analysed 24 h after their respective treatments. Meat texture was determined in a TA-HDi texture analyser (Texture Technologies, Scarsdale, New York/ Stable Micro System, Surrey, England) equipped with a 50 kg load cell and a Warner-Bratzler device. Meat samples were compressed at 2 mm/s constant speed rate and the maximum force detected was reported as Warner-Bratzler shear force. Calcium chloride was determined colorimetrically with the AOAC Official Method No. 983.19 (AOAC, 1999). A total of three replications were processed and analysed. Results obtained were statistically analysed with ANOVA in the SAS Statistical Software v. 8.0 (SAS Institute, Cary, North Carolina). Significant differences between means was determined with Duncan mean test.

Results and discussion

Samples for T1 showed higher calcium chloride concentrations ($P<0.01$) than others (Table 2), probably due to the longer contact with $CaCl_2$ solution (48 h). Final calcium concentration was significantly different ($P<0.01$) between the treatments. Calcium chloride concentration was higher for immersion samples (0.91 mg Ca^{+2}/100 g), as compared to control (0.11 mg Ca^{+2}/100 g) or massaging samples (~0.70 mg Ca^{+2}/100 g in average). Control sample (T0) had the lowest calcium concentration. In the tumbling samples, no significant differences ($P>0.05$) were detected. It means that the calcium chloride absorption is independent of the number of tumbling per minutes, and marination time could be reduced until to 2 h instead the 48 h required in the immersion process. There was a significant difference in the meat tenderness, reported as WB shear forces values, by the treatment employed (Table 2). Calcium chloride had effect on meat tenderness (Morgan et al., 1991; Steen et al., 1997; Wheeler et al., 1997; Pérez-Chabela et al. 1998), activating proteolytic enzymes, calpains, which induce myofibrillar structure breakdown, since control sample was tougher than the marinated samples. In same way, no significant differences were detected ($P>0.05$) among marinated samples, despite the process employed.

Conclusions

Meat tenderness induced by calcium marination can be achieved with short massaging times (2-4 hours), where the calcium concentration achieved by tumbling was enough to promote calpains activation, modifying myofibrillar structure and improving meat tenderness in this relatively tough and low value muscle.

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Table 1. Experimental design of the treatments

Sample	Treatment
T0	Control
T1	Immersion 48 h at 4°C
T2	Tumbling, 1000 rpm
T3	Tumbling, 2000 rpm
T4	Tumbling, 3000 rpm
T5	Tumbling, 4000 rpm

Table 2. Duncan's mean test for shear force and calcium concentration results

Response	T0	T1	T2	T3	T4	T5
$CaCl_2$ concentration (mg Ca/ 100g)	0.12^{d}	0.91^{a}	0.64^{b}	0.74^{b}	0.70^{bc}	0.74^{b}
Warner-Bratzler shear force (kg)	26.45^{a}	14.20^{b}	14.01^{b}	13.67^{b}	11.57^{b}	11.48^{b}

[a, b] Means with same letter in same row are not significantly different (P>0.05)

DISTRIBUTION OF RELATIVE HUMIDITY IN DEPENDENCE OF THE INLET AIRFLOW DIRECTION IN DRY SAUSAGE RIPENING CHAMBERS

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Background

Due to quality and safety issues the ripening phase is the most critical stage in the production process of dry sausage. In contrast to the traditional ways of manufacturing, dry sausages nowadays are ripened and dried in modern multifunctional chambers under defined conditions (temperature, relative humidity, airflow velocity etc.) and a defined period of time, which depends on the product [1]. However, one of the main issues in the design and operation of modern ripening chambers is to achieve a homogeneous drying flow over the chamber length and height and knowledge about it's influence on the ripening process from an engineering point of view. The influence of different airflow directions on the local velocities at the sausages surface already has been discussed [2]. However, the mass transfer also depends on the water vapor partial pressure difference between the sausage surface and the surrounding air and is limited by the effective diffusion from the core to the surface [3]. The difference between the surface activity of water and the relative humidity (RH) of the surrounding air is the driving force of the drying process. To avoid drying errors this difference should not exceed 5 % during the whole process [4, 5, 6].

Objectives

In this project a method for quality maintenance in dry sausage production through the analysis of airflow and water transport in ripening chambers is investigated. Therefore, a method of measuring the relative humidity (RH) during the regular production in ripening chambers was developed. This study discusses the effect of different airflow directions on the distribution of the RH in a newly developed type of ripening chamber. Furthermore, the effect of natural convection on RH was investigated by cyclically interrupting the inlet air flow.

Materials and methods

Ripening chambers

The investigations were conducted in two different types (type 1 and type 2) of ripening chambers. Both chambers contained 60 trolleys (three in a row) loaded with 12 tons of the same sausage in total.

Conventional ripening chambers (type 1) work on a basic principle: Inlet air flow enters the chamber via two separate nozzles batteries, located at both sides of the ceiling. A rotary valve periodically varies the amount and ratio of air exiting the two feeding ducts. The inlet air flows descend vertically along the side walls and are diverted at the chamber bottom into a horizontal flow. Both jets merge over the bottom. The merging point is shifted periodically over chamber width by the variation of the ratio of the air inlet flows between both sides. In the following upward zone, the air stream flows through the sausages towards an exhaust channel located on the ceiling midsection.

In this flow type, dry air gets transported to the lower chamber areas with high velocity. Passing the sausages, the flow decelerates and due to water transfer from the sausages the saturation level of the humidity rises simultaneously. This results in an inhomogeneous drying over the height of the trolleys.

For this reason, a new type of ripening chamber (type 2) was developed (Ness & Co. GmbH, Remshalden, Germany), which uses two additional inlet nozzles batteries installed horizontally (Fig. 1). The airflow here is also distributed periodically between the two feeding ducts by a valve. The jets merge in the headspace and pass the sausages from the top to the bottom of the rack. Afterwards the air stream flows along the floor towards an exhaust duct located at the bottom of the left side of the chamber. Through a periodic change between these two flow types (vertical and horizontal), drying errors can be avoided.

Measurement of relative humidity RH

The RH and the temperature were measured with capacitive RH sensors (Model FH A646, Ahlborn, Holzkirchen, Germany). Eight sensors were fixed on a square steel bar at intervals of 20 cm (Fig. 2). This bar was attached in the middle of a trolley. The data were recorded by a data acquisition unit (Almemo 8990-8, Ahlborn, Holzkirchen, Germany). The trolley with the sensors was placed in different positions inside the chamber, in the case shown here, in the middle of the first row.

Results and discussion

Figure 4 shows a typical trend for RH values over the height of a trolley in chambers with vertical inlet flow. Sensor 1 was below the sausages, sensors 2-7 were arranged over the height, sensor 8 was in the head space. There has been a stratification from the bottom, with prevailing dry air from the climatisation, to the top, where the air has accumulated water from the sausages surface. The cyclical variations in the diagrams are due to the rotary valve in the feeding duct. Corresponding to the valves' position the RH values differ from 6 up to 12 %. This large difference causes an inhomogeneous drying within the trolley (Fig. 3). The sausages on the top of the trolley showed a bright reddish color, which indicates a low drying rate, in the lower positions the sausages were glaring red, caused by a high drying loss.

For the investigation of the relative humidity without forced convection, the fan has been stopped for 15 minutes. In this period the RH values increased asymptotically. RH should not be increased too much, otherwise a micro-climate will develop, where moulds and yeasts can grow on the sausage surfaces. The RH difference between the lower and the upper sensors has been reduced, because the values of the lower positions increased stronger (Fig. 5). After restarting the fan the RH values rearranged themselves quickly. Stopping the fan or reducing the fan speed cyclically can therefore be used in the later ripening phase to achieve a more homogeneous drying of the sausages and to save electrical energy. Figure 6 shows the influence of changing the inlet airflow direction from horizontal to vertical inlet airflow on the distribution of the relative humidity. After changing the inlet airflow direction at a run time of 25 min, the RH values of the lower positions increased while the values of the sensors on the top decreased. The result was a "cross-over" of the charted humidity values. So the change from horizontal to vertical inlet airflow direction had a positive influence of reducing the RH values differences over the trolley height. For this reason the periodic change from horizontal to vertical inlet airflow is an adequate means to achieve a homogeneous drying rate over the height in the whole chamber.

Conclusions

The inlet air flow direction in ripening chambers influences the drying rate, not only by convection but also by the distribution of relative humidity. In chambers with vertical inlet flow (type 1), the RH is stratified with the effect of an inhomogeneous drying over the trolley height. Cyclically interrupting the inlet air flow can be used to achieve a slightly better drying result. In newly developed ripening chambers (type 2) the problem of an inhomogeneous drying by RH layers has demonstrable been improved by a periodical change of the flow direction.

Acknowledgements

This work was supported by the FEI (Forschungskreis der Ernährungsindustrie e.V., Bonn), the AiF and the Ministry of Economics and Labour. Project No.: AiF-FV 12519 N

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Fig. 1: Newly designed ripening chamber with vertical and horizontal inlet airflow

Fig. 2: Arrangement of the RH sensors

Fig. 3: Trolley with inhomogeneous dried sausages

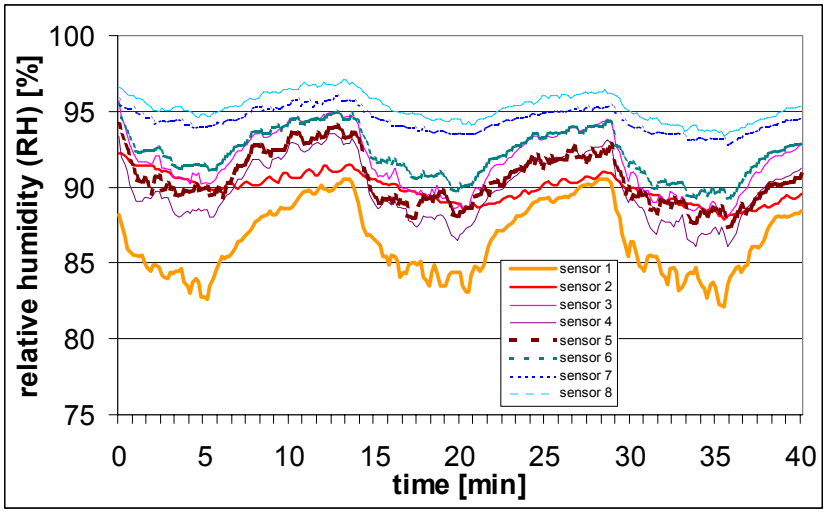

Fig. 4: Distribution of RH in chamber type 1

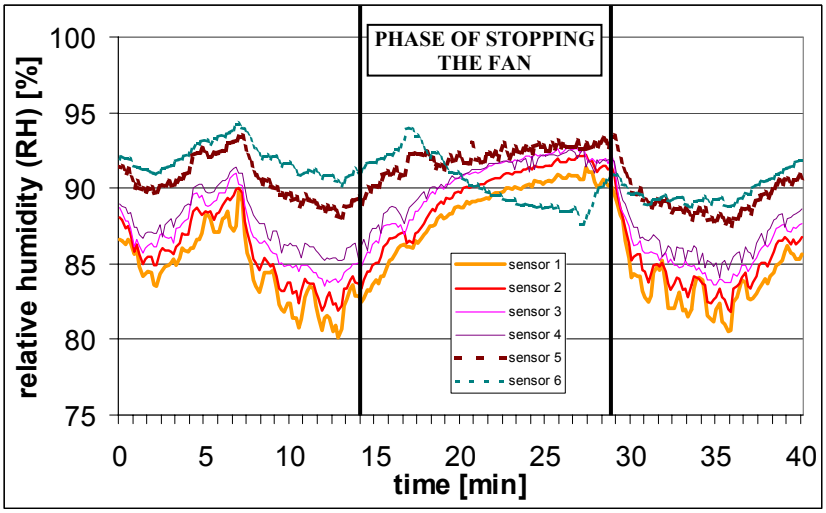

Fig. 5: Distribution of RH in chamber type 1 with stopped fan

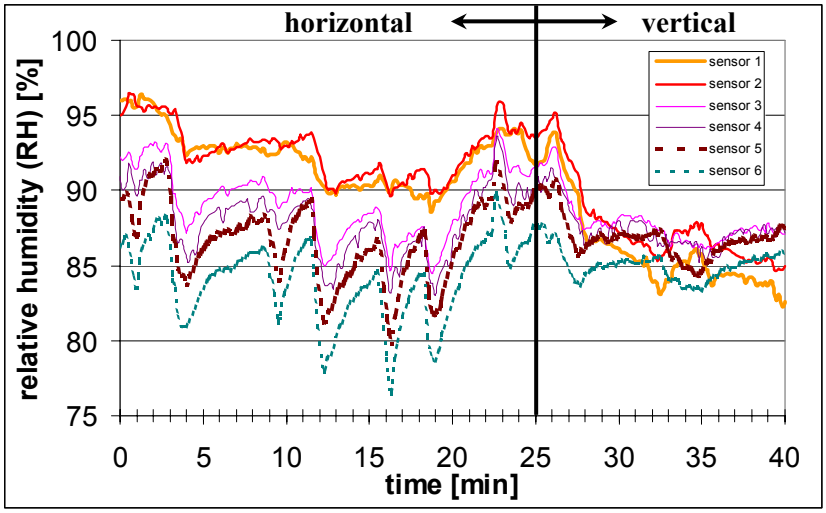

Fig. 6: Distribution of RH in chamber type 2 with change in the airflow direction

EFFECTS OF COOKING TEMPERATURE AND TIME ON GEL FORMATION ABILITY AND COLOR OF SURIMI-LIKE PORK

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Background and objective

Recently there has been considerable interest in manufacturing surimi-like materials (SLM) from the muscle of animal species. The characteristics of SLM from poultry meat, beef, pork, and sheep meat and also from meat by-products, such as beef or pig hearts have been studied (Kang et al., 2003; Lesiów and Xiong, 2003; Park et al., 1996). When the red meat is used to produce SLM, the high fat content of red meat, the more heme pigment and the high concentration of collagen cause several problems. Moreover, gel-forming properties are the most important attribute because surimi is formed and then cooked to make products. Several studies have been made to assess the effect of different temperatures on the gelation properties of SLM. Park et al. (1996) insisted that heating rate, endpoint temperature, protein level and source are important factors in developing gel texture of SLM. However, there is a little information on the gel forming characteristics of surimi-like pork. Understanding the gel forming mechanism of SLM by heating could increase the value of unpopular cuts or by-products of pork, increasing profitability. The objective of this study was to evaluate the effect of various cooking temperatures and times on gel properties of SLM made from pork.

Materials and methods

The *semimembranosus* pork muscle was obtained by hot boning, commercial vinyl pack-packed and stored during 3 days at cold room (2□4℃). After aging, removal of caps, vessels and external fat tissue, the lean muscle was diced into approximately 2 ㎝ cubes, and ground through a 4.7 ㎝ diameter orifice with a Kitchen Aid mince, then frozen at -60℃ until processed. The diced muscle was thawed at 4℃ for 24 hr. The lean muscle was chopped in a silent cutter with five volumes of iced water and the resulting slurry was filtered through a metal sieve of 1 mm mesh to remove connective tissue and filtered through in a metal sieve of 500 one more time. After filtered slurry was centrifuged for 15 min at 2,220 g at 4℃ and the supernatant discarded. The residue was re-washed with five volumes of water. A final wash was done in 2.5 volumes (v/w based on original weight of mince) of cold water. The washing procedure was repeated a third time. The resulting residue was centrifuged for 10 min 2,220 g at 4℃ and the supernatant discarded. For washed pork muscle, wet samples were stand upside down centrifuge bottle (500 ml) at cold temperature room for 10 min then removed free water of excess. Finally, SLM were re-mixed with 3% NaCl w/w, 0.5% tripolyphosphate w/w and 4% sorbitol w/w in a silent cutter for 4 min. The mixed SLM was stored in deep freezer at -60℃ and functional property by various cooking temperature were evaluated after 1 week. Stored SLM were kept overnight at 4℃ then stuff into a 62 mm diameter PVDC (polyvinylidene chloride) casing. SLM was heated for 20 min in a water bath at a constant of 65, 70, 75, 80 and 85℃, and heated at 75℃ for 15, 20, 25 and 30 min. Color (CIE L* and Hue) of cooked SLM was measured using a Minolta Chromameter CR-301 (Minolta Co., Japan) and Gel strength was measured with Sun Rheo Meter (COMPAC-100, Japan).

Results and discussion

The values of lightness, yellowness, chroma and hue were significantly ($p<0.05$) increased, whereas redness was significantly ($p<0.05$) decreased of cooked surimi-like pork by increasing of cooking temperature. pH and moisture % were significantly ($p<0.05$) increased as increasing of cooking temperature over 75℃. Also, hardness, springiness and gel strength were increased linearly as increasing of cooking temperature. Gel properties were evaluated by cooking times at cooking temperature 75℃. There was no significant difference in moisture % of cooked surimi-like pork among cooking time treatments. However, water-soluble protein solubility was decreased as increasing of cooking times. The values of lightness, hue and chroma were significantly increased with increasing cooking times, but lightness value was not changed over cooking of

25 min. Hardness and gel strength values of cooked SLM at cooking time of 15 min were significantly higher compared to cooking times over 20 min. However, panel evaluated the surimi cooked for 15 min as having a poor gel formation ability because it was too sticky.

Sarcoplasmic protein fraction pattern in SDS-PAGE showed that various enzymes were decreased as increasing of cooking times. The A band (phosphorylase) was remained at cooking of 15 min, but it was disappeared over 20 min cooking. The B (about 60 kDa) and D band (about 32 kDa) were remained until 30 min cooking, and they were dim at 35 min. Intensity of C band (about 46 kDa) was decreased at 20 min, and then it was disappeared. These results suggested that enzymes remained in sarcoplasmic could attribute to dark color and undesirable gel formation ability of cooked surimi-like pork. Especially it was assumed that approximately 46 kDa protein might be related to gel formation ability and color of cooked surimi-like pork.

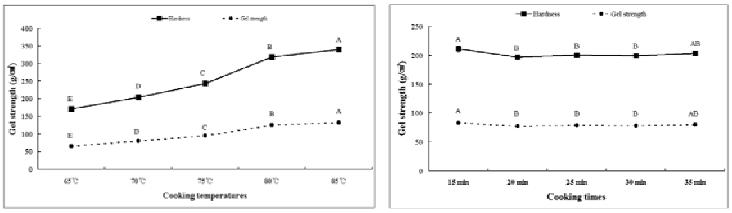

Fig. 1. Effect of cooking temperature and time on gel strength of cooked surimi-like pork.
Mean±S.E. $^{A-E}$Different letters are within a column indicate significant differences between mean values ($p<0.05$).

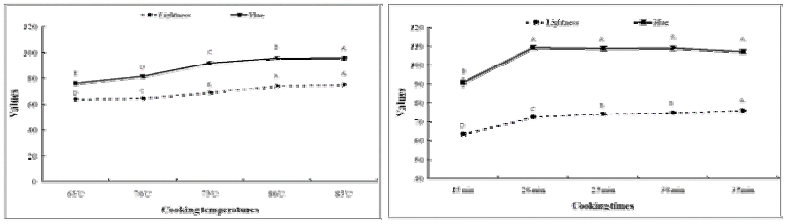

Fig. 2. Effect of cooking temperature and time on color values of cooked surimi-like pork.
Mean±S.E. $^{A-E}$Different letters are within a column indicates significant differences between mean values ($p<0.05$).

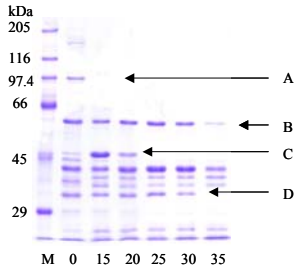

Fig. 3. Changes in protein fraction patterns of surimi-like pork by cooking times at at 75℃.

Conclusions

Gel strength and color of cooked SLM were improved as increasing of cooking temperature. Cooking time of 15 min at 75℃ was not enough for a desirable gel formation and color of SLM. Sarcoplasmic proteins that were not disappeared by cooking might be a reason for undesirable gel formation ability and color of cooked surimi-like pork.

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EFFECTS OF HIGH-PRESSURE TREATMENT ON INTRAMUSCULAR COLLAGEN MOLECULE

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Background

High-pressure treatment is an attractive application for foods, because that make possible non-heat food processing. High-pressure treatment is also one of the new technologies for tenderizing meat or accelerating meat conditioning (SUZUKI *et al*., 1998). Meat tenderness was determined by both actomyosin toughness attributed to myofibrillar proteins and background toughness attributed to connective tissue proteins mainly composed of collagen. It has been well known that high-pressure treatment causes the structural changes in a number of myofibrillar proteins. However, little is known about the effects of pressurization on connective tissue. We reported that intramuscular connective tissue was tenderized by high-pressure treatment and thermal stability of intramuscular collagen was decreased with pressure applied (ICHINOSEKI *et al.*, 2003). We also observed the structural weakening of perimysium and of perimysial-endomysial junction by high-pressure treatment.

Objectives

The purpose of this study was to confirm whether intramuscular collagen molecule could be degraded by high-pressure treatment. In this study, SDS-PAGE and differential scanning calorimetry of pepsin-soluble collagen and determination of collagen-derived peptide were investigated.

Materials and methods

Lean meat was removed from the shoulder of 76-86 months old Holstein cows 1 day after slaughter and stored at –20˚C. As required, it was tempered overnight in a cold room at 4˚C.

1. Isolation of intramuscular connective tissue

Intramuscular connective tissue was isolated by the method of FUJII & MUROTA (1982). After minced, muscle sample was homogenized in 10 mM Tris-maleate buffer, pH 7.2 containing 0.1 M KCl. Fibrous material in suspension was collected by passing the homogenate through a sieve with 1.0-mm pore. The residue was extracted with Hasselbach-Schneider solution and then 0.6 M KI-0.06 M $Na_2S_2O_3$. The insoluble residue containing collagen fibril was washed with distilled water. A part of the isolated connective tissue was lyophilized, and remainder was used for the following analysis.

2. Preparation of pepsin-soluble collagen

The isolated connective tissue was suspended in 0.5 M acetic acid containing 1 mg/ml pepsin and stirred overnight. The supernatant was collected by centrifugation at 100,000 *g* and precipitated by 2 M NaCl. After centrifugation, the pellet was resuspended in 50 mM acetic acid, extensively dialyzed against 5 mM acetic acid and then lyophilized.

3. Pressurization

Intramuscular connective tissue or pepsin-soluble collagen was packed in a polyethylene bag, sealed with cold distilled water and pressurized at 100-500 MPa for 5 min at about 8˚C using an isostatic press apparatus (Nikkiso KK, Tokyo).

4. SDS-PAGE of collagen

SDS-PAGE of collagen was carried out according to the method by HAYASHI & NAGAI (1979). Pepsin-soluble collagen was dissolved to a final concentration of 2 mg/ml in sample buffer (0.01 M Tris-HCl, 3.6 M Urea, 1% SDS, 1% β-mercaptoethanol, 0.01% BPB, pH 6.8). After heating at 100℃ for 3 min, the sample was subjected to SDS-PAGE analysis. Gels were stained with Coomassie brilliant blue R-250.

5. Differential scanning calorimetry (DSC)

Lyophilized pepsin-soluble collagen with or without high-pressure treatment was swollen in distilled water and then was put into the DSC stainless container. DSC was carried out using Setaram micro DSC VII at a heating rate of 0.5˚C/min and temperature range from 0 to 100˚C.

6. Determination of collagen-derived peptides

After pressurized, intramuscular connective tissue was suspended in twice volume of distilled water, and then centrifuged at 10,000 *g*. The supernatant was used for determining hydroxyproline content of collagen-derived peptides obtained by addition of trichloroacetic acid solution (final conc. 5%) (Fig.1). Hydroxyproline concentration was measured according to BERGMAN & LOXLEY (1963).

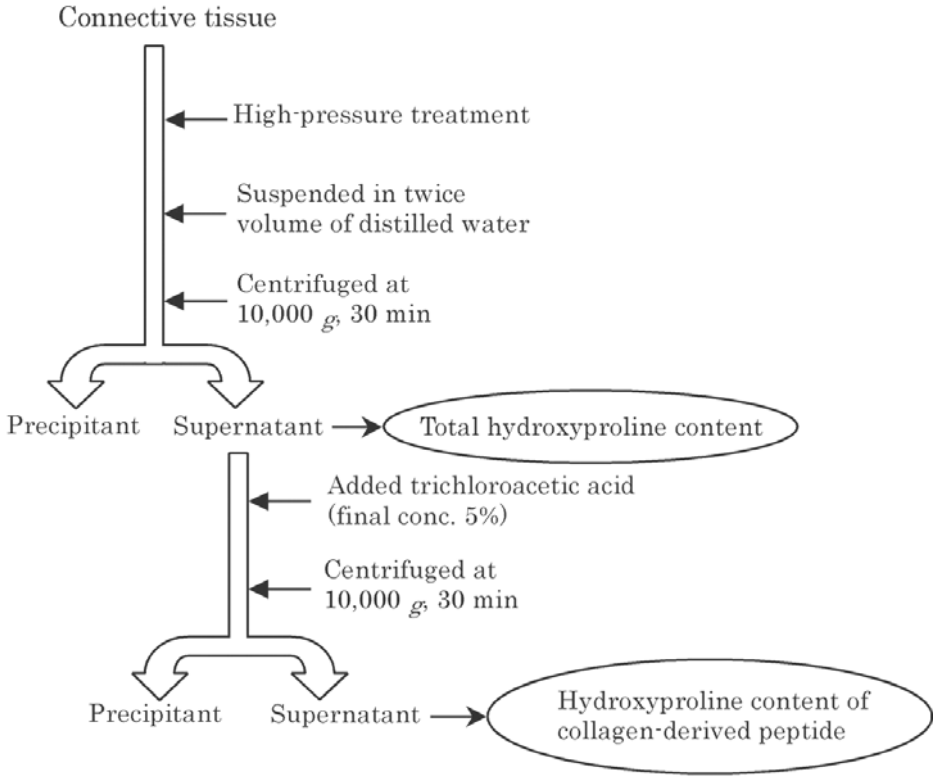
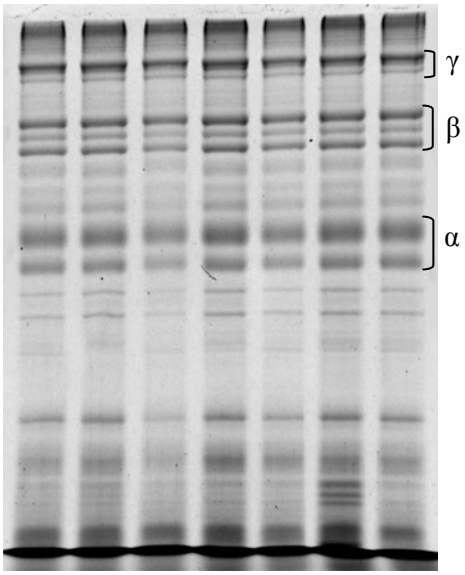


Fig.1 Scheme of determination of collagen-derived peptide.

Results and discussion

SDS-PAGE profile of pressurized pepsin-soluble collagen was shown in Fig.2. It observed that α, β and γ chains of collagen had no significant changes with pressure applied, which suggests that high-pressure treatment could not degrade collagen molecule.



(0.1) (100) (150) (200) (300) (400) (500)

Fig.2. SDS-PAGE profile of pepsin-soluble collagen.
(0.1) untreated, (100) pressurized at 100 MPa, (150) pressurized at 150 MPa, (200) pressurized at 200 MPa, (300) pressurized at 300 MPa, (400) pressurized at 400 MPa, (500) pressurized at 500 MPa.

Denaturation temperature of pepsin-soluble collagen was shown in Table.1. Pressure-induced significant changes in the denaturation temperature suggesting the denaturation of collagen molecule were not observed.

Table.1. Effect of pressurization on denaturation temperature of pepsin-soluble collagen.

Pressure applied (MPa)	0.1	100	300	500
Denaturation temperature (˚C)	42.9	42.7	42.0	42.3

Total hydroxyproline content in the supernatant (See Fig.1) increased with an increasing pressure applied, while hydroxyproline content of peptide fraction did not change. The proportion of collagen-derived peptide in exudation decreased from about 50% at 0.1 MPa (untreated) to about 20% at 400 MPa. These results suggest that high-pressure treatment could not degrade collagen molecular structure but could dissociate collagen super molecular structure, e.g. collagen fibers or fibrils, to collagen molecule.

Conclusions

From these results, it is suggested that the pressurization may cause some changes in the intramuscular collagen. The changes may not be involved in the degradation of collagen molecules but dissociation of collagen fibers or fibrils.

Acknowledgment

This research was supported in part by Grand-in-Aid for Scientific Research (A: 15208025) from Japan Society for the Promotion of Science (JSPS).

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TEXTURE PROPERTIES OF SAUSAGE EMULSIONS ANALYSED BY EXTRUSION METHODS AS PREDICTION INDEX OF SAUSAGE TEXTURE

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Background

In the last few years there has been a strong increase in the request and development of light products, that is, those of low-fat contents. In conventional cooked sausages, animal fats are an essential ingredient easily representing a 20-25% of the global composition. Reduction of the fat level has negative repercussions on the sensory quality of the final product, affecting mainly its texture.
The adequate combination of several compounds such as proteins, starches and hydrocolloids allow achieving a low-fat product with similar characteristics of texture the conventional product has. (Glicksman, 1991). There are a broad group of compounds with high functionality, but it is very important its adequate combination since sausage emulsion is a complex matrix where synergetic actions or complementation of properties of ingredients bring about (Dziezak, 1991; Keeton, 1994, Ordoñez et al., 2001).
In low fat product design, extrapolation of texture properties of final product from texture measurement of emulsion characteristics would be very useful. There are two instrumental methods to study semi-solid foods as emulsions, dough, jellies, etc. Both of them give a measurement of the compression force when product is extruded. Their differences are based on method of extrusion: Direct and back extrusion.

Objectives

The aim of this work was to find an easy instrumental measurement of sausage emulsion characteristics that can predict texture properties of final product, especially to be used in low fat sausages design.

Materials and methods

Emulsion texture analysis: Three different meat emulsions were used to establish analysis conditions. These emulsions differ with respect to protein and fat contents. Emulsion A was made with 13% protein and 13,3% fat, emulsion B 10% protein and 18% fat, emulsion C 8% protein and 13% fat. The rest of ingredients (water, additives, starch, etc) were added in the same amount to the three formulations.
The three experimental emulsions were evaluated by direct extrusion (probe A/BE) and back extrusion (probe HDP/FE5) with a texturometer Stable Micro Systems TA.XT2 (Haslemere, England). Maximum force along extrusion were taken and considered related to viscosity of emulsions. According to SMS Application Studies (1995), the maximum peak force (obtained from the F/t curve) is correlated with the viscosity of the product. Differences between both tests are based on how the product is extruded. Using the A/BE probe, the product is extruded up and around the edge of the disc plunger. However, with HDP/FE5 the product is extruded through a standard size outlet in the base of a sample container. Each data correspond to three measurements on 100 g emulsion at 20ºC in standardised containers. Compression percentages of 10, 15 and 20% and speeds of 0.8, 1 and 1.2 mms^{-1} were tested with both probes to determine the analysis conditions. Final conditions were 20% of compression and cross-head speed of 1 mms^{-1}. (Severiano Pérez, 2002).

Sausage texture analysis: Texture Profile Analysis (TPA) were performed with a probe of 50 mm of diameter using a Texturometer Stable Micro Systems TA.XT2 (Haslemere, England). Analysis conditions were level of compression of 55% with respect to initial height of sample and 1 mms^{-1} cross-head speed. Sausages, vacuum packed, were heated in water at 75ºC for 15 minutes. Samples were obtained by cutting off the ends of sausage, then the remaining sausage were divided into pieces of 10 mm length. Samples were held at 70ºC during instrumental evaluation. An average curve of consecutive measurements on ten portions of each sample were obtained. Texture parameters hardness, chewiness, adhesiveness, springiness and cohesiveness were deduced from the resulting force-time curve (Breene, 1975).

Sensory evaluation of texture was performed by a trained panel, constituted by twelve members. The selection and training of panel was done according to Severiano Pérez (2002). Texture attributes elasticity, hardness, chewiness, cohesiveness, adhesiveness, juiciness and fat perception were evaluated using a nine point scale. Samples were prepared identically than for instrumental analysis.

Experimental sausages: Five sausages were prepared with different fat contents and hydrocolloids added: carrageenan and xanthan gum and Genugel®, type CHP-200 (HERCULES, Copenhaguen, Denmark), a commercial mix of carrageenan and xanthan gum. Table 1 shows the content of these ingredients added with a view to modified emulsion properties and texture of light sausages. In all formulations, sausages were made with pork meat and fat and included 2% salt, 2% soya protein, 1.3% starch, polyphosphates, nitrite, ascorbic acid, flavourings and colouring.

Sausages were manufactured following a conventional process; sausage batter stuffed in 22 mm cellulose casings (Viscofan, Pamplona. Spain) was cooked until 72ºC internal temperature. Sausages were vacuum packed and kept under refrigeration until analysis in the same week they were manufactured. Two batches were made.

Results and discussion

First at all, it was verified there was no significant difference between batches for any parameter analysed. No differences were detected neither in both types of extrusion tests nor in sausage texture parameters.

The effect of reduction of fat content was remarkable on viscoelastic properties; emulsion A presented the highest force value compared with low fat emulsions (B, C, D and E). Therefore, decrease of percentage of fat lead to lower values of extrusion force, and this parameter could be considered an index of emulsion viscosity (Figure 1). The influence of fat level on emulsion viscosity could be determined both by direct extrusion and back extrusion. On the other hand, results of back extrusion show lower force of emulsion B (0.6% commercial mix Genugel®), than the other low fat emulsions. Emulsions C, D and E have very similar viscoelastic properties. Bigger differences were expected in emulsion characteristics according to previous studies (Severiano Pérez, 2002). However, there is enough variability to be able to study the possible correlation between textural properties of emulsion and texture of sausage.

Figure 2 shows mean values of instrumental texture parameters of the two batches. No significant differences between sausages were found in springiness, cohesiveness and adhesiveness. It was expected bigger differences between sausages, especially those made without hydrocolloids. Concerning to springiness and cohesiveness, these results do not agree with Mittal and Barbut (1993), who found higher springiness and cohesiveness in low-fat products (12-14% fat) than in conventional sausages (26% fat). Sausage A (conventional content of fat) is considerably harder and presents a higher chewiness than low fat sausages, being sausage B and E sausages with medium hardness and chewiness and sausages C and D are situated in the lowest extreme with respect to both parameters. There is disagreement in previous references about texture of low fat sausages and the effect of functional ingredients, with no modification of texture (Mittal et al., 1993, Bloukas and Paneras, 1996) or decrease or increase of hardness (Hand et al., 1989, Decker et al., 1986, Ordoñez et al., 2001).

Results of sensory evaluation of texture are similar to those found in instrumental measurement as it was expected. Parameters with significant variability between sausages were only hardness, chewiness and cohesiveness. Sensory texture profile follows the same pattern described in instrumental texture, corresponding the highest and lowest values of hardness and chewiness to sausages A and B, respectively, as described above. Sensory evaluation showed significant differences in cohesiveness, but the only two sausages clearly different are A and E. No differences between sausages were detected in elasticity, adhesiveness and juiciness.

Concerning to correlation between force resulting of extrusion tests on meat emulsion and sausage texture, irrespectively of method used (direct or back extrusion) significant correlations were found only with hardness and chewiness and cohesiveness sensory evaluated (Table 2).

Conclusions

Measurement of characteristics of emulsion by extrusion tests give an easy and quick estimation of texture of sausages after processing with respect to hardness and chewiness, two of the main constituents of texture. This measurement as an index of sausage texture is also useful in low fat products and results of both direct and back extrusion tests are comparable. However, further research on emulsions and sausages with bigger differences in other texture parameters as elasticity, adhesiveness and juiceness is necessary to determine the accuracy of extrusion force to predict a wide texture profile of sausages.

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Table 1. Sausage formulations. Percentages of ingredients differing in experimental sausages.

	Sausages				
Ingredients	A	B	C	D	E
Fat	22	14	14	14	14
Protein	12	12	12	12	12
Lactate	----	1.5	1.5	1.5	1.5
Genugel®		0.6	----	----	----
Carrageenan	----	----	0.4	0.2	----
Xanthan Gum	----	----	0.2	0.4	----

Table 2. Significant correlation coefficients between emulsion properties and texture parameters.

	Direct extrusion	Back extrusion
Sensory Hardness	0.690	0.685
Instrumental Hardness	0.542	0.596
Sensory Chewiness	0.736	0.766
Instrumental Chewiness	0.736	0.766
Sensory Cohesiveness	0.655	0.665

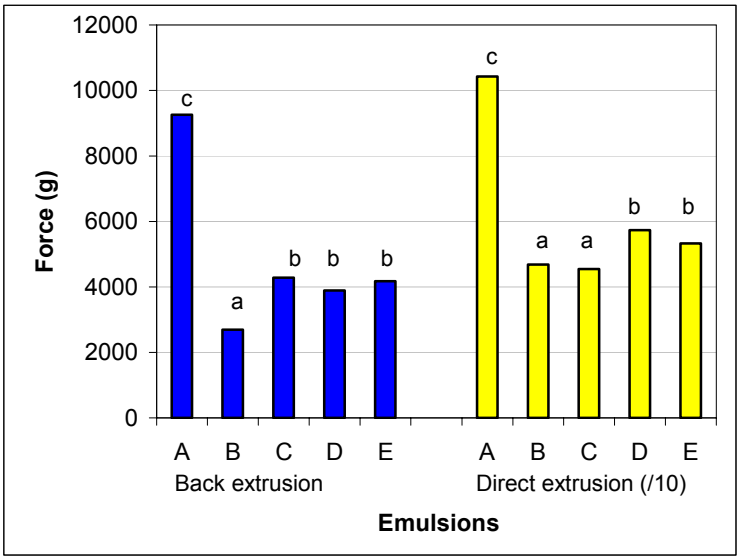

Figure 1. Evaluation of viscoelastic properties of different sausage emulsions (expressed as extrusion force). [a, b, c] For each type of extrusion columns with different letter are significantly different.

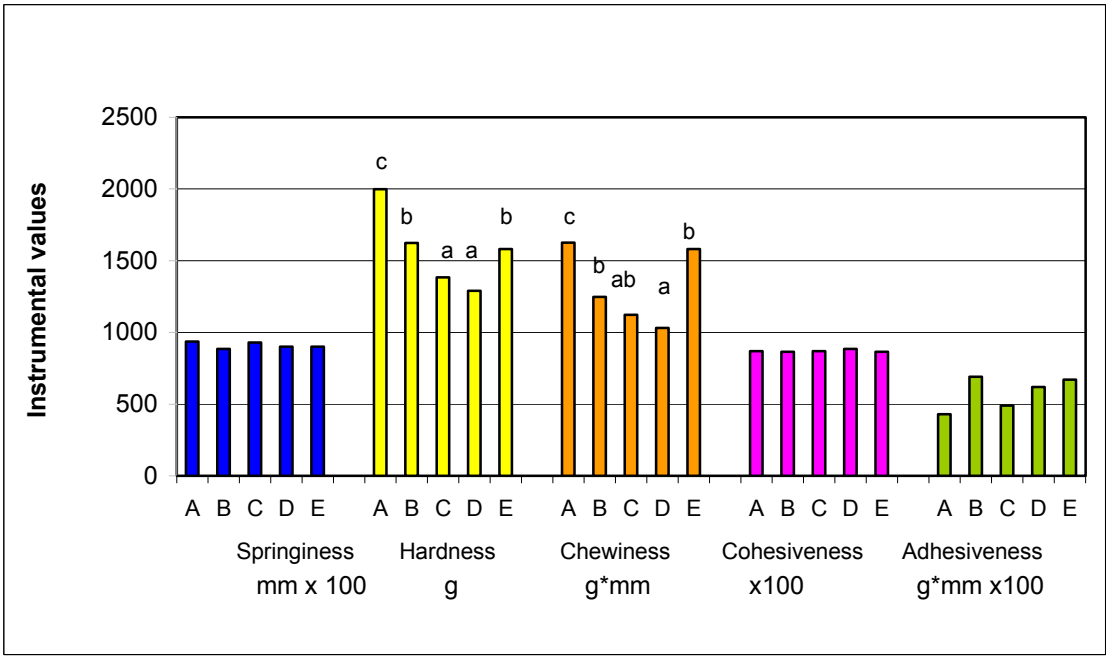

Figure 2. Instrumental evaluation of texture of experimental sausages. [a, b, c] For each texture parameter columns with different letter are significantly different.

GEL PROPERTY OF SURIMI-LIKE MATERIAL FROM PRE- OR POST-RIGOR PORCINE MUSCLE

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Background and objective

Surimi-like material from muscle of other animal species has been hypothesized to have similar properties to surimi from fish (Park et al., 1996). However, there is a little information on the preparation of surimi-like material from beef or pork. Pre-rigor beef is generally recognized to have superior functionally when used in the manufacture of processed meat products (Hamm, 1981). Salting of pre-rigor beef is known to promote maintenance of high binding quality (water and fat stabilization, texture development) during short-term refrigerated storage, presumably by solubilization the protein prior to a tight association of actin and myosin. Park et al. (1987) reported use of cryoprotectants to stabilize functional properties of pre-rigor salted beef during frozen storage. Also Park et al. (1993) reported cryostabilization of functional properties of pre-rigor and post-rigor beef by dextrose polymer and phosphates. The objective of this study was to evaluate the effect of rigor conditions of porcine muscle on gel properties of surimi-like material (SLM).

Materials and methods

Pigs were slaughtered at a Meat Plant of Gyeongasng National University, Jinju, Korea. The *semimembranosus* muscle was obtained by hot boning. After removal external fat tissue, the lean muscle was divided into three portions. For pre-rigor sample, one of the three portions was diced into approximately 2 ㎝ cubes, and ground through a 4.7 ㎝ diameter orifice with a Kitchen Aid mince, and SLM was manufactured. The others samples are commercial vinyl pack packaged and stored at cold room (2 ˚C 4 ˚C) until processed at *post mortem* 24 or 72 hrs. The *post mortem* 24 hr (rigor-mortis) and 72 hr (post-rigor) samples were also used for SLM manufacture, and the SLM manufacture procedure was modified to method of Park et al. (1996).

SLM yield % and color (CIE L*, Chroma and Hue) of SLM gel were measured using a Minolta Chromameter CR-301 (Minolta Co., Japan). Moisture content of uncooked and cooked SLM, and water-holding capacity (WHC) and water–binding capacity (WBC) were measured. Gel strength of cooked SLM was measured with Sun Rheo Meter (COMPAC-100, Japan) and SDS-PAGE was applied to investigate changes in sarcoplasmic and myofibrillar proteins of SLM.

Results and discussion

All SLM from muscles at pre-riogor, rigor-mortis and post-rigor was a light, opaque material with a dough like consistency. When additives (salt, TPP and sorbitol) were mixed with surimi-like pork, the material became comparatively clear and sticky. Specially, SLMs of pre-rigor and rigor-mortis muscles were lighter than that of post-rigor muscle after cooking. When pre-rigor muscle was used, more color was removed with the first washing step, resulting in lighter water washed material. After cooking of SLM, there were significant differences in Chroma and Hue values among treatments (Table 1). This color effect had been reported previously by Lan et al. (1993) and Park et al. (1996). However, yield % of pre-rigor muscle was sigificantly decreased compared to rigor-mortis and post-rigor muscles. Results suggested that sarcoplasmic proteins including pigments such as myoglobin and residual hemoglobin in pre-rigor muscle could be removed easily by water washing, resulted in having decreased yield of SLM. This was confirmed in sarcoplasmic proteins fraction of SDS-PAGE. Intensities of some sarcoplasmic enzymes such as phosphoylase were dim in rigor-mortis and post-rigor muscles (Fig. 2.). The sarcolasmic enzymes were still remained with myofibrillar proteins fraction in SLM.

Although WHC of SLM from post-rigor muscle was significantly lower (Table 2), gel strength and hardness of cooked SLM were significantly ($p<0.05$) stronger and harder in post-rigor muscle than those of other muscles (Fig. 2). Gel forming ability could be influenced by differences in WHC, WBC, protein concentration, ultimate pH and heating condition of SLM (Park et al., 1996). The lower moisture % in SLM

from post-rigor muscle might be related to gel strength and hardness. The lower moisture % in SLM implied that more concentration of proteins would be in SLM. Therefore, the harder gel of post-rigor muscle might be due to a higher concentration of protein in SLM without regard to the lower WHC. These results indicated that strong gel could be obtained with post-rigor porcine muscle because of higher concentration of protein in SLM. The post-rigor muscle, however, could produce dark color that would be avoid for surimi products. It was assumed that the dark color of SLM from post-rigor muscle might be due to a high concentration of sarcoplasmic proteins, oxidation of lipid and protein and the small space of gel matrix resulted in stronger absorption nature than reflection nature of light.

Table 1. Yield % and color of cooked SLM from different rigor conditions of porcine muscle

Muscle conditions	SLM yield %	CIE L*	Chroma	Hue
Pre-rigor	82.33 ± 7.42^{B}	78.51 ± 0.22^{B}	3.16 ± 0.07^{C}	159.61 ± 1.73^{A}
Rigor mortis	107.50 ± 2.17^{A}	80.30 ± 0.37^{A}	3.55 ± 0.07^{B}	126.96 ± 0.81^{B}
Post-rigor	100.77 ± 2.75^{A}	78.75 ± 0.21^{B}	6.61 ± 0.06^{A}	108.37 ± 0.70^{C}
SEM	3.79	0.18	0.17	2.34

Mean±S.E. $^{A-C}$Different letters within a column indicates significant differences between mean values ($p<0.05$).

Table 2. Moisture, free water, WHC and WBC % of SLM from different rigor conditions of pork

Muscle conditions	SLM moisture %		WHC %	WBC %
	Uncooked	Cooked		
Pre-rigor	89.10 ± 0.06^{A}	80.30 ± 0.70^{A}	64.61 ± 0.70^{A}	90.12±1.24
Rigor mortis	88.34 ± 0.93^{A}	81.33 ± 0.27^{A}	65.77 ± 2.32^{A}	91.53±1.05
Post-rigor	86.68 ± 0.31^{B}	77.18 ± 0.25^{B}	58.35 ± 0.83^{B}	88.51±0.70
SEM	0.36	0.43	1.01	0.63

Mean±S.E. $^{A-C}$Different letters within a column indicates significant differences between mean values ($p<0.05$).

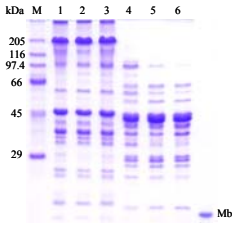

Fig. 1. SDS-PAGE patterns of obtained myofibrillar and sarcoplasmic proteins from rigor conditions of pork. (M : standard marker, lane 1: myofibrillar protein of pre rigor muscle, lane 2: myofibrillar protein of rigor-mortis muscle, lane 3: myofibrillar protein of post -rigor muscle, lane 4: sarcoplasmic protein of pre-rigor muscle, lane 5: sarcoplasmic protein of rigor-mortis, lane 6: sarcoplasmic protein of post-rigor muscle, Mb: horse heart myoglobin).

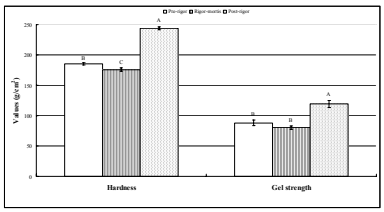

Fig. 2. Gel strength and hardness of cooked SLM from rigor conditions of pork. $^{A-C}$Different letters are within a column indicates significant differences between mean values ($p<0.05$).

Conclusions

A bright and white surimi could be obtained with pre-rigor porcine muscle, whereas a strong and hard surimi could be obtained with post-rigor muscle. However, post-rigor muscle can produce a dark color surimi and pre-rigor muscle can decrease yield % of surimi.

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METHOD AND DEGREE OF MECHANICAL TENDERISATION OF BEEF MUSCLES FOR USE IN RE-FORMED JOINTS MADE WITH A COLD-SET BINDER

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Background

The purpose of producing reformed beef products is to effectively market less valuable carcass components. These contain high levels of connective tissue and therefore require tenderisation for such products (Rolan et al, 1988). Tenderness is the single most important factor affecting consumers' perception of taste in beef (Morgan et al, 1991). Beef round and chuck are traditionally marketed in the form of low priced steaks or roasts. The present study is part of a project designed to increase the value of some muscles from these cuts through tenderising and re-forming treatments. Glitsch (2000) found that for European consumers, tenderness and flavour were the most important factors in eating quality of beef. Numerous reports have indicated that mechanical tenderisation reduces shear force values (Jeremiah et al, 1999). It has also been established that beef steaks and pork chops are more juicy and tender when injected with a phosphate and salt-containing solution (Robbins et al, 2003). Tumbling or massaging treatment is also used in the industry to increase tenderness. In this study several tenderising methods were assessed in conjunction with preparation of re-formed beef joints using Activa ™ (Ajinomoto Ltd.). The active ingredient in Activa is transglutaminase cross-bonding enzyme which catalyses the polymerisation and cross-linking of proteins (Kolle and Savell, 2003).

Objective

The objective was to compare the effects of three different methods of tenderisation on the processing characteristics and tenderness of two muscles selected from the beef chuck and two from the round.

Materials and methods

Beef muscles used were from the chuck (*M. pectoralis profundus* and *M. supraspinatus*) and round (*M. semimembranosus* and *M. vastus lateralis*) of R4L grade steers 4 days post-slaughter. *Semimembranosus* muscles were seamed out of the topside round and *Vastus lateralis* muscles were seamed out of the knuckle. The heavy central piece of connective tissue was removed from the *Supraspinatus* and the *Pectoralis profundus* was used whole. Muscles were trimmed of all visible external fat and connective tissue. Sufficient quantity of each muscle was pooled together to make twenty 19 x 13 x 7.5 cm joints (approx. 2kg weight) for steak cutting, a quarter being assigned to one of four treatments- 1) non-tenderised, 2) blade tenderised, 3) needle tenderised and 4) injected + vacuum-pulsed, giving five replicates of each treatment. For roasting joint production, which was an additional product made in the case of the injected + vacuum-pulsed treatment, five more such joints were made for each muscle group and these were compared with five commercial silverside round roasts. Blade tenderised (BT) muscles were passed once through a commercial roller-blade tenderiser. With this tenderiser, meat is passed between two rollers of 305mm length, with 78 blades per roller (blade width 1mm, 3.9mm interval between blades). Each blade contains 20 teeth of 9mm width with a 10mm interval space. Blades from opposing rollers overlap by 10mm. Needle tenderised (NT) muscles were passed once through a commercial Tender Star Model TSE tenderiser with solid stainless steel chisel-shape needles (needle diameter 1mm, at chisel end 2mm). The needle bank area of 250mm x 80mm contained 574 needles. Injected and vacuum pulsed muscles (VP) were injected with brine using a Dorit PSM-21 Inject-O-Mat brine injector at 16% to give a concentration of 0.5% salt and 0.3% sodium tripolyphosphate (STPP) in the meat, chopped to give pieces of ~200g weight and vacuum-pulsed for 14 hours in a Rühle vacuum tumbler/ mixer (Model MKR 150-600, Rühle GmbH) using a "Tender beef " programme which alternated between 90% and 10% vacuum (100 and 900 hPa). BT and NT muscles were also cut into ~200g pieces following treatment. Activa ™TG-RM at a level of 1% (% of raw meat weight) was whisked with 4% water until homogenous, and the suspension was then mixed with meat chunks, ensuring each piece was fully coated. Meat pieces were then layered in a polythene-lined rectangular-shaped mould (1.5-2.0kg per mould), vacuum packed and held at 2ºC overnight to allow completion of the protein

cross-linking reaction. Controls (CO) were treated in the same way but omitting the tenderisation step. The formed meat was removed from moulds and liners, and cut into steaks of 2.54cm width. Steaks were vacuum packaged and held at 2ºC pending analysis, with the exception of steaks for taste testing which were frozen at –20ºC for a minimum of one week. In the case of the injected and vacuum-pulsed treatment, joints for roasting were made in the same way but not cut up into steaks. They were then vacuum packaged in Cryovac cooking bags and following overnight holding cooked in a Jugema steam/ air cooker to an internal temperature of 70ºC using a cabinet temperature of 82ºC and a relative humidity of 99%. Five commercial round roasts made from the silverside, averaging 1kg in weight were cooked for comparison. Steaks were tested for cooking weight-loss, Warner-Bratzler shear force (WBS), colour (L*a*b*) and eating quality. Total viable bacteria counts (TVCs) at 30ºC were determined for steaks from one forequarter muscle (*Pectoralis*) and one hindquarter muscle (*Semimembranosus*) on day 0 and day 7. Cook loss was measured on the day steaks were cut and WBS on the following day. Cook loss was measured by weighing both prior to and after cooking to a core temperature of 70ºC. Shear values of 7 cores of 13mm diameter sheared perpendicular to meat fibre direction were measured. Colour was measured using a HunterLab spectrophotometer (Ultrascan XE) on the cut surface of PVC film-covered steaks after 1 hour blooming in air at 2ºC on the same day that steaks were cut. Measurements were taken on 10 locations per steak and averaged. Steaks for taste panels were thawed using cold running water. Taste testing was carried out on steaks grilled to a core temperature of 70ºC by a panel of eight people experienced in tasting beef. The panellists graded the samples for tenderness, chewiness, residual connective tissue, juiciness, overall flavour and overall acceptability on a scale of 1 (worst) to 6 (best).
Roast joints were tested for cook loss, texture (Kramer Shear Force), sliceability and eating quality. Cook loss was measured by weighing beef joints before and after cooking. Kramer Shear Force (KSF) was measured by placing approximately 30g of 1mm-thick roast beef slices in the bottom of a 10-blade Kramer Shear cell attached to an Instron model 4464 texture meter. Because of unavoidable weight variability between samples, measurements are expressed as Newtons force per g of meat sample. Sliceability was determined as the percentage of broken slices upon cutting 10 x 1mm-thick slices from a roast joint. Panels assessed taste and appearance of slices of the roast beef for colour acceptability, tenderness, juiciness, overall flavour, binding/ cohesion, overall acceptability and saltiness, on a scale of 1 (worst) to 6 (best). In the case of saltiness 1 represented not salty, 6 being extremely salty.
Analysis of variance of the results was carried out using the Genstat 5 Release 3.2 (Rothamsted Experimental Station). For total viable counts the analysis used was a split plot design.

Results and discussion

WBS results and taste panel ratings showed that type of tenderisation treatment significantly affected resulting steak tenderness (Table 1). For all except one muscle, CO samples, as expected, had higher WBS values than all other treatments, the exception being *Semimembranosus*, for which CO and BT samples were not different. Sensory panel tenderness ratings showed CO samples were significantly less tender than all treatment samples for 3 of the 4 muscles. Also in the case of 3 of the 4 muscles, BT had higher WBS than VP, and for both forequarter muscles, WBS values were higher for BT than NT. *Pectoralis* CO samples had higher WBS values than BT, NT and VP ($p<0.001$). This was also reflected in taste panel results where CO samples were rated less tender than BT and NT samples ($p<0.05$) and less tender than VP samples ($p<0.001$). CO samples were also rated more chewy than BT ($p<0.05$), NT ($p<0.01$) and VP ($p<0.001$) samples. Panellists also rated CO samples as having higher residual connective tissue content than BT ($p<0.05$), NT and VP samples ($p<0.01$). Overall acceptability ratings showed CO samples to be less preferable to NT and VP samples ($p<0.01$). BT samples had higher WBS values than VP samples ($p<0.001$), and were also rated less tender ($p<0.05$), more chewy ($p<0.01$) and less juicy ($p<0.001$) in sensory panels. *Supraspinatus* CO samples also had higher WBS values than all other treatments ($p<0.001$). This was reflected in sensory panel tenderness ratings for BT ($p<0.05$), NT and VP samples ($p<0.001$); chewiness ratings for NT and VP samples ($p<0.001$); residual connective tissue ratings for NT and VP samples ($p<0.01$) and overall acceptability ratings for NT and VP samples ($p<0.001$). BT *Supraspinatus* muscles had higher WBS values than NT and VP samples ($p<0.05$) and this was also reflected in tenderness, chewiness, residual connective tissue content and overall acceptability ratings for both muscles and in juiciness ratings for VP samples only. *Semimembranosus* CO samples had higher WBS values than NT ($p<0.01$) and VP ($p<0.001$) samples, but did not differ from BT samples. This corresponds to sensory panel ratings for tenderness, chewiness and overall acceptability, although panellists did differentiate between CO and BT samples, rating BT samples

better for tenderness, chewiness and overall acceptability. BT *Semimembranosus* samples had higher WBS values than VP samples ($p<0.01$) but this was not reflected in sensory results. *Vastus lateralis* CO samples had higher WBS values than BT, VP ($p<0.01$) and NT samples ($p<0.001$) but again, this was not reflected in sensory panel ratings. Overall, for all 4 muscles, VP samples were rated significantly more juicy than all others. They were also more tender in the case of all forequarter CO and BT samples, but panellists did not differentiate between these treatments in hindquarter muscles. WBS values also showed VP samples to be more tender than CO and BT samples for three of the four muscles. These results show that the superiority of the VP treatment over other tenderisation treatments used is evident in the forequarter muscles only, these having higher shear values than the hindquarter muscles in non-tenderised samples. These results correspond with a number of previous findings which indicated that brine-injection improves eating quality of beef e.g. Vote et al, 2000. Cook loss from steaks was not affected by treatments. Colour readings showed that for both forequarter muscles, NT samples were significantly lighter in colour, i.e. had higher L values than VP samples. There was, however, no difference in the lightness value between these two treatments in hindquarter muscles. *Supraspinatus* NT samples also had higher L values than BT ($p<0.01$) and CO samples ($p<0.05$). *Semimembranosus* CO samples also, had a lower a value than the corresponding NT samples (i.e. CO samples were less red than NT samples). Both hindquarter CO and BT samples had higher values than VP samples. Even though there were some differences in b values, no patterns emerged. From these results, overall, tenderisation treatments were not shown to have a negative impact on re-formed steak colour when compared to non-tenderised samples. Results of TVCs showed that for *Pectoralis* on day 0, the VP samples had a higher count than CO, BT and NT samples (VP=$5.1x10^5$ versus CO=$2.5x10^4$, BT=$7x10^4$, NT=$1.8x10^4$). On day 7, VP samples still had a higher count than all other treatments (VP=$1.7x10^6$ versus CO=$1.9X10^5$, BT=$4.9x10^4$, NT=$4.1x10^5$). Also, BT samples had higher counts than NT samples ($p<0.05$). *Semimembranosus* day 0 samples showed no differences between treatments, but on day 7 VP had higher counts than CO ($p<0.001$) and BT and NT samples ($p<0.01$). These results indicate that the injection and vacuum pulsing process allows for greater microbial contamination than the other tenderisation treatments used.

The comparison of roast beef slices from commercial silverside roast joints with those from the reformed joints showed (Table 2) that 3 out of 4 of the latter were as tender or more tender than the retail beef. Sliceability was better for reformed joints from all four muscles than for the commercial roasts. *Pectoralis*, *Semimembranosus* and *Vastus lateralis* reformed joints gave 100% sliceability and *Supraspinatus* gave 80%, while commercial silverside roast gave 67% sliceability. There was no significant difference in cook loss between commercial roasts and any of the reformed roasts.

Conclusions

All three tenderisation treatments used in this trial significantly reduced WBSF values, and in most cases these reductions were reflected in sensory panel ratings. Needle tenderisation and injection + vacuum-pulsing treatments gave about equal levels of tenderisation, while blade tenderisation had a lesser effect on tenderness. Injected + vacuum-pulsed samples retained more juiciness than those from other treatments for all four muscles. Tenderisation treatments were shown not to impair colour of re-formed steaks. Total viable bacteria counts indicated that injection + vacuum-pulsing results in higher counts than other treatments. Texture analysis of roast beef joints indicated that the commercial silverside roasts used for comparison, along with the *Pectoralis* roasts were both less tender than those from the other three muscles. The study indicates that needle tenderisation and injection + vacuum pulsing treatments are more effective than blade tenderisation for tenderisation of beef for use in reformed joints. Account needs to be taken of the possible higher bacterial numbers arising from the injection + vacuum-pulsing treatment.

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Table 1. Effect of tenderisation treatments on shear force values and taste panel ratings of re-formed steaks.

	Pectoralis profundus				*Supraspinatus*				*Semimembranosus*				*Vastus lateralis*			
	CO	**BT**	**NT**	**VP**	**CO**	**BT**	**NT**	**VP**	**CO**	**BT**	**NT**	**VP**	**CO**	**BT**	**NT**	**VP**
WBSF *	80.8^{a}	61.8^{b}	46.1^{c}	40.4^{c}	54.6^{a}	37.7^{b}	30.7^{c}	31.0^{c}	49.2^{ab}	44.3^{b}	38.4^{bc}	33.7^{c}	47.2^{a}	34.5^{b}	32.8^{b}	35.7^{b}
Tend.**	1.73^{a}	2.88^{b}	3.08^{b}	4.03^{c}	2.38^{a}	3.25^{b}	4.70^{c}	5.03^{c}	2.85^{a}	4.22^{b}	4.38^{b}	4.93^{b}	4.00	3.93	4.90	4.55
Chew.$^{\#}$	1.73^{a}	2.55^{bc}	2.93^{cd}	3.70^{d}	2.48^{a}	3.08^{a}	3.88^{bc}	3.93^{c}	2.78^{a}	3.70b	3.85^{b}	4.10^{b}	3.43^{a}	4.05^{ab}	4.08^{ab}	4.52^{b}
RCT §	2.28^{a}	2.95^{b}	3.28^{b}	3.48^{b}	2.83^{a}	3.08^{a}	4.08^{b}	4.00^{b}	3.45	4.22	4.18	4.07	4.18	4.43	4.70	4.13
Juicin. ¤	3.43^{a}	3.38^{a}	3.60^{a}	4.90^{b}	3.85^{a}	3.58^{a}	4.05^{a}	5.25^{b}	3.78^{a}	3.95^{a}	3.80^{a}	4.90^{b}	4.00^{a}	2.05^{b}	3.90^{a}	5.16^{c}
O. acc.«	2.35^{a}	3.03^{ab}	3.35^{b}	3.45^{b}	2.93^{a}	2.90^{a}	4.15^{b}	4.25^{b}	3.25^{a}	3.73^{bc}	4.18^{d}	4.00^{cd}	3.95^{ab}	3.50^{a}	4.18^{b}	4.31^{b}

a-d Treatment means with different superscripts, for each muscle, are significantly different ($p<0.05$)
* Warner Bratzler Shear Force (N)
** Taste panel tenderness
# Taste panel chewiness
§ Taste panel residual connective tissue
¤ Taste panel juiciness
« Taste panel overall acceptability

Table 2. Comparison of shear force values and taste panel results of vacuum-pulsed and injected beef muscles with silverside round roast

	Silverside round roast	**Pectoralis**	**Supraspinatus**	**Semimembranosus**	**Vastus lateralis**
KSF *	69.5^{a}	62.1^{a}	49.7^{b}	47.7^{b}	49.9^{b}
Tend. **	4.70	4.65	4.65	4.38	4.50
Juicin. $^{\#}$	4.33	4.38	4.48	4.53	4.65
O. acc. «	4.10	4.35	4.43	4.08	4.53

a-b Treatment means with different superscripts, for each muscle, are significantly different ($p<0.001$)
* Kramer Shear Force (N)
** Taste panel tenderness
# Taste panel juiciness
« Taste panel overall acceptability

HEAT-INDUCED CHANGES IN THE MECHANICAL PROPERTIES OF PERIMYSIAL CONNECTIVE TISSUE FROM TWO BEEF BREEDS

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Background

The structural origins of variations in meat tenderness, arising from various treatments or animal characteristics, are far from clarified. Results from macroscopic mechanical methods can lead to conflicting conclusions. Micro-mechanical tests performed on isolated structures (Lewis and Purslow 1989) allow clear understanding of the contribution of theses structures to the overall meat tenderness. Theses techniques have been used to elucidate the mechanical changes in connective tissue and muscle fibres associated with contraction state, ageing or cooking conditions (Christensen, Purslow and Larsen 2000). Only few data are available on the variations in the mechanical properties of these structures associated with different animal characteristics.

Objectives

The purpose of this study was to quantify heat induced changes, in mechanical properties of isolated perimysium sheets from beef muscles from two breeds.

Materials and methods

Muscles : *Semimembranosus* muscles were taken from cows (6 -7 years old) Holstein (12) and Salers (12). The muscles were removed from each carcass at 24h *post-mortem*. They were divided into 2 parts which were vacuum packed and stored at 4°C for 14 days and then frozen. One part was for measurements on raw meat and the other for measurements after 90 min cooking at 70°C.

Measurements :
All the samples were thawed in water at 10°C.
Measurements on raw meat:
The resistance of muscle fibres was measured in compression according to the method of Lepetit and Buffiere (1995) using an Instron 4501 testing machine. Sheets of perimysium (approximately 10 mm long x 4 mm wide) were dissected and tested with a micro-tensile device described below, for the determination of breaking stress, breaking strain, Young's modulus, breaking energy and total energy. The width of the samples were determined using a microscope and the thickness determined with a Mitutuyo micrometer under a force of 0.2N applied on the whole surface of the sheets. During the tensile test the samples were immersed in meat drip.
Measurements on cooked meat :
The cooking was done in water bath on 10x10x4 cm samples, the length in the direction of muscle fibres being 10 cm. Cooking losses were determined. The breaking stress of cooked samples was measured in compression with an Instron 4501 (Lepetit, Grajales, and Favier 2000). Sheets of perimysium (approximately 10 x 4 mm) were dissected from cooked meat and tested with the micro-tensile device. Width and thickness were measured as above. During the tensile test the samples were immersed in the liquid lost during cooking.
Micro-tensile test :
Tensile test was carried out on a micro-tensile device developed in this laboratory (figure 1). The procedure for handling samples is similar to that described by Lewis and Purslow (1989). After dissection, strips of perimysium were glued on aluminium foil frames with cyanoacrylate glue. The samples on the aluminium frames were then fixed on the micro-tensile device so that the direction of tensile testing was in the direction of collagen fibres.
The extension rate was 130 µm/s. The software applied a slack toe correction to the force – displacement curves to remove the part of the displacement where collagen fibres are just unfolded. The actual lengths of

the samples were determined from the rapid increase in force which happened when the perimysium structure entered into tension. The Young's modulus was calculated as the slope in the straight region of the stress – strain curves.

Statistical analysis :
For all mechanical variables means were obtained from 10 measurements. Data were analysed using the general linear model procedure of SAS Software (SAS/Stat Cary, NC: SAS Institute Inc., 2000).

Results and discussion

Meat from both breeds was not fully aged after 14 days of storage at 4°C as the resistance of muscle fibres were higher than the limit of 4 N/cm2 (Lepetit and Buffiere 1995) as seen in Table 1. Maximum compression stress of raw meat did not show any differences between breeds, but drip was significantly higher in meat from Salers than in Holstein. The breaking stress of cooked meat from Salers was significantly higher than from Holstein and cooking losses were similar between breeds (Table 2). The mechanical properties of perimysium sheets in the raw and cooked states are given in Table 3. The breaking stresses of perimysium sheets did not differ between breeds when compared raw or cooked, but there was a 36% reduction of the mean stress by cooking for 90 min at 70°C. The breaking strain was similar for both breeds in raw samples. For cooked samples breaking strain was slightly higher in Holstein than in Salers. Breaking strain increased, on average by 31% by cooking. Young's modulus decreased by 52% from raw to cooked. Breaking energy showed no significant variation neither between breeds nor from raw to cooked, whereas the total energy for the disruption of perimysium sheets showed a 25% reduction by cooking. The values of drip found for both meats (Table 1) include the drip occurring during 14 days of ageing and also the exudation due to thawing which explain why these values are quite high, but they are in agreement with values given by Offer and Knight (1988). The cooking loss agrees with values observed previously for the same muscle cooked in same conditions (Lepetit, Grajales and Favier 2000).

The breaking strain of perimysium sheets increased with cooking as collagen is progressively denatured and becomes rubber-like (McClain, Kuntz and Pearson 1969). Our values of breaking strain are much lower than those found by Lewis, Purslow and Rice (1991) for two reasons. First, in the present study, a slack toe correction was applied to the force-displacement curves to remove the displacement which corresponds to the unfolding of the collagen fibers. Secondly, the perimysium sheets in the present study, are cut parallel to one of the main directions of the collagen ply whilst samples in the study of Lewis, Purslow and Rice (1991) were cut perpendicularly to the direction of muscle fibers. Under those conditions, there is not only an unfolding of collagen fibers but also a reorientation of collagen fibers in the direction of the strain during the tensile test. In our samples no reorientation of collagen fibers occurred because they already are in the direction of strain and therefore a lower breaking strain is expected.

Values of the breaking stress from the present study are about four times higher than those reported by Christensen, Purslow and Larsen (2000) but here also precise comparisons are difficult due to the different type of samples. The present study concerned perimysium sheets from cull cows (6 – 7 years old) whereas the previous study concerned perimysium from young (2-2 ½) heifers and the resistance of connective tissue changes significantly with the age of animal as shown by adhesion measurements (Bouton and Harris 1972). Also differences in breaking stresses between the two studies may come from differences in sample shape and collagen fibre direction which both affect the number of collagen fibres contributing to the stress. Comparisons of Young's moduli lead to similar conclusions as for the breaking stress as they are highly correlated ($r=0.93$, $n=24$, $P<0.01$ for raw samples ; $r=0.87$, $n=24$, $P<0.01$ for cooked samples). Breaking energy, which represents the work done up to the maximum stress, was not affected by cooking. This results from opposing variations of breaking strain and breaking stress with cooking. There is a decrease in breaking stress and an increase in breaking strain due to cooking which lead to an almost constant breaking energy from raw to cooked. The total energy needed to separate perimysium sheets was significantly reduced by cooking. The energy which is developed after breakage is due to shear between collagen fibres. It represents about 60% of the total energy in raw samples and about 45% in cooked samples. It is this energy of shear which is decreased by cooking. In a fibrous composite material the energy of shear is supported by the matrix, which, in the case of perimysium, is composed of proteoglycans.

The maximum stress of compression of raw meat reflects mainly variations in connective tissue and therefore was not expected to vary between breeds as the data on perimysium sheets did vary between breeds. The small differences between breeds in maximum stress of cooked meat could not be linked to the minor variations between breeds in mechanical properties of cooked perimysium sheets.

Conclusions

Tensile tests on perimysium sheets isolated from *semimembranosus* muscles of cows from two breeds show significant changes with cooking (90 min at 70°C) in breaking stress, breaking strain, Young's modulus and total energy. On raw perimysium sheets no differences between breeds was observed which is in agreement with compression data on raw meat. The small differences between breeds in maximum compression stress of cooked meat cannot be linked to the minor variations in mechanical properties observed on cooked perimysium sheets.

Acknowledgements : These data are a part of a program supported by the FNADT (Massif Central Specific plan, 99/000144).

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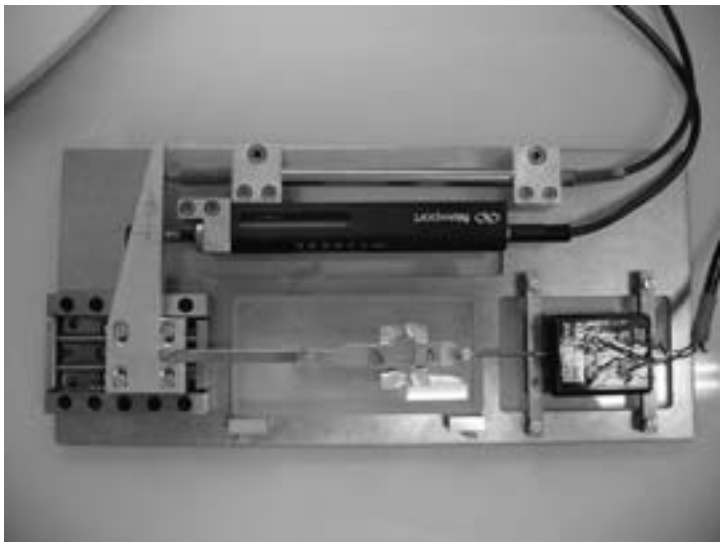

Figure 1 : Micro-tensile device. Total length 28 cm.

Breed	Resistance of muscle fibres (N/cm2)	Maximum stress (N/cm2)	Drip (%)
Holstein	8.1 *a*	85.1 *a*	7.6 *a*
Salers	7.0 *a*	82.5 *a*	8.6 *b*

Table 1 : Mechanical properties and drip of raw meat
In each column the values followed by different letters are significantly different at a 5% level.

Breed	Maximum stress (N/cm2)	Cooking loss (%)
Holstein	204.7 *a*	21.8 *a*
Salers	226.8 *b*	21.2 *a*

Table 2 : Mechanical properties and loss of cooked meat
In each column the values followed by different letters are significantly different at a 5% level.

	Breed	Breaking Stress (MPa)	Breaking strain	Modulus (MPa)	Beaking energy (mJ)	Total energy (mJ)
Raw	Holstein	10.3 *a*	0.36 *c*	41.0 *a*	8.5 *a*	20.0 *a*
Raw	Salers	12.5 *a*	0.35 *c*	52.4 *a*	8.6 *a*	20.5 *a*
Cooked	Holstein	7.6 *b*	0.49 *a*	22.1 *b*	9.2 *a*	15.9 *b*
Cooked	Salers	7.1 *b*	0.44 *b*	22.4 *b*	7.9 *a*	14.5 *b*

Table 3 : Mechanical properties of raw and cooked perimysium sheets
In each column the values followed by different letters are significantly different at a 5% level.

DEVELOPMENT OF TECHNOLOGY FOR PRODUCTION OF MEAT-PLANT EXTRUDATES

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Background

Extrusion technology is one of the advanced methods for obtaining high quality foods. The range of products manufactured using this technology includes more than 400 items: dry breakfasts, products for nutrition of children, cracker-type bread, potato chips, corn and wheat flakes, etc. /1/.
The advantages of extrusion technology consist in flexibility of its technological systems, continuity of the process, possibilities of simultaneous homogenization of raw materials and their thermal treatment which allow to solve many important problems in foods production, i.e. : preservation of biological and food value, inhibition of development of pathogenic microflora, formation of prescribed structural and mechanical properties and organoleptical characteristics.
These advantages indicate good prospects of use of extrusion technology in meat industry for development of meat-plant extrudates. It should be noted that the problems associated with peculiarities of formation of structure in the process of thermoplastic extrusion have been little studied.

Objectives

The purpose of this work was to investigate the influence of technological parameters of extrusion process (temperature, frequency of screws rotation) on structural and mechanical properties and quality characteristics of meat-plant extrudates.

Materials and methods

As objects of investigations were chosen products of animal origin: beef of 2nd grade, dry animal broth, light food grade albumin; of vegetable origin: corn starch and wheat flour. Second grade beef was comminuted (d = 2-3 mm) and mixed with other components in different ratio: 1) 2^{nd} grade beef, dry animal broth, corn starch; 2) 2^{nd} grade beef, wheat meal, corn starch; 3) 2^{nd} grade beef, light food grade albumin, corn starch. The mixtures were maintained during 12 hours at 4^0C for moisture level stabilization. Extrusion treatment was carried out on an experimental laboratory double screw extruder with the selected profile of configuration of screw elements of shafts for treatment of meat containing mixtures with the capacity of 40 kg/hr.
During investigations standard methods of determination of microstructure, mass fraction of moisture, fat, protein, carbohydrates were used. Digestibility of meat-plant extrudates was determined by the method (2). Structural-mechanical properties of extrudates were determined on the INSTRON - 1140 apparatus. Bulk mass p (kg/m^3) was determined by weighing in the capacity 1000cm^3. Coefficient of explosion $K_в$ was found as the ratio between bulk weights of mixture and final product.

Results and discussion

Quality of final products obtained as a result of extrusion treatment of selected recipe mixtures depends on quality of raw materials and parameters of the process: temperature of treatment of mixture and rotation frequency of screws.
Analysis of influence of extrusion treatment temperature shows that with temperatures <150 ^{0}C the extrusion process doesn't reach completion (Fig.1): biopolymeric mixtures don't melt to the end. At the exit from the extruder matrix there is no explosion of starch grains; the product is low-porous with low coefficient of explosion. Increase in the temperature from 150 to 180^{0}C results in $K_в$ increase; denaturation of native proteins and gelatinization of starches occur. In this case crystalline areas of biopolymers melt and the amorphous ones change from disordered high elastic state to a viscous-flow one, and as a result of sharp pressure drop - "decompression shock" - the extrudates have good porous structure /3/.
Temperature increase to higher than 180^{o}C leads to a decrease of the coefficient of explosion. This can be explained by more intensive Maillard reaction, the extrudates are strengthened, their porosity is reduced, and

hence, the coefficient of explosion is reduced, the extrudates acquire pronounced dark color and bitter off-flavor.

The investigations of the influence of rotation frequency of screws on extrudates quality have shown (Fig.2) that with low values of rotation frequency (12.7 s^{-1}) the shear stress of extrudates is not large. This occurs because the mixture is for a long time in the chamber of the extruder and undergoes strong destructive changes.

Proteins are destroyed to amino acids, polysaccharides to dextrins which interact forming different complexes. However, as a result of long effect of temperature, "block-dextrin" complexes are destroyed which results in low value of shear stress.

When the rotation frequency of screws increases to 13.7 –14.7 s^{-1} destruction of complexes "protein-dextrin" takes place not so intensively, but rather strongly. As a result the product is stronger, and the shear stress increases.

If the extrusion process is carried out with frequency rotation of screws 15.7 s^{-1}, the obtained extrudates are porous, easily crumble, don't have burned flavor.

With frequency rotation higher than 16.7 - 18.7 s^{-1}, the extrusion mixture is subjected to the effects of large shear characteristics with the result of destruction of mixture components, and hence shear stress increases.

The obtained extrudates (Table 1) are the products with high level of protein (8.9 – 15.9%) and low level of fat (0.35 – 0.58%).

The investigations on digestibility of these products have shown high degree of availability of proteins for proteases, which indirectly proves a deep destruction of proteins of meat-plant extrudates as a result of thermoplastic extrusion.

Investigations of microstructure of the comminuted extruded product (Figs. 3,4) (x 12) made it possible to establish that the main mass of particles in extrudates has plate-like corrugated structure and flowing internal structure. Methylene dye has clearly determined linear arrangement of protein component in the volume of the investigated particles. However, during the investigation mutually perpendicular orientation of protein and carbohydrate components was revealed. Muscle fibers that occur in the product consist of destroyed bundles of myofibrils with lost sarcolemma; there was a cross striation in some of their parts.

Conclusions

Thus, the experiments have shown that the technology of thermoplastic extrusion is suitable for manufacture of new kinds of meat products. To obtain extrudates of required quality meat-plant mixtures should be treated at technological regimes, as follows: matrix temperature – 180^0C, rotation frequency of screws – 15.7 s^{-1}.

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Table 1. Chemical composition of meat-plant extrudates

Index, %	Extrudate		
	1	2	3
Moisture	8.9	7.8	7.7
Protein	8.9	8.6	15.9
Fat	0.35	0.58	0.40
Carbohydrates	77.65	81.22	74.4
Ash	4.2	1.8	1.5
Digestibility, %	77.1	74.89	78.76

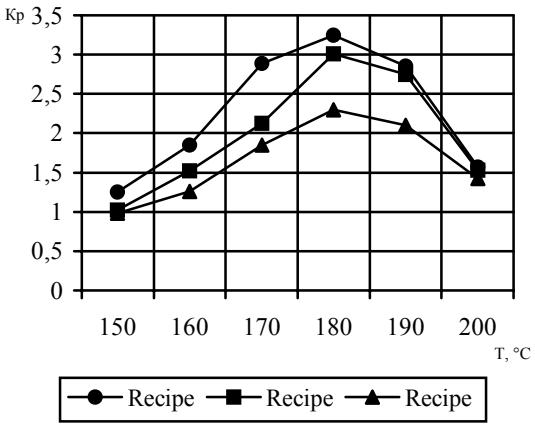

Fig.1 Dependence Кв from temperature of treatment at screw rotation frequency 15,7 s^{-1}

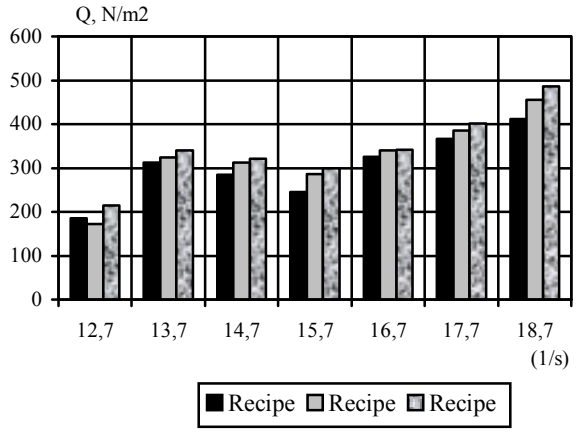

Fig.2 Dependence of shear stress Q ($N/м^2$) from screw rotation frequency at 180 °C

Figure 3. Microstructure of the comminuted extruded product. (x12)

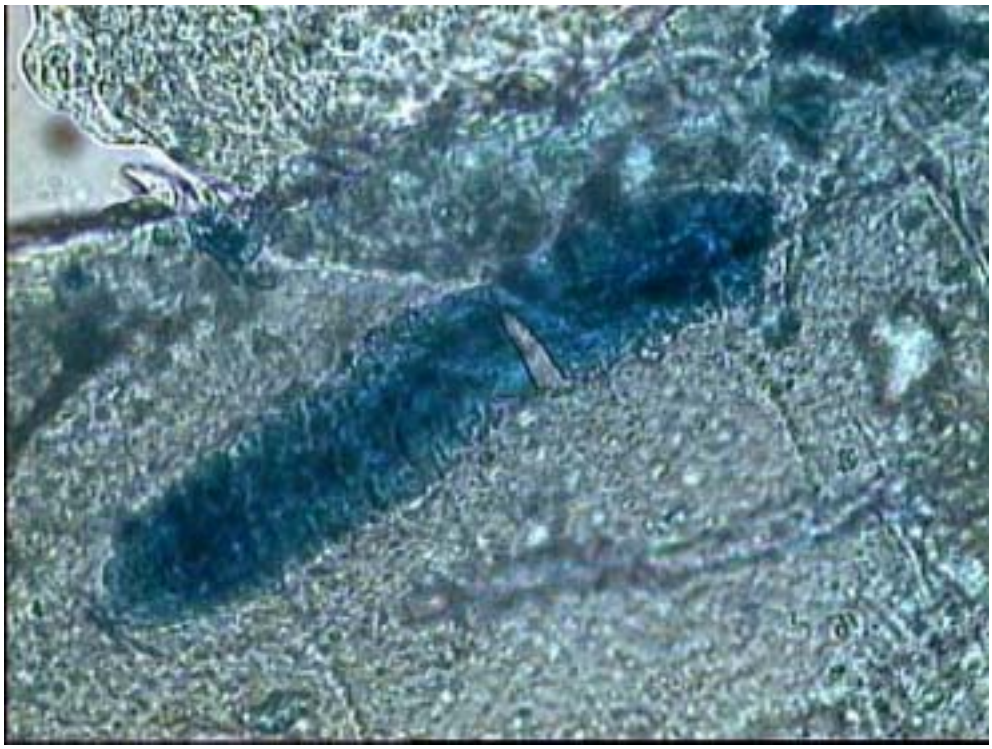

Figure 4. Microstructure of the comminuted extruded product. (x12)

INFLUENCE OF ANATOMICAL ORIGIN OF RAW MEAT ON THE SENSORY AND CHEMICAL CHARACTERISTIC OF DRIED BEEF "CECINA DE LEON"

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Background

Spanish "Cecina" is a salted, dried, and smoked beef meat product manufactured traditionally in the province of Leon (north-western Spain). It is an intermediate moisture product meat, made from three possible different anatomical retail cuts: "babilla" (composed of by *M. rectus femoris* and *vastus lateralis*, *vastus. medialis* and *vastus intermedius*), "tapa" (M. s*emimembranosus*, *sartorius*, g*racilis*, *adductor*, *pectineus*, *quadratus femoralis* and the external extra pelvic portion of M. *obturatorius externus*) and "contra" (*M. semitendinosus* and g*luteobiceps*). The preparation method is similar to that used in dry-cured ham manufacture. The final product has a typical red colour, smoked flavour and a characteristic slight salty taste. "Cecina" is highly prized in Spain and has recently been exported to the rest of Europe. Despite this, few studies have been carried out on the "Cecina de Leon".

Objectives

The objective of this research was to evaluate physicochemical and sensory characteristics of the three different types of "Cecina de Leon" ("babilla", "tapa" and "contra").

Materials and methods

Ten pieces of "babilla", ten of "tapa" and ten of "contra", provided by Protected Geographical Indication (PGI) "Cecina de Leon" were analyzed. All samples were vacuum packed and refrigerated at -4ºC until analyses. The analytical determinations were carried out in triplicate and the results expressed as dry matter.

The samples were subjected to the following analyses: pH, water activity (a_w), moisture, protein, fat and ash content. These physicochemical parameters were determined following the Official Methods of Analysis (BOE 29/8/1979) and the ISO recommended methods (ISO R-1442, ISO R-937, ISO R-1443, and ISO R-936 respectively). The following analyses were also performed: total carbohydrates (Luff-Schoorl method), hydroxyproline (Bonnet and Kopp, 1984), nitrate (brucine method), nitrite (ISO 2918) and NaCl content (Carpentier-Volhard method).

Fatty acid composition was carried out on total of fat extract according Bligh and Dyer (1959) after methyl-derivatization (Morrison and Smith, 1964). Gas chromatographic analysis was performed with a Perkin-Elmer Auto syst-X.L equipped with an Omegawax 320 column. Results are presented as g/100g fatty acids (% by weight) of oleic acid, saturated, monounsaturated and polyunsaturated acids. Nutritional quality is described by the polyunsaturated:saturated ratio and ω_6:ω_3 ratio.

Surface colour of the pieces was measured using a reflectance spectrophotometer (Minolta CM-2002). Colour coordinates were determined in the CIE-LAB system and the results were expressed as lightness (L*), redness (a*) and yellowness (b*).

Instrumental Texture Profile Analysis (TPA) (Breene, 1975) was used to evaluate the instrumental texture. All the measurements were made using Texture Analyzer TA-XT2 (Stable Micro Systems Ltd.) with cylindrical probe. A uniaxial compression test was carried out using a cross-head speed of 1 mm/min and the level of compression was 50% of sample thickness. From the TPA curves hardness, springiness, chewiness and cohesiveness were the parameters obtained.

Sensory evaluation was carried out on "Cecina" slices by an trained 8-member sensory panel. The parameters studied were: external appearance (colour homogeneity, colour intensity, marbling, intermuscular fat and yellowness), odour intensity, texture (hardness, chewiness, juiciness, and pastiness) and flavour (flavour intensity and after taste). The sensory attributes were scored using 5-points hedonic scales, 5 denoted extremely high and 1 denoted extremely low.

Statistical analysis of data was carried out by one-way analysis of variance, and means were separated by Tukey honest significant difference test using at 5% level (Statistica software package).

Results and discussion

The chemical composition and physicochemical parameters of the three types of "Cecina" are shown in Table 1. No differences ($p>0.05$) were observed in pH and a_w. The values of a_w (0.882-0.903) are characteristic of an intermediate moisture meat product, which give microbial stability and, hence, help to preserve meat. In relation to the proximate composition, except for moisture content, "contra" was significantly different ($p<0.05$) from "babilla" and "tapa". The results obtained for protein, fat, ash and hydroxyproline contents in "babilla" and "tapa" were basically in line with data published by Gutierrez et al. (1998) for "tapa". The small differences ($p<0.05$) existing in moisture content between "babilla" (52.4%) and the other pieces, "tapa" and contra", could be attributed to its lower size since for the same ripening time the water loss is higher. Although "Cecina de León" is characterized by a high protein and low fat content, "contra" presented a high ($p<0.05$) fat content (28.6% dry matter) resulting in a low ($p<0.05$) protein, carbohydrate and ash contents. Differences in histochemical composition between muscles could explain the differences found in fat content between retail cuts used (Hunt and Hedrick, 1977; Renerre, 1984; Kirchoefer et al., 2002). In this sense, some muscles included in "contra" (M. *gluteobiceps* and *semitendinosus*, overall its inner part) are characterized by a higher percentage of red fibbers, with greater fat contents, than those included in "tapa" (M. s*emimembranosus* and *gracilis*).

Table 1.-Chemical composition and physicochemical parameters (mean ± S.D.) of "babilla" (n=10), "tapa" (n=10) and "contra" (n=10).

Parameters	***"Babilla"***	***"Tapa"***	***"Contra"***
pH	^{a} 5.9 ± 0.28	^{a} 5.8 ± 01	^{a} 5.9 ± 0.12
a_w	^{a} 0.890 ± 0.021	^{a} 0.903 ± 0.011	^{a} 0.882 ± 0.020
Moisture (%)	^{a} 52.4 ± 3.8	^{b} 56.0 ± 2.0	^{b} 57.0 ± 3.0
Protein (% DM)	^{a} 72.8 ± 7.8	^{a} 70.8 ± 3.6	^{b} 60.0 ± 8.4
Fat (% DM)	^{a} 13.2 ± 3.0	^{a} 12.56 ± 3.0	^{b} 28.9 ± 9.0
Ash (% DM)	^{b} 14.8 ± 1.26	^{b} 16.6 ± 1.5	^{a} 12.26 ± 1.5
Carbohydrate (% DM)	^{b} 0.53 ± 0.31	^{b} 0.92 ± 0.15	^{a} 0.13 ± 0.05
Hydroxyproline (% DM)	^{a} 0.45 ± 0.07	^{a} 0.4 ± 0.07	^{b} 0.65 ± 0.25
Oleic acid (%)	^{a} 35.9 ± 2.6	^{b} 40.6 ± 2.2	^{b} 42.9 ± 2.0
Saturated (%)	^{b} 42.5 ± 2.6	^{ab} 41.5 ± 3.2	^{a} 38.7 ± 2.1
Monounsaturated (%)	^{a} 39.1 ± 2.7	^{b} 47.8 ± 3.7	^{b} 50.0 ± 3.1
Polyunsaturated (%)	^{a} 6.3 ± 2.4	^{a} 4.8 ± 0.9	^{a} 4.4 ± 1.0
Unsaturated (%)	^{a} 45.4 ± 2.0	^{b} 53.0 ± 3.1	^{b} 54.4 ± 3.1
Polyunsaturated/Saturated	^{a} 0.15 ± 0.06	^{a} 0.11 ± 0.02	^{a} 0.11 ± 0.02
ω_3	^{b} 1.8 ± 1.4	^{a} 0.6 ± 0.2	^{a} 1.0 ± 0.3
ω_6	^{a} 4.3 ± 1.3	^{a} 3.3 ± 1.0	^{a} 3.4 ± 0.9
ω_6/ω_3	^{a} 3.5 ± 2.1	^{b} 6.2 ± 2.5	^{a} 3.5 ± 1.1
NaCl (% DM)	^{a} 8.6 ± 1.7	^{b} 13.0 ± 1.2	^{a} 9.6 ± 0.9
Nitrate (ppm DM)	^{a} 120.0 ± 46	^{b} 163.0 ± 40	^{b} 160.7 ± 40
Nitrite (ppm DM)	n.d.	0.9 ^{a} ± 0.1	2.8 ^{b} ± 0.8

$^{a, b, c}$ Means with different letters indicate significant differences (Tukey test: $p<0.05$).
DM: dry matter; n.d.: not detected.

Regarding fatty acid composition, "babilla" showed a lower ($p<0.05$) content than "tapa" and "contra" in oleic acid (35.9% vs. 40.6 and 42.9% respectively), and in consequence in monounsaturated and unsaturated fatty acids. "Babilla" also presented the higher polyunsaturated fatty acids contents, however no differences ($p>0.05$) between pieces were found for this parameter. The amount of saturated fatty acids was significantly different ($p<0.05$) in "babilla" and "contra". The ratio between polyunsaturated and saturated fatty acids was below to that recommended for human diet (>0.4). The highest ($p<0.05$) ω_6/ω_3 was found in "tapa" (6.2), "babilla" and "contra" presented a more favourable balance (3.5) between ω_6 and ω_3 polyunsaturated acid. A dietary ratio of 4 or 5:1 for ω_6/ω_3 polyunsaturated acids is desirable. Higher ratios are less desirable. The proportion is especially important in relation to the incidence of cardiovascular disease (Warriss, 2000).

Concerning the curing agents, the salt content was higher ($p<0.05$) in "tapa", significant differences ($p<0.05$) were also found in nitrite and nitrate content. These values are under their legally established limits (Directive 2001/5/CEE).

Results of colour measurement are shown in Table 2. No differences ($p>0.05$) in L* value were detected among the three pieces. "Babilla" had lower ($p<0.05$) a* and b*, as compared to those of "tapa" and "contra".

Table 2.- Values (mean ± S.D.) of colour parameters in "Cecina".

CIE –LAB coordinates	*"Babilla"*	*"Tapa"*	*"Contra"*
L* (lightness)	^{a} 30.0 ± 2.2	^{a} 29.3 ± 1.8	^{a} 31.7 ± 2.6
a* (redness)	^{a} 7.8 ± 2.5	^{b} 11.6 ± 3.0	^{b} 10.0 ± 2.3
b* (yellowness)	^{a} 1.9 ± 1.4	^{b} 5.7 ± 2.4	^{b} 5.8 ± 2.0

$^{a, b, c}$ Means with different letters indicate significant differences (Tukey test: $p<0.05$).

The values of texture descriptors are shown in Figure 1. No differences ($p>0.05$) were found in cohesiveness and springiness among the three type of "Cecina". On the contrary, "contra" exhibited higher ($p<0.05$) values, both in hardness and chewiness than "babilla" and "tapa". The hydroxyproline content has been previously fitted as indicator for hardness (Rodriguez-Lazaro *et al*., 2001). In this sense, "contra" was the piece that presented a higher ($p<0.05$) hydroxyproline content, this is explained by the higher hydroxyproline content in M. *semitendinosus* (included in "contra") than M. *semimembranosus* (included in "tapa") (Pedersen *et. al*., 1996).

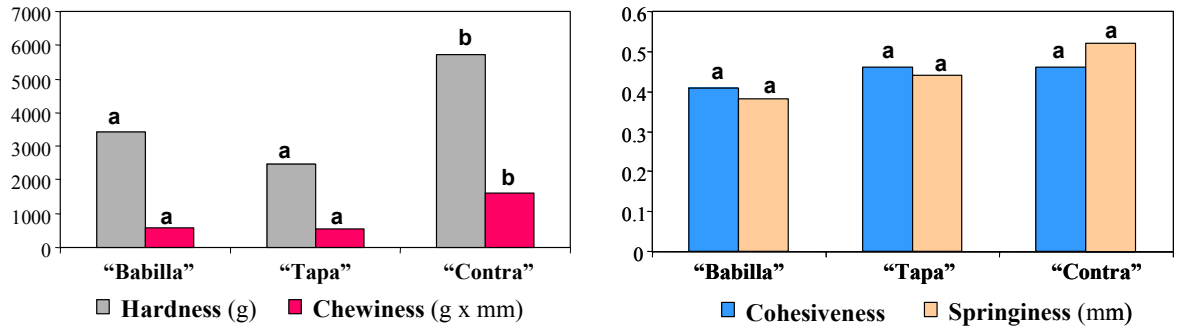

Figure 1.- Mean values of Instrumental Texture Profile Analysis (TPA). (^{abc} Columns with different letters indicate significant differences (Tukey test: $p<0.05$).

Table 3.- Results (mean ± S.D.) of sensorial analysis of the three types of "Cecina".

	"Babilla"	*"Tapa"*	*"Contra"*
Colour homogeneity	^{a} 3.7 ± 0.2	^{a} 3.6 ± 0.4	^{a} 3.4 ± 0.9
Colour intensity	^{a} 3.5 ± 0.5	^{a} 3.5 ± 0.4	^{a} 3.8 ± 0.5
Marbling	^{a} 2.9 ± 0.4	^{a} 2.9 ± 0.8	^{a} 2.5 ± 1.3
Intermuscular fat	^{ab} 3.0 ± 0.5	^{a} 2.2 ± 0.4	^{b} 3.7 ± 1.2
Yellowness	^{a} 1.3 ± 0.4	^{b} 1.8 ± 0.2	^{a} 1.3 ± 0.4
Odour intensity	^{a} 3.0 ± 0.3	^{a} 3.0 ± 0.3	^{a} 3.1 ± 0.6
Hardness	^{a} 2.4 ± 0.8	^{a} 2.3 ± 0.5	^{b} 3.0 ± 0.9
Chewiness	^{a} 2.0 ± 0.3	^{a} 2.1 ± 0.3	^{b} 3.5 ± 0.5
Juiciness	^{a} 3.1 ± 0.2	^{b} 3.5 ± 0.4	^{a} 2.8 ± 0.4
Pastiness	^{a} 1.2 ± 0.1	^{a} 1.2 ± 0.1	^{b} 2.4 ± 0.4
Flavour intensity	^{a} 3.0 ± 0.4	^{a} 3.1 ± 0.3	^{a} 3.1 ± 0.6
After taste	^{b} 3.8 ± 0.1	^{a} 2.6 ± 0.5	^{b} 3.1 ± 0.4

^{a,b,c} Means with different letters indicate significant differences (Tukey test: $p<0.05$).

Results of sensory analysis of "Cecina" are presented in Table 3. No differences ($p>0.05$) were found in colour (homogeneity and intensity), odour intensity and flavour intensity. Concerning fat, marbling was similar ($p>0.05$) between the evaluated pieces, however the presence of intermuscular fat was higher ($p<0.05$) in "contra" than "tapa", which presented the highest yellowness scores ($p<0.05$). On the other hand, the highest values ($p<0.05$) for hardness, pastiness and chewiness obtained in "contra" confirm the instrumental texture results. Finally, the judges considered that "tapa" was more juicy ($p<0.05$) than "babilla" and "contra" although its aftertaste was lower ($p<0.05$).

Conclusions

"Babilla" and "tapa" seem to be the most similar between the types of "Cecina" studied. Both pieces of "Cecina" presented a higher protein content and a lower fat content than "contra". However, "contra" showed the highest acid oleic percentage and a good favourable balance between ω_6 /ω_3 polyunsaturated acids. By the other hand, instrumental Texture Profile Analysis (TPA), as well as sensory evaluation, indicated that "contra" was the hardest piece of "Cecina" studied, but also it had higher chewiness than the other.
Further research is needed for a better understanding of the relationship between anatomical origin of raw meat and final meat product.

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Acknowledgments.

The authors thank the PGI "Cecina de Leon" for providing the samples used in this work. This research was supported by the "Instituto Tecnologico Agrario de Castilla y Leon" project (2003/01001). Molinero C and Rubio B gratefully acknowledges of a grant from Junta de Castilla y Leon-Spain.

EFFECT OF DIFFERENT CONCENTRATIONS OF NATURAL COLORANTS ON THE COLOUR OF FRESH PORK SAUSAGES PACKAGED IN MODIFIED ATMOSPHERE

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Background

Colour is the most deleterious factor as regards the appearance of meat and meat products during storage. It is also the most influential on the consumer purchasing decision. The attractive bright cherry red colour of freshly cut or comminuted meat is used by consumers as an indication of freshness. The preferences of the consumers for natural colorants has been increasing during these last years, because "natural" is often associated with an image of healthy and good quality. Consumers tend to perceive synthetic colorants as undesirable and harmful, and some are considered to be responsible for allergic and intolerance reaction (Blenford, 1995). Stability has been a problem with the use of natural colorants which are often very sensitive to heat, light, acidity, air or water activity changes (Attoe and von Elbe, 1981; Carnevale et al., 1980; Von Elbe et al., 1974). The combined use of colorants and modified atmosphere packaging for meat represents a realistic and attractive strategy to increase the shelf life of fresh meat and fresh meat products. Monascus species produce red colorants through solid state fermentation and have been used as a general food colorant and medical agent for centuries (Went, 1895). Recently, these pigments were tested for their ability to colour different foods, including meat products (Fink-Gremmels and Leistner, 1989). The red beet root (*Beta vulgaris*) is a rich source of red pigments known as betalains (Mabry and Dreiding, 1968).

Objectives

To study the effects of natural pigments of mould *Monascus purpureus* and red beet root juice, compared to betanin (E-162), on the shelf-life of fresh pork sausages packaged in modified atmosphere, in order to select the most appropriate natural colorant for improving their quality.

Materials and methods

Preparation of samples. Four pork forelegs were obtained at 48 h post slaughter from a local supplier (MARBE, Zaragoza, Spain), trimmed of external fat, and ground using an industrial grinder machine. Minced meat was divided in nine batches, which were mixed with NaCl (to a final concentration of 2%) and with either: 1) Control (no colorants), 2) *Monascus purpureus* (0.05%), 3) *Monascus purpureus* (0.1%), 4) *Monascus purpureus* (0.2%), 5) Red beet root juice (0.5 ml / kg meat) , 6) Red beet root juice (1 ml / kg meat), 7) E-162 (0.03%), 8) E-162 (0.05%), 9) E-162 (0.07%). The fresh sausages were stuffed into collagen casings, Colfan F (Viscofan S.A., Caseda, Spain), placed on polypropylene trays, introduced in a pouch made of a polyethylene and polyamide and filled with 80% O2 + 20% CO2 gas mixture. Sausages were stored for 16 days at 2 ± 1°C in the dark.

Red beet juice preparation. Fresh red beet roots (*Beta vulgaris*) were purchased from a commercial store. Samples were washed, dried and cut into cubes of about 1cm x 1cm, which were boiled for 5 min for blanching (Han et al, 1998). After rapid cooling, beet juice was extracted with a standard kitchen food processor. The crude juice was boiled at 100ºC for 1 min. After rapid cooling, it was filtered stepwise (MN 640w, Machinery Nagel GmbH & Co. KG, Düren, Alemania). The clear beet juice was stored at refrigeration until use.

Colour measurement. Meat colour was measured at the sausage surface using a reflectance spectrophotometer (Minolta CM-2002; Osaka, Japan), 30 min after of opening the packing. CIE L*, a*, b* (CIE, 1978) parameters were recorded. The h* value and C* were calculated $h^* = \tan^{-1}(b^*/a^*)$ and $C^* = ((a^*)^2+(b^{*2}))^{0.5}$. The spectra curves were determined over the range of 400-700 nm at 10 nm intervals with Minolta CM-2002. Each value was the mean of 30 determinations.

Statistical analyses. The significance of differences among samples at each day of storage was determined by analysis of variance using the least square difference method of the General Linear Model procedure of

SPSS (SPSS 11.5, 2002). Differences were considered significant at the $p<0.05$ level. Data were also analysed using canonical discriminant analysis (CDA) multivariate statistical method of SPSS (SPSS 11.5, 2002).

Results and discussion

The evolution of different colour parameters is shown in Table 1. Lightness. The use of colorants caused a decrease of L* values in all treatments ($p<0.05$) throughout the whole study. All samples with colorants added were darker than control fresh pork sausages, the higher the concentration the lower the lightness. Bloukas et al. (1999) found that betanin level significantly affected lightness, the higher the betanin level the lower the L* values. Stuempel (1997) also reported lower L* values for frankfurter-type sausages with higher betanin levels; Pipek et al. (1996) found that Monascus extract caused a decrease of brightness in sausages and frankfurters.

Redness. Samples with natural colorant added had higher a* values ($p<0.05$) than control sausages, the higher the concentration the higher the redness. Sayas-Barberá et al. (1987) also found a relationship with a* values and Monascus concentrations. Pipek et al. (1996) found that Monascus extract caused an increase of a* values. The behaviour of red beet root might be explained by betanin degradation by air and light. Bloukas et al. (1999) found that redness of frankfurters steadily increased with the betanin level; similar results were reported by Stuempel (1997).

Yellowness. Samples did not present variations in b* values along the 16 days of storage ($p>0.05$). Neither Klettner (1993), Pipek et al. (1996) nor Sayas-Barberá et al. (1987) found relevant variations in b* values. However, Bloukas (1999) found that use of betanin in frankfurters increased b* values.

a*/b* ratio. Changes of this ratio were very similar to those of a* values.

Chroma. All samples showed a slight increase of C* values at 4th day of storage; after that values decreased ($p<0.05$) along all the storage period. E-162 (0.07%) showed the higher chroma values, near 19, during the first 8 days after that they suffered a very significant decrease. Only Monascus 0.05% presented lower values throughout the experiment ($p<0.05$). Decrease or increase in C* has been associated to increase or decrease in h*, respectively. The typical pink-red colour of fresh pork meat minced is related to low h* and high C* values this colour changed to oxidized brown-grey meat surface (high h* and low C* values).

Hue. As the colour attribute hue increased, the shade of colour changed from red to brown, a gradual rise of hue angle indicated increasing metmyoglobin during storage (Isdell et al., 1999). Changes in hue angles confirmed the results obtained by measuring only a* values. Sayas-Barberá et al. (1987) found that the hue value decreased significantly by using above 40 ppm of Monascus.

Reflectance spectra of control fresh pork sausages and meat with *Monascus purpureus* (0.05%), E-162 (0.05%) and red beet root (1ml) are shown in Fig. 1. The reflectance spectrum of meat with red beet root was very similar to that of the fresh sausage used as control. The use of E-162 gave rise to a spectrum similar to the control, but was lower between 440 and 540 nm and from 590 nm to 700 nm. The spectrum of Monascus was the most different, presenting the lowest reflectance values. Palombo and Wijngaards (1990) pointed out that the shift of the spectrum towards higher reflectance values was due to a remarkable increase of L*, a decrease of h* and a decrease of C* values.

Canonical discriminant analysis is shown in Fig. 2. The two fist canonical functions accounted for 96.2% of variability of fresh pork sausages, therefore, they are a valuable tool for discriminating the different treatments. The most different treatments were Monascus (negative region) and control (positive region); red beet root at different concentrations had an intermediate behaviour between Monascus and the control samples.

Conclusions

The use of natural food colorant improved some colour characteristics of the fresh pork sausages. All samples with *Monascus purpureus*, betanin (E-162) and red beet root presented lower L*, b* and h* values

than control sausages, and higher a*, a*/b* and C* values than control. If red colour is used by consumers as freshness indicator and as willingness of purchasing, the use of natural red colorant enhanced the shelf life of this product. Reflectance spectrum of sausages with red beet root was very similar to control; so this colorant appeared to be most suitable for improving the natural colour of fresh pork sausage.

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Acknowledgements

The authors wish to thank the Commisión Interministerial de Ciencia y Tecnología for research funding (grant AGL2000-1407); Abelló Linde S.A. (Spain), which provided the packaging equipment and gas mixtures; Viscofan S.A (Spain)., which kindly supplied sausage casings; Hela S. L (Navarra, Spain) which kindly supplied Monascus purpureus extract; Sofral (Strasbourg, France) which provided E-162, the Fondo Social Europeo and the Gobierno de Aragón (grant B072/2001, fellowship of author L. Martínez; grant B063/2002; fellowship of author I. Cilla) and Mrs. A. Martínez for skilful technical assistance.

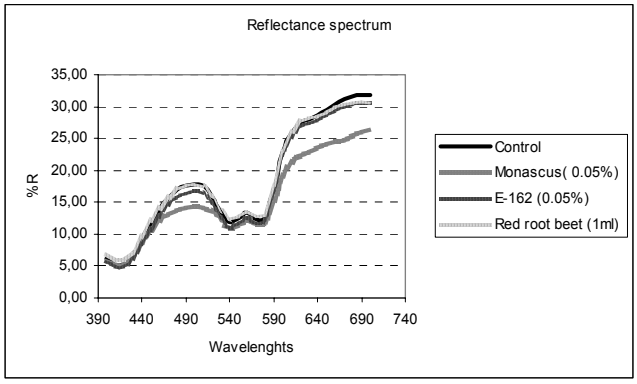

Fig.1. Reflectance spectra of control fresh pork sausages and meat with *Monascus purpureus* (0.05%), E-162 (0.05%) and red beet root (1ml).

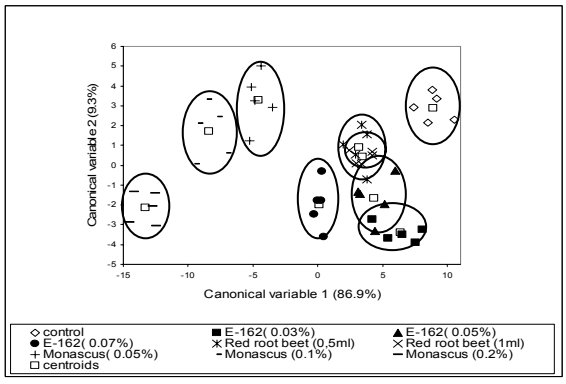

Fig2. Canonical discriminant analysis of control fresh sausages and meat with different concentrations of Monascus purpureus (0.05%, 0.1%, 0.2%), E-162 (0.03%, 0.05%, 0.07%) and red beet root (0.5; 1ml).

Table 1. Evolution of different colour parameters of fresh pork sausages packaged in modified atmosphere with different concentrations of natural colourants.

		Days of storage				
	Treatments	**0**	**4**	**8**	**12**	**16**
L*	Control	43.33aw	43.68aw	44.27aw	45.13avw	47.86av
	E-162 (0.03%)	38.10cw	38.15cw	38.44cw	39.60cvw	41.93cv
	E-162 (0.05%)	38.83cw	37.55cw	38.85cw	40.26bcv	41.21cv
	E-162 (0.07%)	38.24cw	36.83cw	37.61cw	39.74cv	40.00cv
	Red root beet (0,5ml)	41.00bw	40.59bw	41.83abw	41.45bw	46.15av
	Red root beet 1ml	40.73bw	40.18bw	41.13bw	41.28bw	44.94abv
	Monascus (0.05%)	40.73bw	41.87abw	43.15av	42.83bvw	44.63bv
	Monascus (0.1%)	39.6bcw	41.15abvw	41.12bvw	40.01bcw	42.58cv
	Monascus (0.2%)	38.40cvw	39.13bv	39.45cv	37.9dw	38.25dvw
a*	Control	9.31dv	10.08ev	9.32ev	6.06dw	1.25ex
	E-162 (0.03%)	10.80cv	11.25dv	10.11dv	8.73cw	3.50cx
	E-162 (0.05%)	13.01bv	13.30cv	12.18bw	11.51bw	6.08bx
	E-162 (0.07%)	14.00abv	14.61bv	14.57av	11.28bw	6.89bx
	Red root beet (0,5ml)	10.91cv	11.42dv	9.97dew	6.88dx	1.67dey
	Red root beet 1ml	11.15cv	11.58dv	10.04dw	8.44cx	2.33dy
	Monascus (0.05%)	10.08cdw	11.60dv	11.18cv	8.61cx	2.49dy
	Monascus (0.1%)	12.63bv	13.03cv	12.58bv	10.70bx	5.68by
	Monascus (0.2%)	14.89av	15.60av	14.94av	14.02aw	12.40ax
b*	Control	13.21av	13.50av	13.40av	13.54av	13.20av
	E-162 (0.03%)	13.00av	13.20av	13.30av	13.30av	12.60av
	E-162 (0.05%)	12.74av	12.97av	13.01av	13.10av	12.48av
	E-162 (0.07%)	12.32av	12.40av	12.39v	12.34av	12.30av
	Red root beet (0,5ml)	12.43av	12.60av	12.67av	12.58av	12.48av
	Red root beet 1ml	12.53av	12.70av	12.75av	12.50av	12.60av
	Monascus (0.05%)	11.24bv	11.35bv	11.40bv	11.22bv	11.47bv
	Monascus (0.1%)	10.60bcv	10.92bcv	10.62bcv	10.98bcv	10.7bcv
	Monascus (0.2%)	9.72cv	10.11cv	9.77cv	10.10cv	10.04cv
a*/b*	Control	0.70fv	0.75fv	0.70dv	0.45fw	0.09fx
	E-162 (0.03%)	0.83ev	0.85ev	0.76dw	0.66dx	0.28dy
	E-162 (0.05%)	1.02cv	1.03cv	0.94cv	0.88bw	0.49cx
	E-162 (0.07%)	1.14bv	1.18bv	1.19bv	0.92bx	0.57by
	Red root beet (0,5ml)	0.88dv	0.91dv	0.79dx	0.55ey	0.13ez
	Red root beet 1ml	0.89dv	0.91dv	0.79dw	0.68dx	0.18ey
	Monascus (0.05%)	0.90dw	1.02cv	0.98bx	0.77cy	0.23dz
	Monascus (0.1%)	1.19bv	1.19bv	1.19bv	0.97bx	0.53cy
	Monascus (0.2%)	1.53av	1.54av	1.53av	1.42ax	1.23ay
C*	Control	16.16bv	16.85bcv	16.32cv	14.83bw	13.26bx
	E-162 (0.03%)	16.90bv	17.30abv	16.70cv	15.91abw	13.08bx
	E-162 (0.05%)	18.21av	18.57av	17.82bvw	17.44aw	13.88bx
	E-162 (0.07%)	18.65av	19.16av	19.05av	16.62aw	13.86bx
	Red root beet (0,5ml)	16.54bv	17.01bv	16.13cv	14.34bcw	12.59bcx
	Red root beet 1ml	16.77bv	17.19bv	16.23cv	15.08bw	12.81bcx
	Monascus (0.05%)	15.10cvw	16.22cv	15.96cv	14.14cw	11.74cx
	Monascus (0.1%)	16.49bv	17.00bv	16.46cv	15.33bw	11.98cx
	Monascus (0.2%)	17.78av	18.59av	17.85bv	17.53av	15.96ax
H*	Control	54.82ax	53.26ax	55.18ax	65.89aw	84.60av
	E-162 (0.03%)	50.28bx	49.57bx	52.77abx	56.72cw	74.49cv
	E-162 (0.05%)	44.39cwx	44.28cx	46.89cw	48.70ew	64.02dv
	E-162 (0.07%)	41.35dx	40.32dx	40.10dx	47.29ew	60.16ev
	Red root beet (0,5ml)	48.71by	47.80by	51.79bx	61.33bw	82.36av
	Red root beet 1ml	48.33by	47.65by	51.76bx	55.96cw	79.53bv
	Monascus (0.05%)	48.12bx	44.38cy	45.57cy	52.48dw	77.76bv
	Monascus (0.1%)	40.01dx	39.96dx	40.16ex	45.75ew	63.27dv
	Monascus (0.2%)	33.14ew	32.95ew	33.19fw	35.20fw	39.01fv

pH, WATER ACTIVITY, AND PROXIMATE COMPOSITION OF *MORCILLA DE LEÓN*, A TRADITIONAL EUROPEAN BLOOD SAUSAGE

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Background

Among the diverse types of meat products in the world, there are some typical products which are elaborated with blood; these are named as "blood sausages". These sausages have rarely been studied, being found some bibliographic references in Germany (Souci, *et al*., 1989; Stiebing, 1990), Spain (Antiduelo, 2002; Santos *et al*., 2003) and Portugal (Roseiro *et al*., 1998) and some countries from Latin American (Bunger *et al*., 1992, Adesiyun *et al*., 1996). Blood sausages are made (besides blood) with ingredients such as fat, offal, onion, cereals, spices, etc., existing a great diversity of ingredients as well as ways of processing and preparation. The *Morcilla de León* is a typical product from Castilla and León (Spain), being onion (65%–75%), pork and/or beef fat (10%–20%), pork and/or beef blood (10%–20%), rice (2%–5%), salt (1%–1.5%) and spices the main ingredients in its composition (Antiduelo, 2002). Process usually consists on precooking the mixed of ingredients, which are then stuffed into a pork casing and cooked in hot water (80-100ºC). Finally, *Morcilla de León* is consumed fried or boiled.

Objectives

This study is aimed to contribute to *Morcilla de León* typification, a typical regional Spanish sausage, by studying its proximate composition and comparing results from this blood sausage with other results from different blood sausages in the World.

Materials and methods

Samples of blood sausages were purchased from the retail market coming from nine different meat industries which were located in León City and other smaller towns nearby. Once acquired, the samples were immediately transported to laboratory in refrigerated conditions. There, samples were homogenised and the following analysis were accomplished. pH was measured in fresh sample by duplicating according to the official method for meats (Presidencia del Gobierno, 1979). Water activity (a_w) was measured by a dewdrop point method with an Aqualab CX-2 (Decagon Devices, Washington, USA). Moisture was determined according to ISO (1973). Fat content of the dehydrated sample was measured by extraction with petroleum ether according to AOAC (1999a). Total protein was determined by the Kjeldahl method according to AOAC (1999b), by using 6.25 as conversion factor. Ash content was measured by incineration sample at 550ºC, according to Presidencia del Gobierno (1979). Finally, digestible carbohydrates extraction was carried out with 52% perchloric acid following the Presidencia del Gobierno (1982) method and quantification was made with the method described by Dubois *et al*. (1956). Relative analyses on composition were determined by duplicate and data were expressed as percentage on fresh and dry matter.

Results and discussion

pH and a_w values for *Morcilla de León* are shown at table 1, in which it is indicated that the pH averages c.a. 6 and the a_w averages 0.97. These parameters, which depend mainly on used ingredients in the sausage, i.e. onion, blood, fat and salt, were similar to those found by Santos *et al.* (2003) in the '*Morcilla de Burgos'*, another typical blood sausage from Spain made mainly with onion, rice, blood and fat. Due to the high pH and a_w of the sausage and the absence of preservatives in the formula, *Morcilla de León* is susceptible to early spoilage, its shelf-life depends on the initial microbial population of the mixture, heat treatment intensity in the boiling stage, handling conditions after boiling, and storage temperature. Anyway, during chilled storage a pH decrease (c.a. 0.5) has normally been observed which could be attributed to microbial growth (non published data).

Proximate composition of *Morcilla de León* is shown at tables 2 and 3. Regarding to moisture content, it was also similar to that of *Morcilla de Burgos* (Santos *et al*., 2003) but 11% lower than blood sausage from Chile (Bunger *et al*., 1992) and 21% and 25% higher than the moisture of *Morcela de Assar* from Portugal (Roseiro *et al*., 1998) and German '*Blutwurst'* (Souci *et al*., 1990), respectively. The reason for this variability could be explained by the differences in the types and amounts of ingredients used in the formulation of each kind of blood sausages. Furthermore, fat is probably the most variable proximate component in meat products. The fat content on dry matter found in different blood sausages has had a range between 28% of Morcilla de Burgos to 70% of Blutwurst (Souci, *et al.*, 1990, Bunger *et al.*, 1992, Roseiro *et al*., 1998, Santos *et al.*, 2003,), and the fat content on dry matter of *Morcilla de León* was into an intermediate place. In the same way, protein percentage on dry matter of *Morcilla de León* was slightly higher than those found in *Morcilla de Burgos,* and lower than those of other blood sausages.
Digestible carbohydrates in the *Morcilla de León*– fraction constituted basically by starch and soluble sugars – were 27% of the dry matter. This percentage was rather lower than in *Morcilla de Burgos* –51% on dry matter– because more rice (20% to 35% of rice) is added to make the last sausage (Santos *et al*., 2003) while the amount of rice or bread that is added to the *Morcilla de León* is about up to 10% (Antiduelo, 2002). The amount of cereals or other vegetables used for making other blood sausages such as Blutwurst, Chilean blood sausage or *Morcela de Assar* is even less and due to it its results of digestible carbohydrate. The presence of more than 1% of fiber in the *Morcilla de León* as well as in other blood sausage (Bunger et al., 1992; Santos et al., 2003) is also due to the addition of vegetables as ingredients, such as the onion, which, as it was said before, is the principal ingredient of *Morcilla de León* (c.a. 70%). Fiber content of cooked onion is approximately 1.5% (USDA, 2003). Finally, ash content of *Morcilla de León* was in the higher part of the range observed for blood sausages (4.3% a 8.4% on dry matter) (Roseiro *et al*., 1998, Santos *et al.*, 2003, Bunger *et al.*, 1992, Souci, *et al.*, 1990).
Table 4 contains the correlations between moisture, fat, ash and protein contents of *Morcilla de León* expressed as dry matter. Only the correlation digestible carbohydrates *vs.* fat –the two most abundant components– was statistically significant ($p<0.05$).

Conclusions

'Morcilla de León' is a cooked product subject to easy microbial spoilage which needs chilled storage. It represents a nutritional source of fat, carbohydrates, fiber and protein –mainly from blood–; nonetheless it has less protein than meat and most of meat products.

Acknowledgements

This research has been financially supported by the University of León and the "Exma. Diputación Provincial de León".

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Table 1. Mean, standard deviation (S.D.), maximum and minimum values of pH and a_w of *Morcilla de León*.

	Mean	S.D.	Minimum	Maximum
pH	6.1	0.4	6.0	7.1
a_w	0.972	0.003	0.969	0.977

Table 2. Proximate composition of *Morcilla de León* expressed as percentage of fresh matter.

	Mean	S.D.	Minimum	Maximum
Moisture	66.2	4.8	59.8	75.0
Fat	14.2	3.0	9.0	17.2
Total protein	5.7	0.8	4.4	7.0
Ash	1.9	0.2	1.7	2.1
Digestible carbohydrates[#]	9.0	1.8	6.4	10.8
Fibre[*]	3.0	---	---	---

[#] Expressed as % of glucose, [*] Estimated by difference.

Table 3. Proximate composition of *Morcilla de León* expressed as percentage of dry matter.

	Mean	S.D.	Minimum	Maximum
Fat	42.1	6.6	32.1	51.3
Total protein	17.1	2.8	13.0	20.8
Ash	6.6	1.4	4.9	8.6
Digestible carbohydrates[#]	27.0	6.1	20.1	38.8
Fibre*	7.2	---	---	---

[#] Expressed as % of glucose, [*]Estimated by difference.

Table 4. Correlations between the parameters of the proximate composition of *Morcilla de León*, expressed in terms of dry matter.

	Moisture	Fat	Ash	Total protein	Digestible carbohydrates
Moisture	1.00	-0.08	0.05	0.63	0.41
Fat	---	1.00	0.20	-0.00	**-0.74**
Ash	---	---	1.00	-0.27	-0.07
Total protein	---	---	---	1.00	-0.14
Digestible carbohydrates	---	---	---	---	1.00

Values in bold were significant ($p<0.05$)

THE INFLUENCE OF WINE COMPONENTS ON THE TENDERNESS AND HISTOLOGICAL STRUCTURE OF COOKED MEAT

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Background

Marinating in wine have long been used as a mean of reducing toughness and consequently to improve tenderness of beef muscles. In the case of typical German cuisine "Sauer Brauten", beef round meat was soaked in red wine for 2-3days and subsequently was cooked. Some authors reported that the tenderizing effect of wine was related to various components of wine (organic acids, sugar, polyphenol and alcohol [1), 2), 3), 4)]. However more studies are necessary to examine the relationship between the components of wine and meat tenderness.

Keywords: Wine, marinating, cooked meat tenderness, histological structure

Objectives

The aim of this study was to find the effect of various components of red and white wine on the meat tenderness and histological structure.

Materials and methods

Beef round meat was obtained from commercial source. After the fat and tendon (perimycium) were removed, the meat was cut into pieces weighing about 150g (2cm thick). Each piece of meat was put into plastic bag(Asahi Kasei H N type) and then soaked in each solution(water, white wine, red wine, 0-50mg Tannin, 0-0.6%tartarir acid, 0-2%glucose, 0-20%ethylalcohol) for 5 hours. These samples were heated at 100□ for 30min in the water bath. Thereafter five 1.27cm diameter cores, taken parallel to the muscle fiber, were removed from one piece per sample. Cores were sheared with Warner-Blatzler Meat Shear Model 2000. Shear force Values were recorded as kg force/1.27 cm sample. Samples for histological observation of meat structure were frozen in the freezer and cut into thin vertical sections. Observations were performed using an optical microscope (Nikon optiphoto).

Results and discussion

Shear force value of cooked meat

Figure 1 shows the mean and individual shear force values obtained for cooked meat marinating in water, red wine and white wine. There were no significant differences between red wine, white wine and water (control).

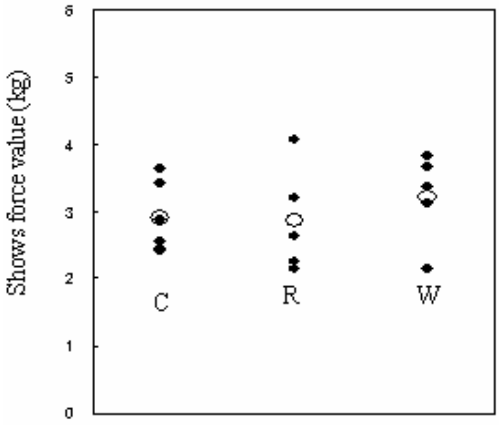

Fig.1

Figure 1 shows the shear force value of cooked meat marinated in C: water, R: red wine, W: white wine

Figure 2-a,b,c,d showed the shear value of cooked meat marinating in tannin, tartaric acid, glucose and ethyl alcohol, respectively.
As, shown in Figure 2-a, the concentration of tannin increased from 0 to 50 mg%, the shear force value of cooked meat increased from 2.4 kg to 3.9kg (correlation coefficient=0.67). There was a similar trend for the increase of glucose concentration (Figure 2-c). Whereas Figure 2-b indicated that there was the excellent correlation (-0.95) between decreased tenderness and increased tartaric acid concentration from 0 to 0.6%. A similar trend was observed in increasing of ethyl alcohol concentration (Figure 2-d).

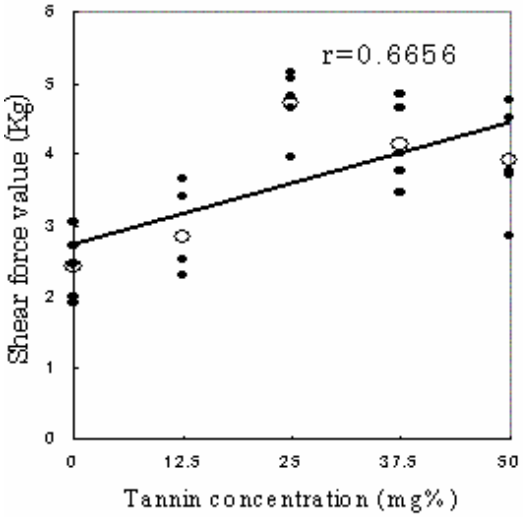

Fig.2-a

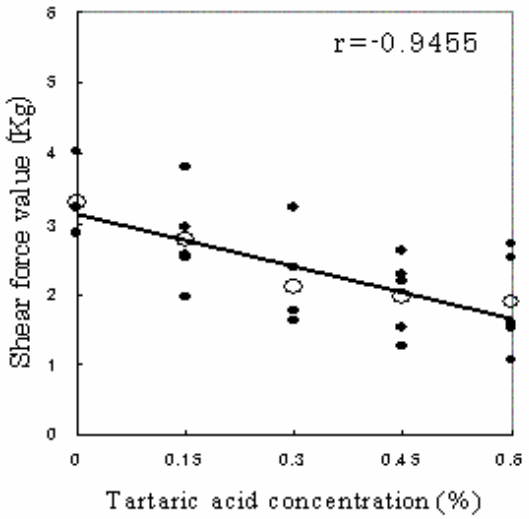

Fig.2-b

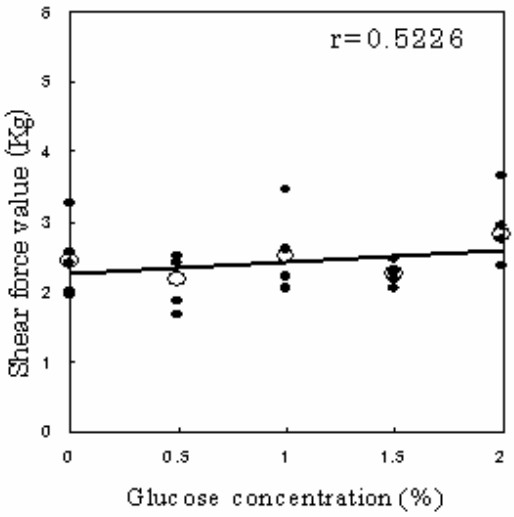

Fig.2-c

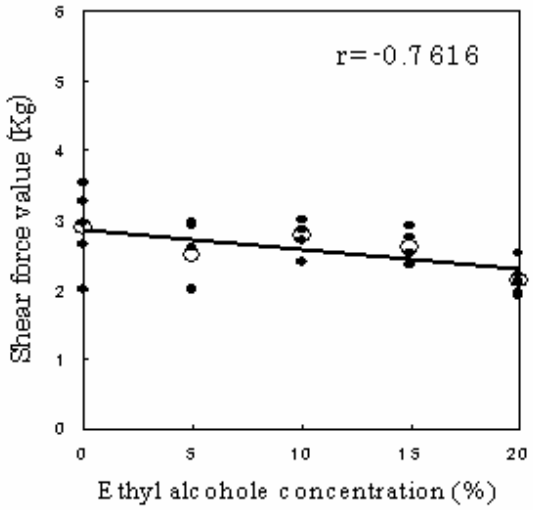

Fig.2-d

Figure 2 compares the shear force value of cooked meat marinated in (a) tannin, (b) tartaric acid, (c) glucose, (d) ethyl alcohol.

Structure of cooked meat

Figure 3-a,b,c,d,e showed the structure of marinated meat in water, tannin, tartaric acid, glucose and ethyl alcohol. The marinating in tannin leads to the remarkable shrinking of muscle fiber, simultaneously, that appearance also observed for marinating in glucose. Whereas in the case of marinating in tartaric acid and in ethyl alcohol no shrinkage occurred, but appeared horizontal cracks in muscle fiber structure.

Fig.3-a Water

Fig.3-b Tannin

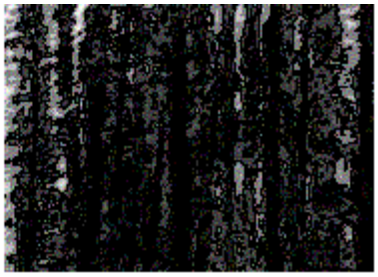

Fig.3-c Tartaric acid

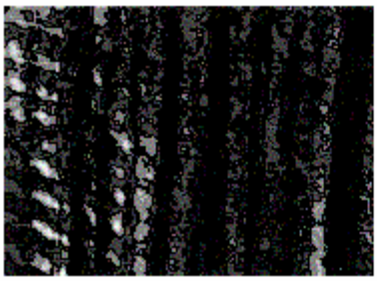

Fig.3-d Glucose

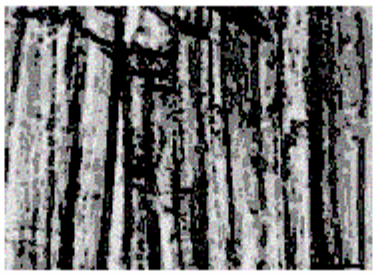

Fig.3-e Ethyl alcohol

Figure 3 showed the microstructure of cooked meat marinated in (a) water, (b) tannin, (c) tartaric acid, (d) glucose, (e) ethyl alcohol.

Conclusions

There were no significant differences in shear force value obtained for cooked beet round meat marinating in red wine, white wine and water. Marinating in tartaric acid was most effective to tenderizing of meat. When the meat soaked in Tannin, the shear force value of cooked meat increased as concentration of tannin increased. This treatment leads to the remarkable shrinking of muscle fiber.
These results indicate that the excellent correlation between increased toughness of cooked meat associated with shrinking of muscle fiber.

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QUALITY OF LOW-FAT MEATBALLS CONTAINING LEGUME FLOURS AS EXTENDERS

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Background

Ground meat is used for the production of a variety of traditional meat products such as meatballs, kebabs and döner in Turkey. The meat is mixed with spices and extenders then formed and cooked. Various extenders and binders such as egg, flours and breadcrumbs are used in ground meat formulations to bind the meat particles. Legumes are economic source of protein and also various minerals. In recent years legumes have been investigated regarding to their potential use in developing functional foods. Inclusion of legumes in the daily diet has many physiological effects in controlling and preventing various metabolic diseases such as mellitus, coronary heart disease and colon cancer (Tharanathan and Mahadevamma, 2003).

Beef sausages extended with common bean flour had higher water holding capacity, lower cooking loss and lower protein content (Dzudie et al. 2002). Modi et al. (2003) investigated the effect of Bengal gram, green gram and black gram flours in meatballs, and gram flour in burgers. Beef patties extended with soy flour and samh flour had lower water activity and moisture content but protein content was not affected by the addition of extenders (Elgasim and Al-Wessali, 2000).

Objectives

The objective of this research was to evaluate the effects of legume flours (blackeye bean, chickpea and lentil) and rusk on proximate composition, fatty acid composition and lipid oxidation of low-fat meatballs.

Materials and methods

Beef as boneless rounds was obtained from the local butcher in Izmir, Turkey. All subcutaneous fat and inter-muscular fat was removed from the muscles and used as the fat source. Lean and fat were ground through a 3 mm plate grinder. Legumes; blackeye bean (*Vigna unguiculata,*23.5% protein, 1.5% fat), chickpea (*Cicer arietinum,* 20.6% protein, 4.5% fat), lentil (*Lens culinary*, 23,5% protein, 1.2% fat) and rusk (12,4 protein, 4.5 fat) used in this study were obtained from a local market. Each legume was soaked (ratio of 1:2 legume to water) for 12 hours and cooked for 1.5 hours in boiling water. Cooked legumes were dried separately in electric oven (100ºC) for two hours and grind in a mill. Similarly rusk was also prepared in the same mill to obtain a fine structure and all above the flours were used as extenders. The minced beef was then mixed with 10% (g/100g) extender, 7% beef fat, 0.3% onion powder, (0.8% spice mix) and 2% salt. Batches of 2 kg of each formulation were mixed with food processor and processed into meatballs (1cm thick and 80 mm diameter) by using a metal shaper.

Moisture and ash content of each meatball were measured by using AOAC procedures. Fat content was determined by chloroform-methanol extraction according to Flynn and Bramblett (1975). Protein content was determined according to Anonymous (1979). Lipids were extracted from 10 g samples with chloroform : methanol (2:1 v/v) (Folch et al.1957) and methylated (Anonymous, 1987). Fatty acid methyl esters (FAME) were analyzed using a gas chromatography (HP5890) fitted with a fused silica capillary column (DB-23, 30 mx 0.25 mm id., 0.25 µm film thickness, J.W.Scientific). The column temperature programmed 100ºC to 220 ºC in 4 ºC/min and 15 min at 220ºC. The injector temperature was set at 220ºC and the detector (FID) temperature was set at 220 ºC. The carrier gas was hydrogen at a flow rate of 1 ml/min. The fatty acids were identified by comparision of the retention times of the sample with those of standards.

Meatball samples were frozen at -18°C for 3 months in polypropylene boxes with lids. On 0 th day and 1st, 2nd and 3rd months of frozen storage , samples were thawed at 4°C and oxidative rancidity of meatballs was determined by thiobarbuturic acid test (TBA) according to Tarladgis et al. (1960). Data were subjected to one-way analysis of ANOVA (Minitab, 2003).

Results and discussion

Mean values for proximate composition of uncooked and cooked meatballs are given in Table 1. For uncooked and cooked samples the moisture, fat and ash contents in the formulations of different extenders were almost the same ($p>0.05$). Uncooked meatballs had a fat content ranging from 8.5 to 9.1%, cooked meatballs had a fat content ranging from 7.9 to 8.8%. Incorporation of legume flours increased protein contents of meatballs ($p<0.05$). Extended with BBF and CF slightly increased the ash content of raw meatballs. Meatballs formulated with rye bran had higher ash content than all meat control (Yılmaz, 2004).

Cooking slightly decreased moisture and increased protein content of meatballs. The protein content ranged from 18.8 to 21.1% for uncooked meatballs, from 19.3 to 23.5% for cooked meatballs. Fat and protein contents of meatballs were within the limits of Turkish Uncooked Meatball Standard (TSE, 1992). Extended with BC resulted lowest (18.8% uncooked, 19.3% cooked) protein content. Several researchers have found that protein content of comminuted meat products increased with the addition of soy proteins (Tömek et al. 1988), cowpea flour (Prinyawiwatkul et al. 1997).

Table 5 shows the changes in TBA values. On 0^{th} day, no differences were observed between the TBA values of meatball samples. In other investigation periods meatballs with BBF and CF had similar TBA values and these values were lower than the TBA values of meatballs with LF and meatballs with R. Antioxidative properties of some fruits and vegetables have been showed in various meat products (Ulu, 2004; Mansour and Khalil, 2000). At the end of the storage period all meatballs had TBA values in consumable limits and were 2.11 mg ma/kg for BBF, 1.99 mg ma/kg for CF and 2.88 mg ma/kg, 2.55 mg ma/kg for LF and R treatments respectively. Ulu (2004) concluded that 0.2% soya protein isolate was effective retarding lipid oxidation in cooked meatballs.

Unsaturated fatty acids were in similar amounts in all formulations. Our results are similar to those determined by Yılmaz (2004), Yılmaz and Dağlıoğlu (2003) for unsaturated and saturated fatty acids. Meatballs extended with BBF and CF had similar concentrations of total saturated and unsaturated saturated fatty acids. Meatballs with LF and R had similar concentrations of total saturated and unsaturated fatty acids. There were significant differences between the amounts of total polyunsaturated fatty acids of meatball samples; samples with rusk had the highest amount of total polyunsaturated fatty acids.

Conclusions

This study suggests that legume flours (blackeye bean, chickpea and lentil) can be successfully used in meatball formulations as extenders. Protein content of meatballs increased with the addition of legume flours. Legume flours are potential source of non-meat protein for meatballs. Blackeye bean flour and chickpea flour slightly retarded oxidative changes during frozen storage. There is significant difference among the meatball samples in respect to fatty acid composition.

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Table 1 Chemical composition of uncooked and cooked meatballs

Sample	Raw Meatballs				Cooked Meatballs			
	Moisture %	Fat%	Protein%	Ash%	Moisture %	Fat%	Protein%	Ash%
BBF	63.1±1.04[A]	8.7±0.28	22.0[b]±0.52	3.6±1.46	58.2±0.43	8.8±0.29	23.2[b]±0.15	2.7±1.16
CF	64.1±0.03	8.5±0.09	21.1[b]±0.34	3.2±0.02	57.4±0.35	7.9±0.71	23.5[b]±0.48	2.6±0.61
LF	65.0±0.54	9.1±0.36	21.1[b]±0.09	2.8±2.76	60.5±1.86	8.7±0.48	23.5[b]±0.43	2.8±1.10
R	63.0±0.42	8.5±0.59	18.8[a]±0.52	2.7±0.05	59.7±0.05	8.3±0.77	19.3[a]±0.26	2.8±1.12
P	NS	NS	0.012	NS	NS	NS	0.082	NS

BBF: blackeye bean flour, CF: chickpea flour, LF: lentil flour, R: rusk, [A]Standard deviation, NS: non-significant, [a-b] Different superscripts in the same column indicate significant differences ($p<0.05$)

Table 2. Fatty acid composition of meatball samples.

Fatty Acids	BBF	CF	LF	R
Saturated	47.9[a]	47.4[a]	39.6[b]	36.0[b]
Monounsaturated	23.9[c]	47.3[a]	37.9[b]	27.5[c]
Polyunsaturated	28.2[b]	5.5[c]	22.5[b]	36.5[a]
Unsaturated	52.1	52.8	60.4	64.0
Unsaturated/Saturated	1.1	1.1	1.5	1.8

BBF: blackeye bean flour, CF: chickpea flour, LF: lentil flour, R: rusk, [a-c] Different superscripts in the same column indicate significant differences ($p<0.05$)

Table 3 Changes in TBA values of meatball samples (mg ma/kg)

Sample	0th day	1st Month	2nd Month	3rd Month
BBF	0.67[x]±0.76[A]	1.02[ay]±0.95	1.67[az]±0.49	2.11[az]±0.63
CF	0.75[x]±0.12	1.13[ay]±0.22	1.55[ay]±0.63	1.99[ay]±0.91
LF	0.82[x]±0.23	1.88[by]±0.67	2.27[by]±0.07	2.88[bz]±0.21
R	0.63[x]±0.88	1.88[by]0.55	2.11[by]±0.56	2.55[by]±0.14

BBF: blackeye bean flour, CF: chickpea flour, LF: lentil flour, R: rusk, [A] Standard deviation, [(a-b)] treatments within the same storage condition with the same superscripts are not different, [(x-z)] storage conditions within the same treatment with the same superscripts are not different.

DEVELOPMENT OF BEEF BURGER MEAT PATTIES ENRICHED IN CLA

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Background

Conjugated linoleic acid (CLA) refers to a group of fatty acids comprised of different positional (placement of the C=C double bonds along the carbon chain) and geometrical (configuration around the C=C double bonds) isomers of linoleic acid (cis-9(Z), cis-12(Z) C18:2). Conjugated fatty acids are predominantly found in the fats and tissues of ruminants (C18:2 and C18:3) with a few plant (C18:3) and marine (C18:4, C20:4, C22:6) sources also known (Parodi, 1977; Chin et al., 1992; Cahoon et al., 1999; Hamberg, 1992). Typically over 80% of the CLA in dairy products is composed of a single isomer, cis-9(Z), trans-11(E) C18:2 (Sehat et al., 1998). Much interest has been generated for CLA in recent years due to the demonstration of a number of health-promoting biological activities in animals, including anticarcinogenic, antiatherogenic, antidiabetogenic, and immunomodulatory effects. In this regard CLA may be a very important functional food ingredient that food industries will need to take advantage of in future developments if the same health benefits are shown to occur in humans.

Objectives

To demonstrate how natural ingredients increase the CLA content in ruminants through dietary intervention and the use of the adipose fat depot as a functional ingredient enriched in CLA for the production of other food products such as burgers.

Materials and methods

Sixty Charolais crossbred heifers (mean initial bodyweight = 333kg, s.d. 39.90) were blocked by initial bodyweight and, within block, were randomly assigned to one of four dietary treatments (n = 15): 1) indoor, silage/concentrates (control, SC); 2) unsupplemented grazing (G); 3) restricted grazing plus 2kg/head/day of linseed oil-enriched meal (LSG) and 4) restricted grazing plus 2 kg/head/day of sunflower oil-enriched meal (SFG). Concentrates and grass allowances were monitored at three-week intervals during a 5-month experimental period to achieve similar carcass weights across the treatments. Animals were slaughtered at a commercial facility, carcasses were chilled for 48 h at 4°C, and the *M. semimembranosus* (SM) and subcutaneous adipose tissue (SC) were excised from each carcass.

The fatty acids (FAs) were extracted and saponified from tissue samples in 6mL 5M KOH in methanol/water (50:50) at 60ºC for 1 hour and methylated using trimethylsilyl-diazomethane in methanol:toluene (2:1 %v/v) at 40ºC for 10 min based on a modified method by Elmore *et al*. (1999). Separation of fatty acid methyl esters (FAMEs) was performed on a Varian CX3400 GC, using a BPX-70 column (120m x 0.25mm i.d., 0.2□m film thickness, SGE, Australia) with a programmed temperature ramp. Injector and detector were set at 270°C and 300°C respectively. The carrier gas was hydrogen set to a flow rate of 1.6ml/min, measured at the initial temperature and using a split ratio of 50:1. FAMEs were identified according to similar peak retention times using standards (Sigma Chemical Co. Ltd., Poole, U.K.), and quantified according to the use of an internal standard ($C_{23:0}$ methyl ester) with its addition prior to saponification. The data was analysed as a randomized block design using MiniTab 14.

Adipose tissue was evaluated for its content of beneficial fatty acids, and, its suitability as an enriched source of CLA that could be used in product development. Beef burgers were produced from the *M. semimembranosus* and the CLA-enriched fat. Fatty acid analysis and sensory evaluation was carried out on these burgers to determine the effect if any on flavour and textural quality attributes

Results and discussion

Fatty Acid Results:

A processed meat product was produced and made available for CLA and fatty acid determinations. The *M. semimembranosous* was used in conjunction with carcass fat to produce beef burgers. Burgers were compared with respect to the initial dietary treatments (table 1). Of interest is the following: 1. the fatty acid content of burgers is quite different to the lean muscle (because of the high percentage of adipose tissue present in the burgers) – long chain polyunsaturates are less abundant (C20:4, C20:5, C22:5, C22:6) but a relatively high amount of C18:1 trans-11 (TVA) and CLA is present; 2. quite marked treatment affects are observed illustrating the production of burgers high in certain fatty acids such as CLA (table 1), diets rich in linoleic acid such as the sunflower oil supplemented diet drive the synthesis of CLA cis-9, trans-11 and C18:1 trans-11 (trans vacenic acid). The results also illustrate a large difference compared to conventional indoor silage-based diets (the content of CLA in silage - control Vs sunflower oil supplemented diet differs by more than 400%!, figure1).

Of the four different dietary treatments the linseed diet contained the most polyunsaturates (PUFA), followed by the sunflower diet and pasture diet with the silage diet containing the least amount of PUFA. For the total saturated fatty acids (SFA) the positions were reversed with the control diet containing the most SFA, followed by the pasture diet and sunflower diet while the linseed diet contained the least amount of SFA. The P:S ratio was highest for the linseed diet and lowest for the silage diet. The n-6: n-3 ratio was lowest for the pasture based diet, followed by the linseed, then silage and finally sunflower oil diets.

Sensory Evaluation:

An inhouse, trained panel of 20 people from different backgrounds was established to access the following sensory attributes: texture (hardness, softness), juiciness, and flavour quality. In addition they were also asked to give an overall acceptability score for the various samples. Samples were graded on a categorical scale from 1 to 6, were 1 is generally least favoured, to 6 the most preferred. In general, higher preference scores were associated with the pasture and oil-supplemented diets. Significant differences ($P< 0.05$) were observed between the silage and the pasture and oil-supplemented diets. It must be stressed however, that these results do not imply that meat from the silage based diet tasted bad or was unacceptable, merely that the panellists showed a trend favouring burgers from the other dietary treatments.

Conclusions

Supplementing grazing animals with plant oil-enriched concentrates resulted in a further beneficial effect on the fatty acid composition of muscle and fat compared to grazing alone. Sunflower oil was more effective in increasing the concentration of CLA and TVA, but had a negative effect on the n-6:n-3 ratio, while linseed oil supplementation had a less pronounced effect on the CLA concentration than sunflower oil.

Beef burgers enriched in CLA were successfully produced and sensory evaluation indicated that there was no detrimental effect on burger flavour and other sensory qualities. In fact, many sensory attributes are improved by pasture and oil supplementation (based on burger sensory analysis).

The results clearly show the suitability of fatty tissue from oil-supplemented diets to be a particularly rich source of CLA. An interesting feature here is the difference in the qualitative fatty acid profile and the relative abundance of individual fatty acids when comparing adipose tissue and lean muscle tissue. The differences presumably reflect the two distinct biological roles these tissues play with respect to their lipid profiles. Adipose tissue is a predominantly storage depot of fat, while muscle adjusts its content of fatty acids (predominantly the phospholipids fraction) to suit the functioning of its membrane systems (calcium ion storage and electrical impulse conduction).

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	Fatty acids (mg 100g^{-1} Burger)				
	SC	G	SFG	LSG	P
Palmitic acid (C16:0)	8417 (613)	6883 (965)	6334 (707)	6427 (810)	***
Stearic Acid (C18:0)	3440 (222)	3596 (594)	3685 (401)	3488 (433)	NS
TVA (C18:1*trans*11)	380 (40)	1051 (165)	2510 (263)	1942 (250)	***
Oleic Acid (C18:1)	9357 (683)	8341 (1126)	8050 (778)	7482 (911)	NS
Linoleic Acid (C18:2)	346 (26)	294 (41)	403 (36)	326 (37)	***
Linolenic Acid (C18:3)	138 (10)	227 (31)	148 (15)	193 (27)	***
CLA cis9,trans11	153 (13)	328 (47)	647 (59)	454 (61)	
PUFA	801	1208	1498	1686	***
SFA	13010	11614	11079	10981	NS
P:S Ratio	0.06	0.10	0.14	0.15	***
n-6:n-3 Ratio	2.30	1.18	2.37	1.56	***

Table 1: Results of selected fatty acids from burgers produced from animals fed different diets. SC = silage/concentrates, G = pasture only, SFG = sunflower oil supplemented, and LSG = linseed oil supplemented. Values are expressed as mean +/- standard deviation.

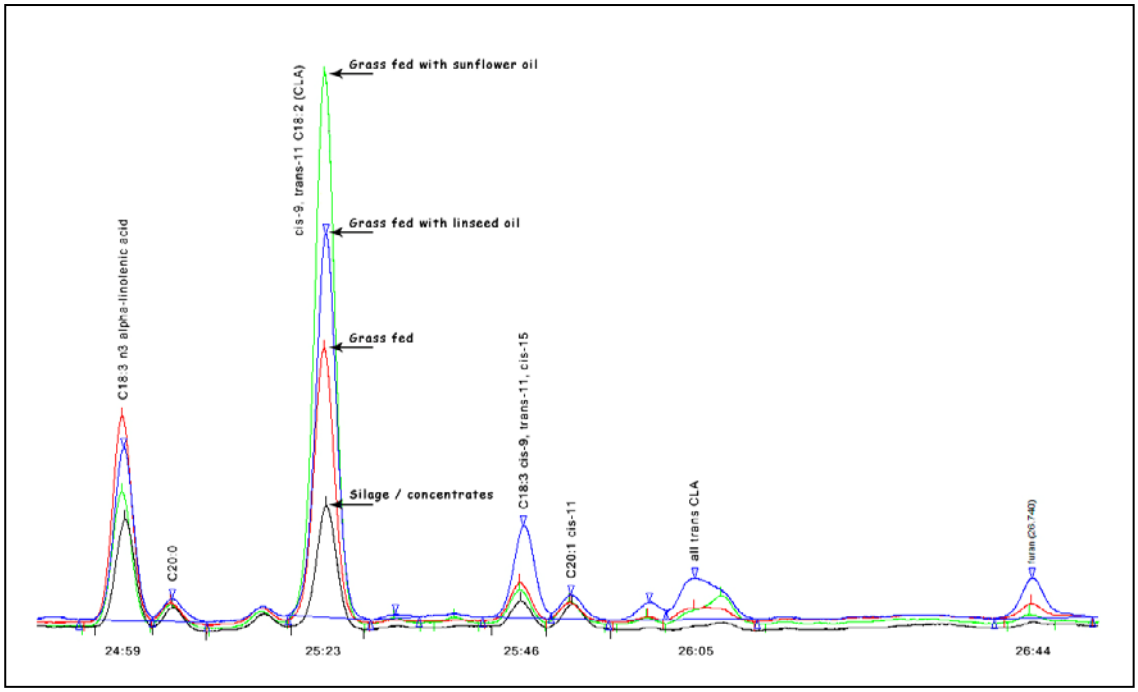

Figure1: Gas chromatogram illustrating the relative differences in the CLA peak (cis-9, trans-11 isomer) from burgers produced from animals on different diets.

EFFECTS OF *POLYGONUM HYDROPIPER* L. EXTRACTS ON THE QUALITY OF CHILLED BEEF STEAK

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Abstract

This study was undertaken to examine the effects of *Polygonum hydropiper* L. extracts on the quality of Australian and Indian beef sirloin steaks. The use of 2000ppm extracts were observed to reduce lipid oxidation during 18 days chilled storage while pH values decreased during storage time in both types of meat, treated (K1 & K2) and untreated (S1 & S2). The application of *Polygonum hydropiper* L. extracts also resulted in the lower hardness values. No significant differences were observed in the L (lightness) value , however there was a decrease in a (redness) value for all samples . As for b (yellowness) value not much difference were detected except for Australian untreated beefsteak which increased drastically after 18 days of chilled storage.

Keywords: *Polygonum hydropiper;* extracts; beef steak; chilled

Introduction

Buffalo meat (Indian beef) are being imported in large volume by Malaysia while higher quality beef cuts are imported in smaller volume from Australia. Steaks from sirloin, striploin and ribeye muscles are generally tender and are quality cuts preferred by the consumers. However restaurants and hotels serving steaks are reluctant to use Indian fillets, referring to poor quality such as toughness, chewiness, low sensory acceptance and poor microbial quality related to meat imported from India. It is with this unconfirmed factors that this study research into the effects of plant extracts to improve certain quality attributes related to the consumers perception of Indian beef. Australian beef fillets are well received by local consumers as well as foreigners who associate beef from Australia as superior and of high quality.

Materials and methods

Preparation of beef steaks

Frozen (-18^{o}C) *longisimmus dorsi* muscles in the form of striploin or sirloin were purchased from a local importer (Lucky Frozen Sdn. Bhd.) in Kuala Lumpur, Malaysia. Beef were cut into pieces and marinated with salt (1.5%) and pepper (2%) for control, namely K1 (untreated Indian beefsteak) and K2 (untreated Australian beefsteak). 2000ppm of *Polygonum hydropiper* L. extracts were applied topically to steaks by dry rubbing the external surface of the steaks on both side for sample S1 (Indian beefsteak) and S2 (Australian beefsteak). The samples were stored at 5 $\pm$ 1^{o}C for 18 days.

pH determination

Duplicate beef samples were periodically removed from storage and homogenized in distilled deionized water (1:10 dilution). Homogenates were filtered through a Whatman No. 1 filter paper to obtain clear filtrate for pH measurement.

Colour measurement

Surface colour of sirloin steaks was determined for L (lightness), a (redness) and b (yellowness) using a Minolta Chromameter CR-100.

Texture Analysis

Textural characteristics of sirloin steaks were analyzed using a Warner-Bratzler shear machine.

Lipid Oxidation Analysis

Thiobarbituric acid numbers (TBA) was determined following the distillation method described by Tarladgis et al (1960) with little modification by Rhee (1978).

Statistical Analysis

The data were analyzed using the Statistical Analysis Systems (SAS) program version 6.12 (SAS 1995). Treatments showing significant differences ($p<0.05$) were subjected to the Duncan's Multiple Range Test.

Results and discussion

Fig 1 shows the pH values for the beef steaks stored at chilled temperature for 18 days. There was a significant decrease in pH values for all samples after chilled storage for 18 days. However there were no significant difference in pH values between the treated and untreated (control) samples for both types of meat. The lower pH values for all samples during the chilled storage could be due to production of free fatty acids from the phospholipids fraction and the separation of free fatty acid from the triacylglycerol (Rhee et al. 1977). The pH of fresh meat can be influenced by the presence of bacteria and may reflect the relative differentiation between the presence of gram positive or gram negative bacteria. Organic acids, produced by gram positive bacteria, decreased the pH of meats, whereas amines produced by gram negative bacteria increase pH (Lefebvre et al., 1994).

Colour changes in beef steaks during chilled storage were shown in Figures 2, 3 and 4.
There were not much changes in the lightness of the samples except for untreated Australian beef steak which increase after 18 days of chilled storage. Values for redness were found to decrease significantly after 18 days of storage in all samples. A loss of surface redness in all samples can be attributed to decrease in dissolved oxygen, due in part to the utilization of oxygen by psychrotrophic bacteria, and the participation in oxidative reactions such as lipid oxidation (Peter & David, 2002). As for yellowness values no significant difference were found in all samples except for K2 yellowness which increased drastically during storage.

Figure 5 showed the results for texture (gF) for the four samples. Warner-Bratzler shear measurement showed an increase in values indicating increase in toughness of all samples. This could be attributed to the moisture loss during the chilled storage.There was significant difference in toughness between the control K1 and K2 whereby K1 was tougher than K2. For samples treated with *Polygonum hydropiper* L. extracts (S1 & S2) the toughness was significantly lower than the control (K1 & K2). Lipid soluble antioxidant from plant extracts can maintain integrity of muscle fibres and reduce moisture loss (Mitsumoto et al., 1995).
Rancidity of meat products are indicated by an increase in the content of malonaldehyde normally measured by TBA method. In this study there was a significant increase in TBA values in all samples as a result of the 18 days chilled storage (Fig. 6). However the values of treated samples (S1 & S2) were significantly lower than the controls (K1 & K2) though K1 showed higher values than K2. Taylor (1987) suggested prooxidants such as iron and copper from water and spices to contribute to increase in rancidity of meat products. Besides, the process of cutting and mixing during preparation of samples also increased oxidation in meat. Lower TBA values in treated samples for both types of meat indicated that *Polygonum hydropiper* L. extract was effective in inhibiting the formation of malonaldehyde, thus delaying lipid oxidation of the chilled beef steaks. The extracts was known to contain ten flavonoid compounds that possess strong antioxidative activity (Zhao et al., 2003).

Conclusions

The addition of *Polygonum hydropiper* L. extracts to the Australian and Indian beef steaks was found to improve the quality attributes such as texture and oxidative changes. The toughness was reduced when compared to the untreated samples for both types of meat. The oxidative process increased at a slower rate when compared to the untreated samples after 18 days of chilled storage at 5^{o}C.

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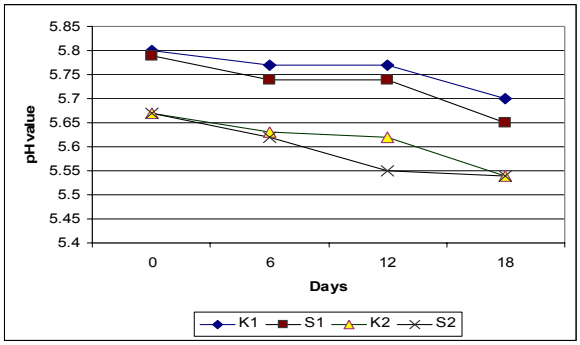

Fig. 1: pH values of chilled storage beef steaks

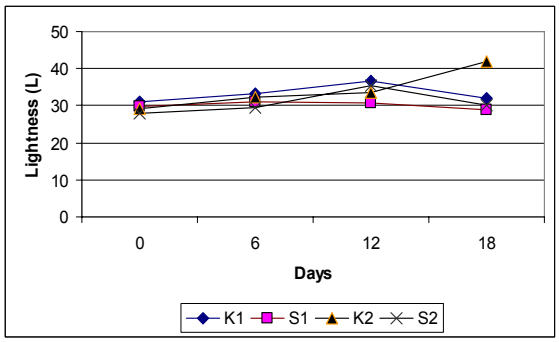

Fig. 2: Lightness (L) values of chilled storage beef steaks

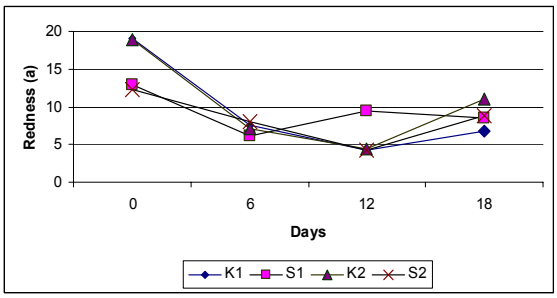

Fig. 3: Redness (a) values of chilled storage beef steaks

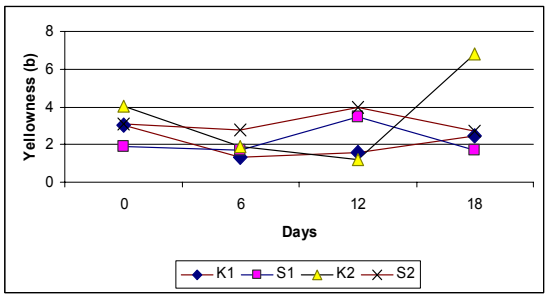

Fig. 4: Yellowness (b) values of chilled storage beef steaks

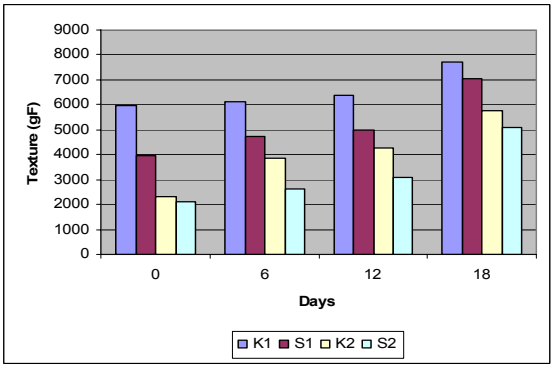

Fig. 5: Texture values for chilled storage beef steaks

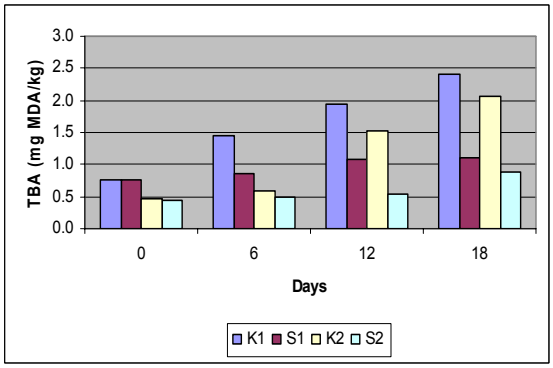

Fig. 6: TBA values for chilled storage beef steaks

EFFECT OF PHOSPHATE, ASCORBIC ACID AND α-TOCOPHEROL WITH THE CONTINUOUS NON-VACUUM OR VACUUM TUMBLING PROCESS ON LIPID OXIDATION OF PRECOOKED ROAST BEEF

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Background

Lipids can be oxidized by enzymatic and nonenzymatic reactions and there are many mechanisms to explain these complex reactions in meat. However, autoxidation is a continuous free-radical chain reaction and is the most important mechanism of lipid oxidation in meat (Pearson *et al*., 1983).
Synergistic antioxidants are the compounds used to improve the function of primary antioxidant and improve lipid stability in food. The functions of synergists are to regenerate the primary antioxidants, to react with oxygen (oxygen scavengers), and to chelate prooxidants (chelators) such as iron and copper (Rajalakshmi and Narasimhan, 1996). Phosphate chelates irons which act as catalysts for lipid oxidation. The antioxidative ability of vitamin E is that it acts as a free radical scavenger and a singlet oxygen quencher (Yang and Min, 1993). Ascorbic acid stabilizes lipid oxidation is due to its functions as an oxygen scavenger (Jadhav *et al*., 1996), and it is used to regenerate activity of primary antioxidants and to inactivate prooxidants (Bauernfient and Pinkert, 1970).
There are two major types of tumbling that includes vacuum and non-vacuum processing. According to tumbling schedules, Ockerman *et al*. (1978) indicated that continuous tumbling is a constant mechanical action during a short period of time from 1 to 3 hours; however, intermittent tumbling is where the tumbler works for 10-15 minutes followed by a rest period each hour for an 18-24 hours period.

Objectives

The objective of this experiment was to test if continuous vacuum or non-vacuum tumbling decreases lipid oxidation of precooked roast beef under a short tumbling period (3 hour of tumbling) after injection.

Materials and methods

Raw beef bottom rounds, which were approximately 7 days post-mortem, were purchased from a local supermarket in Columbus, Ohio. The beef round primal cuts were trimmed of visible fat and connective tissue. The water-soluble antioxidants, such as sodium tripolyphosphate and ascorbic acid, were dissolved in distilled water. The α-tocopherol, a fat soluble antioxidant, was dissolved in propylene glycol. All beef bottom rounds were cut into uniform roasts (8 x 8 x 8 dimensions). The tumbling was a continous schedule` for 3 hrs. A non-vacuum tumbler and a vacuum (Hobart, Model HVM 30, Troy, Ohio) of 15 in (381mm) Hg were drawn and the tumbler was rotated at 12 rpm in a 4°C cooler.
Moisture content (Oven Dry Method; Ockerman, 1985) was measured in a drying oven at 100°C for 18 hours. A Corning pH meter (Model 7) measured the pH values of the samples. A modified extraction of the TBARS method was used to analyze lipid oxidation (Pensel, 1990). The shear values of cooked roast beef were evaluated using a 2.54 cm core by the Warner-Bratzler (G.K. Electric Mfg. Co., Kansas) instrument (Ockerman, 1985). Total iron was followed the ferrozine assay from Stookey, 1970 and Clark *et al*, 1997. Total pigment test was measured by the modified method from Ockerman (1985). Heme iron was calculated by the modified technique of Clark et al. (1997); the iron content is calculated with the factor of 0.0882 μg/μg hematin (Merck, 1989).

Heme iron (ppm; μg/g) = total pigment (ppm; μg/g) x 0.0882
Nonheme iron of sample = Total iron content – Heme iron content

Total aerobic, psychrotrophic, and thermophilic bacteria tests were utilized to detect contamination of various bacteria in precooked roast beef. Total aerobic plate count was evaluated using aerobic plate count (APC; Difco Laboratory, Detroit, MI) agar at an incubation temperature of 25°C for 4 days (Speck, 1984). Psychrotrophic bacteria were tested using APC agar at 4°C for 10 days and also "thermophiles" were determined on APC agar at 35°C for 48 hours. The number of bacteria was converted to $\log_{10}$ colony forming units per gram ($\log_{10}$CFU/g).

The control treatment was a non-injected roast beef.
PAT (phosphate, ascorbic acid and tocopherol) was a roast beef injected (based on cooked meat weight) with sodium tripolyphosphate (0.5%), ascorbic acid (550ppm) and α-tocopherol (200ppm).
PATT(phosphate, ascorbic acid, tocopherol and nonvacuum tumbling) was a 3 hr non-vacuum tumbled roast beef injected (based on cooked meat weight) with sodium tripolyphosphate (0.5%), ascorbic acid (550ppm) and α-tocopherol (200ppm).
PATV (phosphate, ascorbic acid, tocopherol and vacuum tumbling) was a 3 hr-vacuum tumbled roast beef injected (based on cooked meat weight) with sodium tripolyphosphate (0.5%), ascorbic acid (550ppm) and α-tocopherol (200ppm).

Results and discussion

There was a significant difference due to treatment for yield. The non-injected (control) roast beef had significantly lower cooking yield than injected treatments probably due to no added phosphate in the control; however, injected roast beef with or without tumbling had the same cooking yield (Table 1). There was no significant difference between the vacuum tumbled roast beef and the non-vacuum tumbled samples. Basically, nontumbling, continuous non-vacuum, or vacuum tumbling (3 hr) with three antioxidants (tocopherol, ascorbic acid and sodium tripolyphosphate) increased cooking yield, but there was a non-significant difference when non-vacuum was compared with vacuum tumbling.
There was no significant difference of psychrotrophs, mesophile or thermophile among treatments (Table 1). For cooked roast beef, the numbers for total plate counts were relative low.
For moisture, there was no significant treatment-time interaction for the roast beef samples. The control, noninjected treatment, had significantly lower moisture due to non-liquid injection compared to other treatments that all had the same values (Table 2). Increase in water-holding capacity with phosphate is due to the unfolding of the three dimensional protein network by the high ionic strength that causes the muscle to swell and the protein to solublize and to retain water before heating (Torley *et al*., 2000). During refrigerated storage, the moisture content was decreased significantly over time as would be expected.
For TBARS, there was a significant interaction due to treatment and storage time. The control (non-injected roast beef) had significantly higher TBARS value than other treatments at all measurement days (Table 3). During refrigerated storage, samples with three antioxidants maintained stable lipid oxidation up to day 14. However, the control significantly increased its TBARS value at day 14 compared to day 0. There was no significant difference of TBARS values among non-tumbled, non-vacuum tumbled and vacuum tumbled samples. Vacuum is used to retard oxygen that attacks muscle but did not significantly decrease lipid oxidation in this study. The reason could be it only prevents oxygen that contacts the surface of whole muscle while tumbling. Also, vacuum tumbling could cause more disruption of the cell membrane of muscle compared to non-vacuum tumbling.
For pH, there was no significant two-way interaction (treatment x storage time). The control had significantly lower pH value compared to other treatments (Table 2). It appears that the 0.5% sodium tripolyphosphate increased pH values in roast beef (Table 2). The antioxidative functions of alkaline phosphates are to increase the pH and ionic strength and tie up some prooxidants (Trout and Schmidt, 1984). There was no significant difference between non-tumbled, non-vacuum and vacuum tumbling treatments in pH values. There was no consistent change in pH values during the storage period
For shear values, there was no significant treatment x storage day interaction. The control and non-tumbled treatments had significantly higher shear values (tougher) when compared to non-vacuum and vacuum tumbled samples (Table 2). The increased tenderness is a primary function of the tumbling process along with increasing uniformity (Krause *et al*., 1978). The tumbling process did increase tenderness of precooked roast beef during 14-day refrigerated storage. The shear values of all samples had significantly ($p<0.05$) higher values at day 14 than day 0.
There was no significant treatment x storage day interaction and main effects (treatment and time) of total iron (Table 2). For heme iron, there was a treatment-time interaction for roast beef. There was no significant difference of heme iron among treatments at day 0 (Table 4). The vacuum tumbled treatment had the lowest heme iron at day 2, but it was the same as the control. There was no significant difference of heme iron among treatments at day 7 and 14. However, there was no treatment x storage day interaction for nonheme iron. The nonheme iron contents of the control had a significantly higher value than that of non-vacuum tumbled roast beef (Table 2). At day 7 and 14, nonheme iron content significantly increased comparing to those at 0, 2 and 4. According to this result, nonheme iron content was increased during storage.

Conclusions

There was no significant difference among non-tumbled, non-vacuum tumbled and vacuum tumbled samples for lipid oxidation. The vacuum tumbling only prevented oxygen from coming in contact with the surface of whole muscle; therefore, it was not significant in decreasing lipid oxidation compared to non-vacuum tumbling.

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Table 1 Effect of phosphate, ascorbic acid and tocopherol with noninjected, nontumbled, continuous non-vacuum or vacuum tumbling on yield, psychrophile, mesophile and thermophile of roast beef

	Treatments			
	T1 Control	T2 PAT%	T3 PATT	T4 PATV
Yield %[1]	59.52[B] (1.10)	63.46[A] (1.74)	64.98[A] (0.97)	64.22[A] (1.79)
TPC7[2] (log_{10}CFU/g)	1.7 (0.2)	0.9 (1.2)	1.5 (1.0)	1.7 (1.1)
TPC25[2] (log_{10}CFU/g)	1.9 (0.4)	0.9 (1.1)	1.1 (0.8)	1.9 (0.3)
TPC35[2] (log_{10}CFU/g)	1.5 (1.0)	0.9 (1.1)	2.1 (0.3)	1.7 (1.1)

Treatments:
Control= non-injected roast beef
PAT= roast beef injected with sodium tripolyphosphate (0.5%), ascorbic acid (550ppm) and α-tocopherol (200ppm)
PATT= 3 hr-nonvacuum tumbled roast beef injected with sodium tripolyphosphate (0.5%), ascorbic acid (550ppm) and α-tocopherol (200ppm)
PATV= 3 hr-vacuum tumbled roast beef injected with sodium tripolyphosphate (0.5%), ascorbic acid (550ppm) and α-tocopherol (200ppm)
[1] Yield= cooked/fresh meat at day 0
[2] Total plate counts was incubated at 7°C for psychrotrophile, 25°C for mesophile and 35°C for thermophile at day 14, expressed as log_{10} CFU/g.
[AB] Means with different uppercase superscripts within the same row are significantly different ($p<0.05$)

Table 2 Main effect of moisture, pH values, shear values, total iron and nonheme iron of roast beef with different treatments during refrigerated storage

Main effect / Treatment	Moisture	pH	Shear values (Kg)	Total iron (μg/g)	Nonheme iron (μg/g)
T1: Control	51.80[B]	5.77[B]	4.74[A]	52.23	26.50[A]
T2: PAT	53.38[A]	5.92[A]	4.57[A]	49.46	22.48[AB]
T3: PATT	54.76[A]	5.97[A]	3.70[B]	48.46	21.86[B]
T4: PATV	53.66[A]	5.96[A]	3.57[B]	50.48	24.26[AB]
Time					
0	56.01[a]	5.96[a]	3.54[b]	50.37	21.73[b]
2	55.19[ab]	5.87[b]	3.85[b]	49.55	20.05[b]
4	54.09[b]	5.91[ab]	4.14[ab]	48.62	22.04[b]
7	52.12[c]	5.87[b]	4.19[ab]	50.06	27.44[a]
14	49.68[d]	5.92[ab]	5.02[a]	52.19	27.67[a]

[AB] Means with different uppercase superscripts within a column, within main effect of treatment are significantly different ($p<0.05$)
[abcd] Means with different lowercase superscripts within a column, within main effect of time are significantly different ($p<0.05$)

Table 3 Means and standard deviations of TBARS values (mg of malonaldehyde /kg of muscle) of roast beef with different treatments during refrigerated storage

	Storage Days				
Treatments	0	2	4	7	14
T1: Control	0.35^{C}	0.38^{C}	0.71^{B}	0.94^{A}	1.00^{A}
	(0.05)	(0.05)	(0.05)	(0.30)	(0.10)
T2: PAT	0.19^{D}	0.22^{D}	0.21^{D}	0.24^{D}	0.20^{D}
	(0.06)	(0.05)	(0.06)	(0.06)	(0.04)
T3: PATT	0.20^{D}	0.16^{D}	0.17^{D}	0.22^{D}	0.25^{D}
	(0.07)	(0.06)	(0.04)	(0.03)	(0.03)
T4: PATV	0.22^{D}	0.20^{D}	0.20^{D}	0.24^{D}	0.21^{D}
	(0.06)	(0.05)	(0.04)	(0.05)	(0.04)

^{ABCD} All means with different uppercase superscripts are significantly different ($p<0.05$)

Table 4 Means and standard deviations of heme iron (μg/g) of roast beef with different treatments during refrigerated storage

	Storage Days				
Treatments	0	2	4	7	14
T1: Control	29.39^{ABCD}	29.54^{ABCD}	24.74^{DEFG}	21.74^{G}	23.24^{FG}
	(2.73)	(2.04)	(3.44)	(3.88)	(2.26)
T2: PAT	29.39^{ABCD}	31.94^{A}	29.84^{ABC}	21.07^{G}	22.64^{FG}
	(4.52)	(1.33)	(1.02)	(2.63)	(1.86)
T3: PATT	27.44^{ABCDEF}	31.64^{AB}	24.14^{EFG}	24.29^{EFG}	25.49^{CDEFG}
	(4.88)	(3.41)	(2.37)	(4.55)	(2.42)
T4: PATV	28.34^{ABCDE}	24.89^{CDEFG}	27.59^{ABCDEF}	23.39^{EFG}	26.91^{BCDEF}
	(2.99)	(3.55)	(1.77)	(2.02)	(1.87)

^{ABCDEFG} All means with different uppercase superscripts are significantly different ($p<0.05$)

EFFECT OF ANTIOXIDANTS ON COLOR CHANGE AND LIPID OXIDATION OF SLICED ROAST BEEF

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Background

Roast beef is a meat product which has gained a lot of popularity in recent years. Its predominately bright red centre is one of its important characteristics. However, the reddening effect does not remain throughout the centre of the meat but fades as the meat is sliced. As a consequence, the marketing of sliced roast beef is very difficult.

Objectives

This research was conducted to elucidate the effect of antioxidants (rosemary + sage extract, grape seed polyphenol, vitamin E, water-soluble astaxanthin, astaxanthin oil, water-soluble catechin, oil-soluble catechin, butylated hydroxy toluene (BHT), oil-soluble rosemary extract + V.E, water-soluble rosemary extract) on color change and lipid oxidation of sliced roast beef.

Materials and methods

Sample : A commercial roast beef (manufactured by ITOHAM FOODS Inc., Japan) was used for the experiments. Topside grass-fed chilled Aussie beef was used for model roast beef as a raw material.
Antioxidants : We selected ten antioxidants with expected strong antioxidant effects. These were as follows : 1) rosemary + sage extract (Stabiloton WS, RAPS Japan Co., Ltd.), 2) grape seed polyphenol (Gravinol, Kikkoman Co., Ltd.), 3) vitamin E (E-mix, Eisai, Co., Ltd.), 4) water-soluble astaxanthin (Astareal, Fuji Chem. Co., Ltd.), 5) astaxanthin oil (Astareal oil, Fuji Chem. Co., Ltd.), 6) water-soluble catechin (Sunkatol W-5, Taiyo Kagaku Co., Ltd.), 7) oil-soluble catechin (Sunkatol No.1, Taiyo Kagaku Co., Ltd.), 8) butylated hydroxy toluene (BHT ; Sustane emulsion, Nikki-Universal, Co., Ltd.), 9) oil soluble rosemary extract + V.E (RM-21E, Mitsubishi-kagaku Foods Co., Ltd.), 10) water-soluble rosemary extract (RM-21A, Mitsubishi-kagaku Foods Co., Ltd.).
Model roast beef preparation : The preparation of model roast beef was as follows : after cutting and trimming of raw material, and then salting with salt and spices (2g/100g meat), and the surface of raw beef material was burnt by a Bunsen burner. The sample placed into polyethylene bag and cooked to an internal temperature of 57°C, for 43 min in a water bath and cooled.
Analysis : Judgements with the naked eye, the sense of smell, and panel test were carried out as far as possible to sliced roast beef sample. The percentage of metmyoglobin (MetMb) formation was measured by the procedure of Trout (1990). Lipid oxidation was determined by the reaction of malonaldehyde with thiobarbituric acid (TBA) as described Siu and Draper (1974) modified of the “filtration method” of Tarladgis *et al*. (1964). The absorbance value measured by the procedure was expressed as TBA reactive substances (TBARS).

Results and discussion

At first this experiment was undertaken for obtaining information on the changes in MetMb formation and TBARS of sliced roast beef during storage. Sliced roast beef about 3 mm thick was placed at 15 cm (about 3.400 Lux.) from the 15W white fluorescent lamp in refrigerator. A comparison to imitate the lighting used by supermarket displays in storage units was hard to accomplish. The changes in MetMb formation and TBARS of sliced roast beef during storage was shown in Fig. 1. The percentage of MetMb formation during storage increased with each additional storage day. The samples after 2 and 3 days storage reached a MetMb level of 38% and 51%, respectively. The TBARS value of samples increased linearly during storage. These

results indicated that the MetMb formation and TBARS value increased under these storage conditions. And the same condition was adopted to get the remarkable difference of sliced roast beef sample during storage.
Screening tests 1 and 2 were carried out to ten kinds of antioxidants. Screening test 1 was as follows : 1% antioxidants were applied to surface of sliced roast beef, and MetMb formation was determined after 3 days storage. Fig. 2 showed the effect of antioxidants on MetMb formation of sliced roast beef after 3 days storage. Grape seed polyphenol and water-soluble rosemary extract treatment samples exhibited a MetMb level under 30% after 3 days storage. Screening test 2 was as follows : The surface of sliced roast beef under the same condition of screening 1 were judged with the naked eye. Table 1 showed that rosemary + sage extract and vitamin E was acceptable but the water-soluble rosemary extract was much better. It was then agreed that the water-soluble rosemary extract (WSRE) from ten different antioxidants was chosen as a result of these screening tests.
Recently, there were several reports [Ahn *et al*. (2002), Coronade *et al*. (2002), Djeane *et al*. (2003), Tanabe *et al*. (2002), Yu *et al*. (2002)] concerning the oxidation effect of rosemary extract on the oxidation stability of meat and meat products. Yu *et al*. (2002) have already reported that rosemary extract acts as an inhibitor of lipid oxidation and color change in cooked turkey products during refrigerated storage. However, WSRE have not been evaluated for their effects to prevent color change and lipid oxidation of sliced roast beef.
The following experiment was conducted to determine the potential benefits of WSRE on color change and lipid oxidation of roast beef. Model roast beef was prepared by dry or wet salting containing 1% WSRE. After slicing, sample was stored under the same condition as previously stated above. Fig. 3 showed the effect of dry- and wet-salting on MetMb formation of sliced roast beef during storage. The percentage of MetMb formation in dry salted sliced roast beef was slightly higher than that in wet sample after 1 day of storage. But after 3 days of storage, the dry salted sample had a much lower level than that of the wet salted sample. The dry salting method was then used in subsequent experiments for roast beef preparation. Fig. 4 showed the effect of salting time on MetMb formation and TBARS value of roast beef. Model roast beef prepared for 0, 1, 3 and 5 days salting containing 1% WSRE, and after slicing, samples were stored under the above mentioned conditions. After 1 day storage, the percentage of MetMb formation of the sample 3 days salting was higher than those of 0, 1 and 5 salting days. But, after 3 days storage, the sample of 3 and 5 days salting were low level of MetMb formation than those of 0 and 1 day salting samples. The TBARS value of samples salting 3 and 5 days were slightly lower than those of 0 and 1 day salting samples.

From separate experiment, the color of sliced roast beef salted sample containing 1% WSRE for 3 days was much better than those of samples added 1% WSRE just after cooking by judgement of naked eye (data not shown).

More detail investigations are necessary to clarify the effect of WSRE on color change and lipid oxidation of sliced roast beef.

Conclusions

The effect of antioxidants on color change and lipid oxidation of sliced roast beef was investigated. The water-soluble rosemary extract was chosen as a result of the screeing tests. Changes were measured in metmyoglobin formation and thiobarbituric acid-reactive substances of roast beef samples containing 1% water-soluble rosemary extract. It was suggested that the water-soluble rosemary extract showed protection of color change and lipid oxidation of sliced roast beef.

Acknowledgements

We gratefully thank Ms. Atuko Sone in our lab., Kobe Univ., who carried out the experiments in this study. We would like to thank Mr. Toru Ito, ITOHAM FOODS Inc., for donating the beef sample. We are also deeply grateful to Mr. Roy Shrigley for his useful comments in reading this paper.

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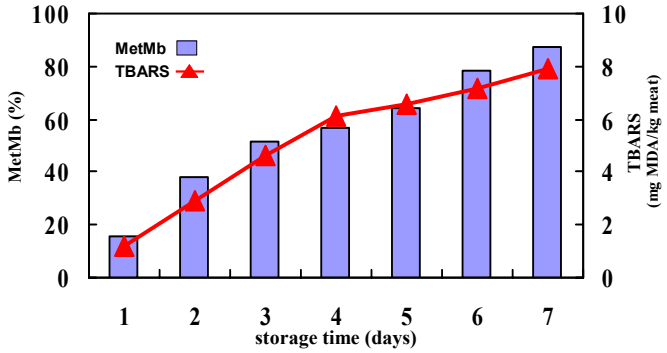

Fig. 1 Changes in MetMb formation and TBARS of sliced roast beef during storage

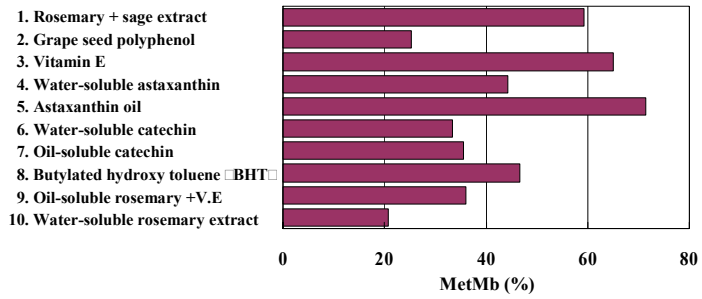

Fig. 2 Effect of antioxidants on MetMb formation of sliced roast beef

Table 1 Effect of antioxidants on surface color of sliced roast beef

Antioxidant	Judgement with the naked eye
1. Rosemary + sage extract	□
2. Grape seed polyphenol	×
3. Vitamin E	□
4. Water-soluble astaxanthin	×
5. Astaxanthin oil	×
6. Water-soluble catechin	×
7. Oil-soluble catechin	×
8. Butylated hydroxy toluene (BHT)	×
9. Oil-soluble rosemary + V.E	×
10. Water-soluble rosemary extract	○

○ □Good, □ □Not bad, × □Bad

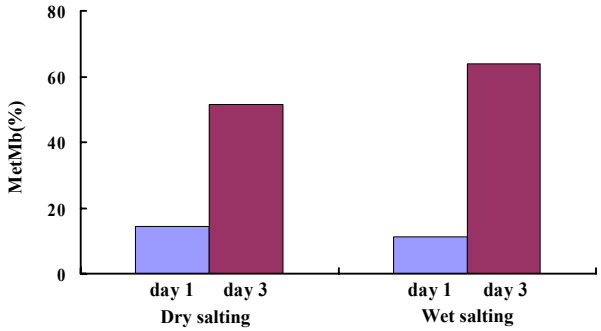

Fig. 3 Effect of dry and wet salting on MetMb formation of roast beef

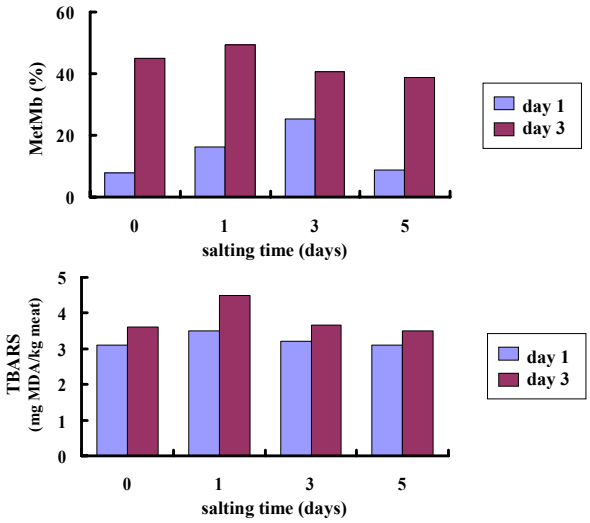

Fig. 4 Effect of salting time on MetMb formation and TBARS of roast beef

IMPROVEMENT OF PROPERTIES OF PRODUCTS MADE FROM PSE MEAT BY ADDITION OF SODIUM CASEINATE AND MTGASE

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Background

PSE meat is characterized by its lowered culinary and processing value. The quality of products manufactured from this meat is worse in comparison with those made from normal meat which are characterized by a lack of appropriate consistency and juiciness, a deterioration of texture, weak binding ability of meat and excessive quantity of thermal loss. One of the common practices aiming to assure high quality of processed meat is the application of certain substances (e.g. phosphates, carbonates) or the incorporation of protein ingredients in the product formulation. The second group of substances includes a variety of isolated proteins, which have been used as functional ingredients in ground or whole muscle foods, of which milk, beside soy proteins, is employed most widely (Pietrasik & Li-Chan 2002, Ramírez-Suárez , Xiong 2003). In recent years, trans-glutaminase (E.C. 2.3.2.13), as a functional additive, has raised much interest (Hammer 1998, Kuraishi et al. 1998). This enzyme promotes polymerization of proteins through intermolecular ε-(γ-glutamyl)lysine cross-links, which form stable covalent cross-bonds between the particles of proteins and peptides and cause modifications in their structure (Kurt & Rogers 1984). It may have an effect on the change in the water-holding capacity of the muscle tissue and improve the texture of the final product.

Objectives

The purpose of the present study was to examine the effectiveness of microbial transglutaminase (MTGase) as a catalyst for the interaction of sodium caseinate and proteins of the PSE muscle under processing conditions. Our hypothesis was that MTGase would induce polymerization and, therefore, properties of products manufactured from PSE muscles might be similar to those made from normal muscles.

Materials and methods

The experimental material used in this study comprised the following ham muscles: *m. semimembranosus* and *m. biceps femoris* which were selected in two quality groups: - normal (RFN) and watery (PSE) meat according to typical critical values used in other studies. They were used for the production of a model ham. Four variants of the model pork ham were produced. The differentiating factors included the type of meat (RFN or PSE) (first two variants – the ham made from RFN meat – first variant that made from PSE meat – second variant). In the case of the PSE and RFN model ham (first and second variants), they were produced with additives, which contained curing brine. In the next variant (third one – PSE1), the MTGase preparation was added to the curing brine and, in the last treatment (fourth variant – PSE2), additionally, 2.0% sodium caseinate preparation was added. Muscles, after coarse mincing, were cured and tumbled. The composition of brine was the following: water – 86.88%, salt – 11.3%, sugar – 1.10%, sodium ascorbate – 0.22%, sodium glutamate – 0.22%, protein hydrolysate – 0.22% and sodium nitrate – 0.055%. The brine was used in the quantity of 30% in relation to the weight of meat. Before tumbling, commercial preparations of microbiological transglutaminase containing 0.5% of the active enzyme ("Active WM"), suspended in maltodextrin as a carrier and / or 2.0 % sodium caseinate preparation were added. Meat was tumbled at the lower pressure in three cycles for 24 hours. Afterwards, some of the meat mass was taken for the analysis of the protein fraction and the remainder was canned and pasteurized until the temperature of 72°C was reached in the centre of the can. The investigation of raw meat comprised determinations of the pH value and electrical conductivity of meat, measurements of its basic composition (total protein, water and fat content), the amount of centrifugal drip from the raw meat and from meat after tumbling, free water content

determined by the filter paper method, by Makała & Olkiewicz (2001), electrophoretic separation of proteins from the fraction of washed myofibrils and the centrifugal drip according to the method of Fritz et al. (1989) with the later modification of Pospiech et al. (2000). This modification consisted in the use of an 8M urea addition in the separating layer of gel. The analyses performed on the final product comprised the estimation of the amount of thermal drip from ham, measurement of slice strength using the UTM Zwick apparatus, model 1445 MOPS (Tyszkiewicz & Olkiewicz 1991) and determinations of rheological characteristics of meat using the CASRA method (Continuously Stress-Relax Analysis) (Tyszkiewicz & Olkiewicz 1997). The experiment was performed in three replications. The statistical evaluation of the results was conducted using the Statgraphic for Windows program ver. 3.1.

Results and discussion

The PSE meat, in comparison with the normal meat, was characterized by a significantly lower pH value and significantly higher electrical conductivity (Table 1). The total water content in the PSE meat was lower than in the normal meat – on average by 1.3%. On the other hand, the free water content was almost double in the PSE meat. The significantly higher content of total protein and higher fat content in the PSE meat were probably caused by the dripping of water from this meat causing a relative increase in the content of the remaining components.

TABLE 1. Characteristics of pork meat

Muscle characteristic	RFN	PSE	*LSD*
water content (%)	75.6	74.3	*1.9*
protein content (%)	19.8 [a]	21.7 [b]	*1.6*
fat content (%)	2.6	2.8	*1.1*
pH	5.89 [b]	5.56 [a]	*0.22*
electrical conductivity [mS]	8.0 [a]	15.3 [b]	*7.3*
amount of centrifugal drip (%)	5.6 [a]	9.0 [b]	*2.2*
free water content (%)	13.9[a]	22.6 [b]	*4.6*

[a, b,] – means in rows with a different superscript are significantly different at $P < 0.05$; LSD – least significant difference

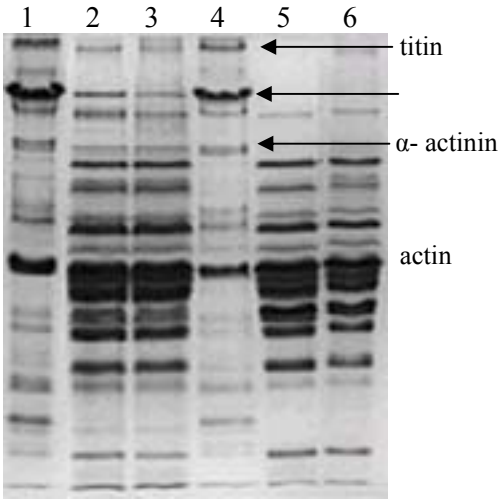

Fig. 1. Protein separation of the washed myofibrils fraction and the fraction of centrifugal drips obtained from diverse quality meat. Band: 1 – washed myofibrils from watery meat, 2 – centrifugal drips from watery meat with 0.1% „Activa WM" preparation (PSE1), 3 – centrifugal drips from watery meat with 2% sodium caseinate preparation (PSE2), 4 – washed myofibrils from meat of normal quality (RFN), 5 – centrifugal drips from watery meat (PSE), 6 – centrifugal drips from meat of normal quality (RFN)

The separations of proteins isolated from the PSE meat were characterized by a somewhat higher number of bands as compared with those from the normal quality meat. In the first case, 30-36 bands were obtained, while in the second – 27 to 30 bands. This may have been connected with more rapid changes of proteins occurring in the PSE meat directly after slaughter - especially in the range below 42 kDa. It is known that in the case of PSE meat, the extraction of proteins is weaker (Fischer et al., 1979; Joo et al., 1999) and the bands of high-molecular proteins (above 200 kDa) are considerably more pronounced. The percentage of native titin (T1) was somewhat higher in the case of myofibrils from the PSE meat as compared with the RFN meat. Double amounts of titin-degraded products were found in normal meat in comparison with the PSE meat. This confirms the more rapid degradation of cytoskeletal proteins in the first type of material. The slower degradation of the cytoskeletal proteins in watery muscles is a typical picture, found not only in pig meat (Boles 1992) but also in turkey meat (Pospiech et al. 1997). It is much easier to observe the liberation of myofibrillar proteins from muscle tissue based on the analysis of the centrifugal drip from the meat (Grześ et al., 1996; Pospiech et al., 2000). If the presence of proteins, which, as a rule, should not be present in the drip, is confirmed, this may be the evidence of protein degradation and their shifting from the muscle fibers to the ambient environment. Observations obtained in this study showed that samples of centrifugal drip from the PSE meat, as compared to the drip from the RFN meat, contained less titin (T1) and products of its degradation (T2) and heavy myosin chains (ca. 200 kDa). These differences may have resulted from the

increased liberation of myosin from the myofibrillar structure and the quicker degradation of the cytoskeletal proteins in the normal meat. According to other authors (Grześ et al., 1996; Pospiech et al., 2001; Taylor et al., 1995), these differences may constitute evidence of better tenderness and water-binding capacity of the normal meat. The use of additives in the PSE meat showed that the electrophoretic separation of proteins of the centrifugal drip was distinctly different from the separation of proteins of the centrifugal drips from the RFN or PSE meat only. In these drips (samples PSE1 and PSE2) were found proteins, which did not appear in the drips from PSE and RFN meat, or were present in them in smaller quantities (Fig. 1). The content of proteins with the molecular weight of 3700 kDa (probably mainly the T1) increased by more than 10 times. The quantity of proteins with the molecular weight of 2400 kDa and 105 kDa was 2-3 times higher than in the PSE and RFN meat drips. The above changes could probably be attributed to the effect of the influence of MTGase and to interactions of sodium caseinate and proteins of PSE muscle under processing conditions. Transglutaminase probably induced their polymerization and, therefore, improved properties of products made from the PSE meat. The data presented in Table 2 indicate a significant improvement of the slice strength in the samples from the PSE meat with the addition of the enzyme. The same observations also concern other reological properties of the model hams i.e. their plasticity, elasticity and fluidity. When evaluating the effect of MTGase on the water-binding capacity (WBC) of the PSE meat, it should be stressed that the process observed as the liberation of proteins and generation of new aggregates, including aggregates with MTGase, affected unfavorably WBC. After the addition of the enzyme, the drip increased, when compared to the sample without this additive, on average by 5.6%. This could be attributed to the participation of MTGase in the formation of a strong covalent cross linking between the particles of proteins and peptides, which caused such a strengthening of protein structures that an additional, mechanical squeezing of water from the product, containing the PSE meat, took place. The addition of sodium caseinate to the meat caused the appearance of one band more in the electrophoretic picture, which was not present in the previous separations. This was a band with the molecular weight of more than 3700 kDa. The studies of Fritz et al. (1992) and Grześ (2000) showed that proteins of such weight might constitute products of aggregation. In the case of both cited studies, titin was present in the composition of the aggregates. Additionally, the study of Grześ (2000) demonstrated that the aggregates also contained myosin. The role of these aggregates in the formation of functional properties of meat and its structure is not fully explained. The studies of Grześ (2000) and Fritz et al. (1992) showed that, together with the prolongation of the tumbling and heating, the incidence of aggregates was more intensive. These results indicate that milk proteins may stimulate the generation of aggregates because the percentage content of this band in the separation of proteins was almost twice as high compared to the remaining samples. The addition of caseinate also caused a 50% decrease of the band responding to T1, almost tripled the increase of the content of the 2400 kDa band and increased, by more than half, the proteins with the molecular weight of 200 kDa. The changes observed in the proteins resulting from the addition of sodium caseinate to the PSE meat favorably affected WBC, which is confirmed by the comparison of the size of the thermal drip. The addition of sodium caseinate to the PSE ham decreased the thermal drip by 3.7% in comparison with the PSE ham manufactured without the caseinate. Whether the water was retained by the generated protein aggregates, or bound by the caseinate alone, requires further studies. The retention of a great quantity of water in the hams caused a lowering of the strength of slices. Plasticity, elasticity and fluidity were worse (table. 2). The conducted study suggests that the addition of any of the employed functional additives individually did not decrease the unfavorable consequences of the PSE meat to any satisfactory degree and only their application together with MTGase resulted in a positive effect.

Table 2. Binding ability and reological characteristics of model hams made of RFN and PSE meat

Type of the sample	Binding ability		Reological characteristics		
	slice strength ($N\ cm^{-2}$)	thermal loss (%)	plasticity ($x\ 10^5\ N\ m^{-2}$)	elasticity ($x10^{-6}\ m^2\ N^{-1}$)	fluidity ($x10^{-8}\ m^2\ N^{-1}\ s^{-1}$)
RFN	$2.65^{c} \pm 0.21$	$11.3^{a} \pm 0.7$	$6.87^{ab} \pm 0.47$	$6.10^{ab} \pm 0.88$	$4.34^{a} \pm 0.17$
PSE	$1.50^{a} \pm 0.04$	$19.1^{c} \pm 0.8$	$6.55^{a} \pm 0.84$	$6.63^{b} \pm 0.82$	$5.05^{a} \pm 0.59$
PSE 1	$1.80^{b} \pm 0.07$	$24.7^{d} \pm 1.7$	$7.72^{bc} \pm 0.50$	$5.56^{ab} \pm 0.26$	$5.56^{b} \pm 0.26$
PSE 2	$1.34^{a} \pm 0.07$	$15.4^{b} \pm 1.39$	$6.46^{a} \pm 0.21$	$5.65^{ab} \pm 0.19$	$4.73^{a} \pm 0.30$
LSD	0.22	2.4	0.96	1.12	1.12

a, b, – means in rows with different superscript are significantly different at $P<0.05$; LSD – Least Significant Difference, RFN – raw meat of normal quality, PSE – raw watery meat, PSE1 – raw watery meat after tumbling with 0.1 % „Activa WM" preparation, PSE2 – raw watery meat after tumbling with 2.0 % sodium caseinate preparation.

Conclusions

1. Transglutaminase, when added to the PSE meat, affected significantly the improvement of consistency. At the same time, the water binding capacity of the product deteriorated.
2. Sodium caseinate, when added to the PSE meat together with MTGase, intensified the aggregation of proteins and improved water binding significantly but, at the same time, a weakening of the structure and consistency of the final product was observed.

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COLOUR PHOTOGRAPHS TO ESTIMATE HETEROCYCLIC AMINE INTAKE FROM PORK OF DIFFERENT RN GENOTYPES

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Background

The Maillard reaction is important to obtain an appetizing surface browning of meat during frying and roasting; however, it also induces the formation of several carcinogenic/mutagenic heterocyclic amines (HCAs) (Jägerstad *et al.*, 1983). Cooked meat and fish are important sources of exposure to HCAs, as are pan residues when used to prepare gravy. The most abundant HCAs in cooked foods include 2-amino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine (PhIP) and 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) (Skog *et al.*, 1998). The formation of HCAs depends on cooking conditions, especially temperature and time, the concentration of precursors, such as free amino acids, creatine/ine and sugar in the food as well as the occurrence of certain modulators, e.g. pro/antioxidants (Skog *et al.*, 1998).

Although eight HCAs have been judged as probable human carcinogens and one as a possible carcinogen (group 2B and 2A respectively, according to (IARC, 1993)) it is difficult to evaluate their impact in the aethiology of human cancer. The most serious drawbacks in epidemiological studies include difficulties in estimating the exposure, for example the intake of HCAs in a population because of large ranges in the reported level of HCAs in cooked food, differences in estimated consumption (e.g. portion size, frequency), as well as difficulties in taking various cooking practices into consideration (Augustsson & Steineck, 2000). One common way to indirectly assess HCA intake is through food frequency questionnaires, sometimes complemented with colour photographs so that the degree of doneness can be estimated (Augustsson *et al.*, 1999; Sinha *et al.*, 1999). It has been suggested that visual aids, such as photographs, will improve exposure assessment in epidemiological investigations of HCA intake (Keating *et al.*, 2000). However, the relationship between cooking conditions (temperature and time), degree of surface browning and concentration of HCAs as judged from colour photographs has limitations. We have shown that pig meat of different genotypes (the dominant RN^- mutation) through a marked variation in glycogen levels, may give rise not only to varying concentrations of HCAs but also degree of browning when fried under identical conditions (Olsson *et al.*, 2002). In that study, a subjective scoring of surface browning correlated negatively with the level of HCA. Thus, the degree of surface browning may be a poor indicator of HCA content, and meat with similar surface browning could contribute very differently to the daily intake of HCAs. Because the colour of the crust is an important factor for judging doneness of the domestically cocked pig meat, we wanted to investigate how these findings affected the estimated intake of HCAs from pork chops.

Pig meat is the most popular type of meat in Sweden and the estimated frequency of carriers of the RN^- allele may be as high as 60-70% in the Swedish slaughter pig population. The *RN* mutation affects several of the precursors for the formation of HCAs, and due to hyperaccumulation of glycogen in glycolytic muscles, meat from carriers of the RN^- allele exhibits markedly higher glycogen levels than the normal meat of non-carriers (Lundström *et al.*, 1996). Further, we have found that the level of creatine and some free amino acids in the meat are affected by the *RN* genotype of the pigs (Olsson *et al.*, 2002).

Objectives

The overall aim of the present study was to contribute to an improvement of HCA exposure assessment. Pork chops from different *RN* genotypes were fried at three different temperatures, photographed, whereupon crust colour was recorded and the crusts analysed for HCAs. The results were combined with a simple questionnaire to study dietary practice and preferences, and a monthly intake of mutagenic HCAs through fried pork chops was estimated (Olsson *et al.*, 2004).

Materials and methods

Two *M. longissimus dorsi* (LD) muscles of pig, weighing approximately 2.5 kg each, were selected from the commercial cutting line at the slaughterhouse in Uppsala, Sweden. Genotyping (carrier (*RN⁻/rn+)* or non-carrier (*rn+/rn+)* of the *RN⁻* allele) was performed following the procedure reported by Milan *et al.* (2000). The contents of free amino acids (FAA), dipeptides, residual glycogen, creatine and creatinine in the meat were analysed as described before (Olsson *et al.*, 2002). The *RN⁻/rn+* and *rn+/rn+* samples were vacuum-packed, stored at +4ºC for 4 days and thereafter used in the frying experiment.

The deboned meat was fried as intact 2-cm-thick chops. Before frying, the edges of the chops were slightly cut to avoid an uneven curving during frying and the meat was tempered until the mean internal temperature was 18.7 ± 1ºC. Three *RN⁻/rn+* chops at a time, followed by three *rn+/rn+* chops were fried at low (160ºC), medium (180ºC) and high (200ºC) temperatures in a cast iron pan on an ordinary household stove with a ceramic top. The chops were fried in 10 g of a commercial vegetable frying fat for 3 min per side and then the chops were left to cool to room temperature, weighed and photographed. Cooking loss was calculated as the percentage change in chop weight before and after frying.

The concentration of HCAs in the crust was analysed using a Blue chitin solid-phase extraction method. In short, the freeze-dried crust was homogenised in NaOH, mixed with diatomaceous earth and packed into a cartridge. The HCAs were extracted with ethyl acetate. The eluate was evaporated to dryness, dissolved in NaOH and applied on a Blue Chitin column. The HCAs were eluted with MeOH/ NH4OH (9:1 v/v), evaporated to dryness, dissolved in MeOH, and then analysed using LC/MS (LCQDECA ion-trap mass spectrometer, with Xcalibur software, Thermo Finnigan, San José, CA, USA), using the electrospray as ion source (Bång *et al.*, 2002). The recoveries ranged between 30-80% depending of type of HCA and recovery corrections were made before presenting the results.

In the questionnaire, the questions were aimed to provide data on consumption habits and household cooking practices for pig meat with special emphasis on the loin (pork chops), *M. longissimus dorsi* (LD). For general questions on the consumption of pig meat the respondents were asked only to consider lean meat and not mixed or further processed products such as cooked ham, sausages or bacon. Six colour photographs showing the surface colour and the interior colour of fried pork chops were presented to the respondents who were asked to indicate which of the photographs corresponded best to their normal frying practices. The six photographs represented *RN⁻/rn+* and *rn+/rn+* meat, fried at three different temperatures described previously. The questionnaire was distributed to a total of 151 people (60 men and 91 women) in Uppsala, Sweden, at three different occasions during the summer of 2002. For the analysis of the questionnaire, the respondents were divided into four age groups: 1 = 15-20, 2 = 21-40, 3 = 41-60, and 4 = 61 years and upwards.

Results and discussion

Three initial pan temperatures, 160, 180 or 200º C were chosen to mimic various domestic frying practices. After frying pork chops from one carrier and one non-carrier of the *RN⁻* allele at these different temperatures, we found the mutagenic HCAs, MeIQx, PhIP and IQx, in low concentrations in the crust. The level of mutagenic HCAs was almost ten times higher in *rn+/rn+* meat fried at 200ºC compared to the *RN⁻/rn+* chops (0.49 vs. 4.13 ng/g cooked meat). IQx was detected only in the *RN⁻/rn+* crust whereas the comutagens, harman and norharman, were detected in the crust of the *rn+/rn+* chops but not in the *RN⁻/rn+* meat. The *rn+/rn+* meat, fried at an initial pan temperature of 200ºC gave more than 20 times the amount of mutagenic HCAs compared to that fried at 160ºC, 4.13 compared to 0.18 ng/g cooked meat. PhIP was the main HCA formed. In a study by Augustsson *et al.* (1997) a 20-fold increase of PhIP levels was typically seen when increasing the frying temperature for several meat dishes from 175ºC to 225º C, and a general temperature dependency of the formation of HCAs has been repeatedly reported (e.g. Skog *et al.*, 1995).

Based on colour photographs, the respondents reported that they preferred fried chops with medium crust colour, rejecting the very pale crust of the non-carrier of the *RN⁻* allele fried at low temperatures as well as the dark brown of the carrier fried at medium/high temperature. Most respondents chose fried chops from the non-carrier, which, based on information given in a questionnaire, would result in an markedly higher average contribution to the monthly HCA intake of 359 ± 402 ng (mean ± SD) compared to 35 ± 60

ng/month for consumers who preferred the *RN⁻/rn+* chops. Overall, the total monthly intake of mutagenic HCAs derived from consuming pork chops was moderate, on average 256 ng, ranging from 0 to 1982 ng/month (Table 1). A mean intake of 256 ng/month would mean a limited daily contribution of about 9 ng from fried pork chops, well in agreement with an earlier Swedish study which estimated a daily contribution of 10.1 ng/day from fried pork chops and gravy (Voskuil *et al.*, 1999). This intake rate should be related to the assessed total daily intake of 200 and 120 ng HCAs for Swedish men and women, respectively (Augustsson *et al.*, 1997). Besides the Swedish study, data on the specific contribution of pork to the intake of HCAs are sparse. According to estimates by Layton *et al.* (1995) the intake of HCAs originating from pork would be 51 ng/day for a man or woman weighing 70 kg. The corresponding figures for beef and chicken would be 542 and 173 ng/day, respectively. The estimated daily intake in this study was however high, 1820 ng/day compared to 160 ng/day in another Swedish study (Augustsson *et al.*, 1997).

Conclusions

To estimate total HCA intake, epidemiologists try to identify markers for the factors cooking time and temperature. Doneness of meat and crust browning have been put forward as a reasonable indirect measurement of mutagenic activity (Steineck *et al.*, 1993; Sinha *et al.*, 1999). However, our data show that care should be taken when using colour photographs to determine cooking preferences for assessment of HCA intake because background factors, such as the occurrence of the *RN⁻* allele in pigs, may influence the results. The *RN* genotype of pigs affects both colour formation and HCA content and thereby makes crust colour inappropriate for estimation of HCA content in cooked pig meat carrying the *RN⁻* allele.

The study involves respondents from a small regional area in Sweden and a relatively homogenous population group. Thus, it is not appropriate to extrapolate its findings to general exposure of HCAs in the Swedish society. However, it does contribute to better knowledge about the level and formation of HCA in domestically prepared pork chops as well as understanding of the complexity of using different exposure indicators, such as the degree of surface browning, in the assessment of HCA intake through diet. The study shows that the raw material composition is important and that small adjustments in the domestic cooking practices may help reduce the formation and intake of HCAs.

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Table 1. *Estimated monthly intake (ng) of mutagenic HCAs from domestically cooked pork chops, based on information on how often pork chops (loin) are fried, serving size and the calculated content of HCAs for the cooked chop in the particular photograph chosen (means ± SD)*

	IQx	MeIQx	PhIP	Total mutagenic HCAs
Men, n = 58	6 ± 20	50 ± 56	189 ± 252	244 ± 305
	max 137	max 206	max 1033	max 1188
Women, n = 90	1 ± 8	52 ± 78	211 ± 327	264 ± 401
	max 80	max 413	max 1570	max 1982
Tot. population, n=148	3 ± 14	51 ± 70	202 ± 299	256 ± 365
	max 137	max 413	max 1570	max 1982

SOUS VIDE COOKED BEEF MUSCLE. EFFECT OF SALT ADDITION ON BIOCHEMICAL PARAMETERS

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Background

Semitendinosus beef muscles injected with salt solutions -sodium chloride (SC) and sodium tripolyphosphate (STPP)- and cooked by the *sous vide* system (research carried out by our group), showed that both salts -alone or in combination- successfully reduced cooking weight losses (Sanchez, *et al*. 2004). The best results were obtained with the following salts combinations (0.25 g STPP + 1.20 g SC, and 0.25 g STPP + 0.70 g SC, per 100 g tissue), reducing cooking weight losses to almost 0%. Salt incorporation modified both, pH and Hunter Lab *a* parameter. Sample pH increased as STTP concentration increased. Hunter Lab *a* parameter was affected by both, salts and temperature. The optimum cooking temperature (CT) range was 60-65 ºC, this temperature range modified structural components of the muscle inducing disintegration of filaments and lateral and longitudinal shrinkage of myofibrils. The magnitude of the alterations depends on CT. The combination of STPP and SC salts and a CT of 65ºC produced the best gel-like structure that preserves cellular structures and increases water holding capacity (Gonzalez *et al.* 2004). Even though the micro and ultra structural studies described certain mechanisms involved in salts and CT effects on these muscles, it was important to evaluate biochemical parameters in order to confirm or further explain these effects on the final quality of the product.

Objectives

To determine tissue salts concentrations and evaluate its distribution and analyse collagen, salt soluble and total protein content, and protein patterns in cooking juice after the application of both, salts injection and *sous vide* cooking, on *Semitendinosus* muscles. To evaluate myofibrillar protein patterns in non-cooked samples.

Materials and methods

Beef *Semitendinosus* muscles were excised from steers carcasses and collected at a beef packaging plant 48 h after slaughter. Fat free muscles (pH average: 5.49 ± 0.26) were injected (10%w/w, hand operated brine pump) with appropriate salt solutions to obtain the final tissue concentrations of STPP and SC indicated in tables I and II. Injected and non-injected samples were then submitted to *sous vide* cooking procedure, temperatures applied were 55, 58, 65, 72 and 75 ºC (Sanchez *et al*. 2004). Tissue phosphate extraction was carried out as in Sanchez, *et al*. (2004) and the determination was accomplished utilizing a Sigma Diagnostics Kit based on the Fiske-Soubarrow reaction. Sodium Chloride quantification was performed following the directions of Ockerman (1985). Total collagen was evaluated following the procedure reported by Hill (1966). Salt soluble protein extraction was carried out as in Saffle & Galbreath (1981), and protein quantification was completed by the Bradford methodology (1976). Total protein content was achieved by the Kjeldahl method (AOAC 1994). Purified myofibrils were isolated following the method described by Molina & Toldrá (1992), and the SDS-PAGE was performed following the procedure of Laemmli (1970). Molecular weight (MW) and relative quantity of protein bands in the gels were analysed with a Bio Rad GS-800 Calibrated Densitometer.

Results and discussion

Table I shows the average and standard deviation (SD) for each salt actual value (experimentally obtained values) expressed as g/ 100 g dried tissue, and the comparison to the nominal one (theoretically injected value). It can be seen that until 0.25% STPP, the nominal and actual values were close similar, and at higher STPP concentrations lower amount of the salt was retained inside the muscle. Instead, SC was almost totally

retained at all concentrations utilized. This result made evident that the procedure selected to incorporate the salts was appropriate.

Figure 1 depicts the slice sampling performed to study salts distribution. Table II illustrates the significant differences of STPP and SC actual values for each slice sampling. It can be seen that the higher the STPP concentration is, the higher is the difference between values of the centre (site of the injection) and the peripheral positions, indicating a restricted diffusion of this large molecule inside the muscular tissue. However, at the same STPP concentration injected, the presence of SC improved STPP distribution, probably accelerating its diffusion by establishing a competence between both ions (increases effective diffusion coefficient). In the case of SC, this table shows that at the lower SC concentration the salt is evenly distributed nevertheless when the salt concentration is increased to ≥ 0.7 (g/100 g tissue) the distribution became less homogeneous. However, when STPP concentration increased it improved SC distribution as was previously described. The overall result indicated that salts distribution was not as homogeneous as we expected. Consequently further investigations related to the present issue were conducted applying multi-needle injection and tumbling (massaging) procedures to improve salts distribution.

There were not significant differences ($p>0.05$) in total collagen concentration due to the different salt treatments and CTs including 72 ºC (data not shown), indicating that this parameter was not affected by the incorporation of the salts and/or by changes in CT between 55-72 ºC. On the other hand at 75ºC, the total amount of collagen in the tissue consistently diminished (no significant differences were found among salt treatments), probably due to the solubilization/gelatinization of the collagen that occurs at temperatures between 75-85 ºC, with the potential loss of this protein in the cooking-juices.

Fig. 2 shows the salt soluble protein content for each salt treatment at the different CTs grouped as Low CT (55 ºC to 58 ºC), Medium CT (65ºC) and High CT (72ºC to 75ºC) expressed as mg/100 g of dried tissue. Non significant differences were found among salt treatments for each temperature range ($p> 0.05$). Instead, when CT increased the salt soluble protein concentrations in the tissue decreased ($p < 0.05$). This tissue reduction was probably due to a greater protein solubilization induced by the higher temperatures, and the consequent loss of the solubilised protein in the cooking-juices.

Non changes in the total protein content of the tissue (data not shown) were found among salt treatments or comparing the different CTs, it seems that the small but significant changes detected in the tissue concentration of the salt soluble proteins were not evidenced in the total protein content of the tissue.

Supernatants (final wash) of the myofibril extraction of non-cooked samples (figs. 3 & 4), and of cooking-juices (65 ºC, data not shown) coming from salt-treated and control samples, were analysed for type and quantity of proteins. These figures depict the protein patterns of the 10% and 7.5% SDS-PAGE of the same sample. It can be seen that three major bands in the range of 43-45 kD, 105 kD and 200 kD appeared in the 5th line (0.25% STPP + 0.70% SC), which 2 to 3 times higher concentration compared to the other salt treatments and the control. Densitometry analysis showed that the MW of these major bands are in accordance to the actine (42-43 kD), α-actinine (100-103 kD), and Heavy Chain Myosin (200 kD) bands. It appeared that these proteins were much more solubilized by this combination of salts. However, when higher SC concentration (0.25% STPP + 1.2%SC) was applied, it seems that a salting out effect reduced protein solubilization. In the protein pattern of the cooking-juices (data not shown), it was also detected an increment in proteins concentration, mainly in those of lower MW, corroborating partially previous results. Again, it appears that 0.7% SC is the more suitable concentration to increase protein solubilization, and the combination of both salts improved this solubilization. The differences found between supernatant (non-cooked) and cooking-juice was probably due to the CT applied, which also increased protein solubilization disregarding of the salt concentration used. As well, the appearance of smaller MW bands could be related to the temperature-induced degradation. The presence of these solubilised proteins could be responsible for the gel-like structure described previously by this group (Sanchez, *et al.* 2004, Gonzalez, *et al*. 2004), where the strongest gel-like structure formed and the lowest cooking weight loss was obtained with the salt combination of 0.25% STPP + 0.70% SC and 65 ºC cooking temperature.

Conclusions

Salts incorporated were not evenly distributed in the muscle suggesting the use of another methodologies to improve incorporation and distribution.

Salt addition (particularly 0.25% STPP + 0.70% SC) induced protein solubilization in the fresh tissue, this result supports the formation of a gel-like structure at 65 ºC that reduce cooking weight loss reported in previous work of the group.

No other biochemical changes were observed with the different salt combinations.

Acknowledgements
This work has been granted by Instituto Nacional de Tecnología Agropecuaria (INTA) & Agencia Nacional de Promoción Científica y Tecnológica de Argentina (Subsidio BID 1201/OC-AR PICT 09-08831), and Facultad de Agronomía y Ciencias Agroalimentarias - Universidad de Morón (Subsidio 06A001/02).

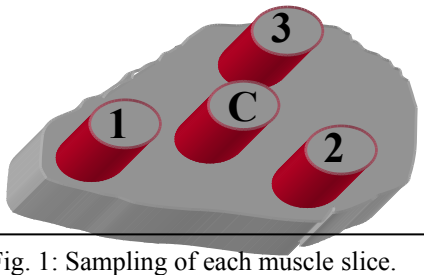


Fig. 1: Sampling of each muscle slice.

Table I: Nominal and Actual value of STPP and SC for each salt treatment

% STPP		% SC	
Nominal Value	**Actual Value ± SD**	**Nominal Value**	**Actual Value ± SD**
***	***	0.70	0.57±0.15
0.10	0.11±0.01	0.20	0.15±0.04
0.10	0.12±0.01	1.20	1.00±0.37
0.25	0.24±0.05	***	***
0.25	0.23±0.03	0.70	0.74±0.11
0.25	0.26±0.05	1.40	1.30±0.18
0.40	0.32±0.05	0.20	0.21±0.07
0.40	0.35±0.05	1.20	1.35±0.35
0.50	0.35±0.05	0.70	0.71±0.15

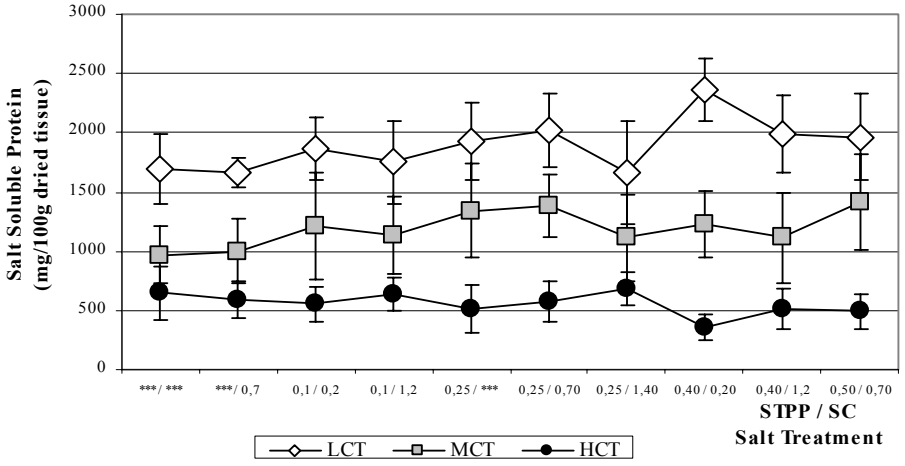
SD: Standard Deviation

Table II: Significant differences for STPP and SC actual values

Nominal Value (%) STPP / SC	STPP				SC			
	1	2	3	C	1	2	3	C
*** / 0.70	*	*	*	*	b	b	b	a
0.10 / 0.20	a	b	ab	ab	a	a	a	a
0.10 / 1.20	a	a	a	a	b	b	b	a
0.25 / ***	a	a	a	b	*	*	*	*
0.25 / 0.70	a	a	a	b	ab	b	b	a
0.25 / 1.40	a	a	a	a	b	ab	b	a
0.40 / 0.20	abc	a	ab	c	a	a	a	a
0.40 / 1.20	ab	a	ab	b	b	ab	ab	a
0.50 / 0.70	a	a	a	b	a	a	a	a

Different letters in the same row (within each salt), indicate significant differences among the positions for each salt treatment

Fig. 2: Salt Soluble Protein (mg/100g dried tissue) for the different salt treatments at each grouped CT



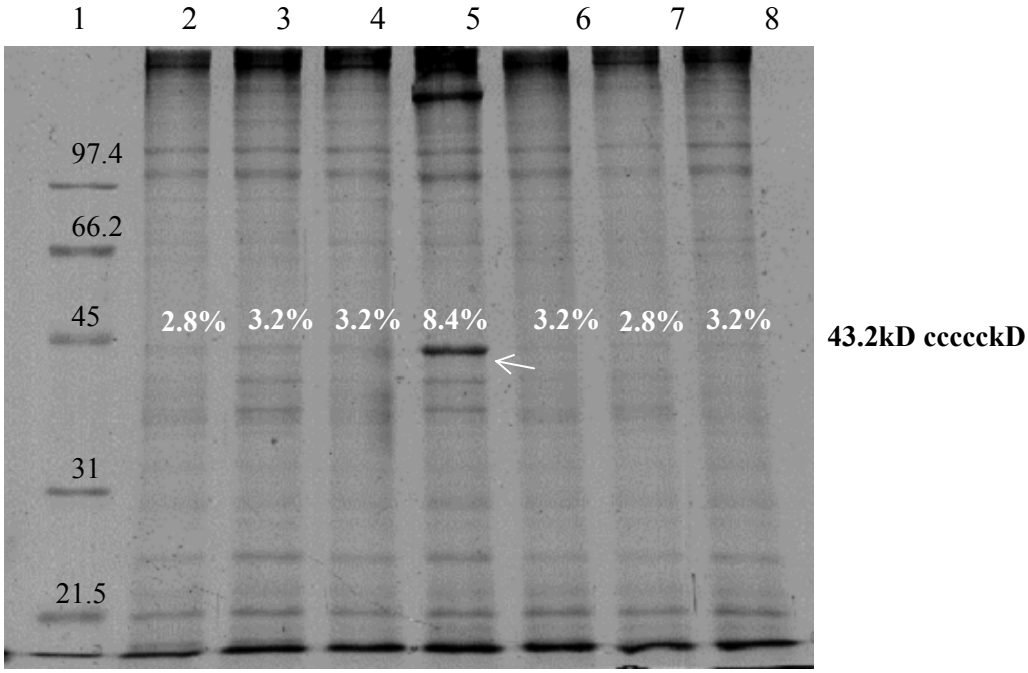


Fig. 3: 10% SDS-PAGE of myofibril supernatants (final wash) Lines: 1= MW standard (kD), 2= SC 0.7%, 3= SC 1.2%, 4= STPP 0.25%, 5= SC 0.7% + STPP 0.25%, 6= SC 1.2% + STPP 0.25%, 7= water injected, 8= control (non injected)

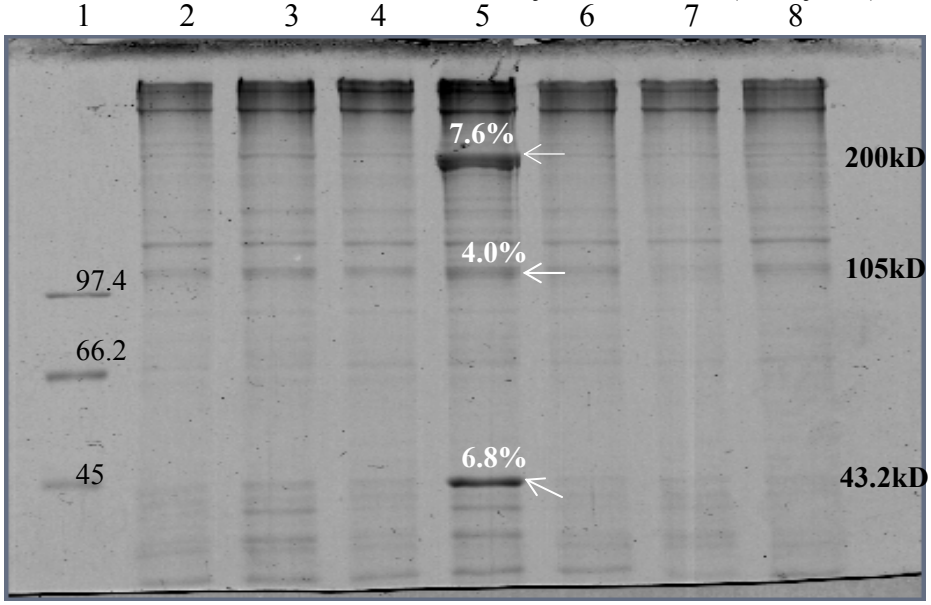


Fig. 4: 7.5% SDS-PAGE of myofibril supernatants (final wash). Lines: 1= MW standard (kD), 2= SC 0.7%, 3= SC 1.2%, 4= STPP 0.25%, 5= SC 0.7% + STPP 0.25%, 6= SC 1.2% + STPP 0.25%, 7= water injected, 8= control (non injected)

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INDONESIAN BAKSO MEATBALL PROPERTIES WITH POSTMORTEM MEAT TIME AND TAPIOCA STARCH CONCENTRATIONS

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Background

Food is an integral part of human society, providing nourishment and cultural enjoyment. Worldwide, more emphasis is being placed on convenient and traditional foods. Meatballs are a convenient meat product for preparation in the home or by commercial eating establishments. Meatballs and fishballs are especially popular in Asia. In Indonesia, meatballs known as bakso are made from prerigor meat or meat before completion of rigor mortis (Purnomo, 1990). The meat is ground and emulsified with salt, tapioca starch, and garlic. The batter is formed into small balls and cooked by steaming, boiling, or deep-frying, depending on the cuisine. Bakso is commonly served with boiled chicken stock or soup and distributed from pushcarts at street corners (Pandisurya, 1983), with consumer preference for tougher, but more elastic, bakso (Yuliati, 1999). Commercial production of bakso has been difficult for Indonesian meat processors because sufficient quantities of prerigor or early postmortem meat materials are not readily available. The use of raw chilled or frozen postrigor meat would provide for consistent supplies of adequate raw materials.

Objectives

The purpose of this research was to evaluate the properties of bakso after substitution of postrigor meat for early postmortem meat in bakso and to determine the appropriate level of tapioca starch for production of bakso with postrigor meat.

Materials and Methods

Local 2 to 4 year old Ongole crossbred grass-fed cattle were slaughtered at RPH Pegirian (Pegirian Slaughter House, Surabaya, East Java, Indonesia). Meat collection was approximately 3 hours in total for deboning of *Semimembranosus* and *Semitendinosus* muscles from carcasses and about 1 hour transportation to the PT. Eloda Mitra meat plant (Sidoarjo, East Java, Indonesia) in a refrigerated truck. The 160 kg of collected meat was chilled immediately after collection (10°C) and randomly assigned to early postmortem and postrigor groups of 80 kg. Early postmortem meat was ground for immediate usage for bakso. Postrigor meat was held chilled at 10°C for approximately 24 hours until processing into bakso. Early postmortem ground meat pH was 5.30-5.79, while the postrigor ground meat had pH of 5.06-5.59. Added ingredients were tapioca starch (National® 7, National Starch and Chemical, Singapore) at 5, 10, or 15% of total formulation, 0.6% sodium tripolyphosphate (STPP, $Na_5P_3O_{10}$, Albert & Wilson Phosphate Groups, Indonesia), 1.6% salt (NaCl) from local markets, 0.6% regular cane sugar obtained from local markets, and 0.8% monosodium glutamate (MSG, PT. Ajinomoto, Indonesia). The meat block was decreased proportionally to increased starch levels. Bakso was manufactured using the equipment, facilities, formulation, and processing procedures of PT. Eloda Mitra.

Ground meat was chopped in a bowl chopper (K.G. Wetter, Germany) for 20 minutes. Salt, STPP, and tapioca starch were added to the early postmortem or postrigor meat batches with crushed ice at 5% to maintain 15°C batter temperature. Batter was formed mechanically into 14 g balls with a meatball former (Chuang Zong Baller, Taiwan). The balls were boiled at 100°C for 20 minutes in an open boiler (PT. Mastrada, Indonesia) or until balls were floating in the boiling water. Cooked bakso balls were drained on perforated aluminum trays to remove excess water (outer surface appeared dry) before packing into limited-low-density polyethylene bags (25 x 160mm, 0.15 mm thick, Top Printing Indonesia Co., Indonesia) with 20 balls/bag for vacuum packaging (Henkelman H-800 Double Chamber, Netherlands) and frozen at -20°C. Each experiment was replicated 3 times.

Bakso balls were stored frozen at -20°C and thawed at room temperature (32°C) for 20 to 30 minutes before analyses. Moisture and fat were determined by methods 950.46 and 960.39 (AOAC, 1990), respectively, at Laboratorium Sentral Pangan (Central Food Lab, University of Brawijaya, Malang, East Java, Indonesia). Texture analysis was with a Lloyd Machine Model Universal Testing Instrument according to Hidayati (2002) and Yuliati (1999) at Pusat Antar Universitas Pangan dan Gizi (Center Inter-University Food and Nutrition, Gajah Mada University, Yogyakarta, Indonesia). The Lloyd Universal Testing machine was warmed up for 10 minutes before reading elasticity, gel strength, and shear force readings with an upper cycle limit of 4-mm, lower cycle limit of 3-mm, compression mode, and 60-mm/min test speed, 20-mm/min chart speed. Sample width was set to 10-mm, depth to 10-mm and gauge length to 10-mm. The bakso sample was a cube of 10 x 10 x 10-mm placed under the slice shear force probe for measurement of texture as minutes/gram for elasticity and newtons for gel strength (hardness) and shear force values. Samples for scanning electron microscope procedure were prepared according to Hidayati (2002) and Yuliati (1999) at UPT Mikroskopi Elektron (University of Airlangga, Surabaya, East Java, Indonesia). Bakso samples were sliced 2 to 3-mm thick with a razor blade, fixed with 2% glutaraldehyde in a phosphate buffer of 7.3 pH, and dried with critical point drying (Sumdri-780 Sample Drying, USA) for 72 hours before placing on a brass plate holder. Samples were coated with 24 carat gold with an ion sputter-fine coater (JEOL-GLE4X, JEOL Technic Co. Ltd., Japan) for 1.5 min, achieving an approximate thickness of 0.25-mm. The coated sample was observed under a scanning electron microscope unit (JEOL GSM-T100 Scanning Electron Microscope, JEOL Technic Co. Ltd., Japan) at 1500X magnification. Statistical analyses were performed by General Linear Model procedures for a completely randomized design (SAS, 1998), with main effects of postmortem condition, tapioca starch concentration, replication, and interactions, and least mean squares differences in analyses of variance at probability value of less than 0.05.

Results and Discussion

Replications in this experiment were different ($p<0.05$) for fat content and shear force, most likely due to animal source, but perhaps due to differences in homogenization of different batches. Moisture, fat, elasticity, and gel strength were not different ($p>0.05$) in bakso from postrigor meat or early postmortem meat. Shear force was higher ($p<0.05$) in bakso from early postmortem beef (21.4 N) than postrigor beef (20.6 N). The bakso met the Indonesian National Standards (70% moisture, less than 2% fat, Board of Nasional Standardization, 1995) with 72 to 75% moisture content and 0.2% fat. Moisture, fat, elasticity, and gel strength did not differ ($p>0.05$) with level of tapioca starch, but trends for increased texture with increased starch were observed. Shear force increased ($p<0.05$) with increased starch. SEM micrographs of bakso from early postmortem and postrigor meat with 5, 10, and 15% tapioca starch illustrated spongy three dimensional structures. The myofibrillar proteins appeared as interconnecting thin protein strands that formed a net-like matrix. The tapioca granules were observed as dense spherical granule aggregates, aw was also reported by Yuliati (1999) in canned bakso. The dense, aggregate, and non-swelled appearance of tapioca starch granules may be an effect of the high vacuum of the scanning electron microscope column (Hood et al., 1974). Micrographs of bakso from postrigor meat showed slightly less complex protein networking and pores appeared to have more large voids than in bakso from early postmortem meat. Bakso from postrigor meat had a spongy structure with less protein network development than bakso from early postmortem meat that appeared to have more extensive matrix development and more and smaller void spaces.

Sufficient proteins were extracted during comminution with 1.6% salt to result in stable batters (Barbut, 1995) and added phosphates reduce processing yield losses and stabilize emulsion structure (Eilert et al., 1996). Starch granule swelling and gelatinization contribute to emulsion stability, viscosity or elasticity, and prevention of syneresis during refrigeration, frozen storage, or thawing (Hood *et al*., 1974). Hidayati (2002) studied the effects of sodium tripolyphosphate and sodium alginate on the rheological properties of bakso and obtained elasticity ranging from 0.5183 to 0.540 min/gram while hardness (gel strength) of bakso ranged between 24.237 to 59.410N. The texture in those studies was relatively uniform, which was also observed with the present results. Sensory panelists indicated that tougher bakso with more elastic properties was more desirable (Yuliati, 1999).

Conclusions

Use of postrigor meat in bakso production gave only slightly different textural properties than early postmortem meat. Bakso with 15% starch had the highest elasticity, which is sought as a bakso rheological trait. Postrigor meat with inclusion of 15% tapioca starch could be used to produce bakso with sufficient textural traits for commercial mass production. This will allow improved raw material procurement and increased efficiencies for bakso processors.

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Yuliati, S. 1999. *Pengaruh perebusan sebelum pengalengan dan lama simpan terhadap kualitas bakso dengan bahan pengisi tepung kentang modifikasi yang dikalengkan* (Effects of boiling before canning and storage time towards modified potato starch substitution in canned bakso). *Skripsi* (Thesis). Fakultas Peternakan, UNIBRAW, Malang.

Table 1. Proximate analysis and rheological means of bakso from early postmortem and postrigor meat with 0, 5, and 15% starch.

Meat rigor Condition	Starch (%)	Moisture (%)	Fat (%)	Elasticity (min/g)	Gel strength (N)	Shear force (N)
Postrigor	5	72.78	0.18	0.439	34.269^{b}	18.505^{f}
Postrigor	10	74.80	0.20	0.503	36.335^{a}	21.401^{cd}
Postrigor	15	72.59	0.27	0.557	38.490^{a}	21.905^{b}
Early postmortem	5	74.45	0.22	0.535	34.901^{b}	19.720^{e}
Early postmortem	10	74.21	0.14	0.582	37.467^{a}	21.817^{bc}
Early postmortem	15	72.39	0.23	0.601	40.871^{a}	22.762^{a}
SEM		0.82^{nd}	0.04^{nd}	0.071^{nd}	1.248	0.186

Data are means and standard errors of mean (SEM) of 3 replicated experiments. Means for a variable with different superscript letters are different ($p<0.05$); nd superscripts indicate no differences ($p>0.05$) among corresponding means.

DESCRIPTIVE EMPIRICAL MODEL OF INSTRUMENTAL HARDNESS CHANGES IN DRY-CURED *SEMIMEMBRANOSUS* AND *BICEPS FEMORIS* MUSCLES AS A FUNCTION OF WATER CONTENT, NACL AND PH

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Background

The dry-cured ham process involves a long drying period that produces a gradient in hardness in the product. Extreme superficial hardness could cause the crust formation, which is one of the most important problems in cured meat products. This problem is related to a high drying rate on the outer part while in the inner part there is a high moisture content. The diffusion rate of water from the inner zone does not compensate the high dehydration rate on the surface and consequently the surface hardens and forms a crust (Flores, 2001). The experimental relationship between hardness and water content has been reported by various authors (e.g. Virgili *et al*., 1995; Ruiz-Ramírez *et al*., 2003) but until now no attempt has been made to describe the effect of NaCl and pH on this relationship. According to Ruiz-Ramírez *et al*. (2003), the hardness-water content relationship can be described by an exponential model.

The effect of pH and NaCl content on the texture of dry-cured meat products have been examined in previous studies. Arnau *et al*. (1998) and Guerrero *et al*. (1999) established higher hardness for normal or low pH hams than for high pH hams. Whereas, Magraner *et al*. (2003) associated high pH levels with harder dry-cured hams. Low NaCl content was related with pastiness and softness in dry-cured ham (Arnau, 1991), while high NaCl content increases the shear force in dried fish products (Iseya *et al*., 1998).

Objectives

The aim of this study was to model the relationship between water content and hardness of dry-cured meat products considering the NaCl and pH effects.

Materials and methods

The experiment was undertaken using 18 hams. Half of the hams had a pH<5.7 and the rest a pH>6.2. The pH was measured on *Semimbranosus* (SM) muscle at 24-h post mortem (pH_{SM}) with a combined electrode (Ingold 406, Ingold, Urdof, Switzerland) attached to a portable pH-meter (Crison 507, Crison Instruments S. A, Barcelona, Spain). SM and *Biceps femoris* (BF) muscles were separated from the hams and manually rubbed with a dry salt-cured mixture of 0.5 g of KNO_3, 0.3 g of $NaNO_2$ and depending on treatment with 20, 50 and 80 g of NaCl per kg of muscle (8.19%, 16.67% and 21.29%) on a dry matter basis (DM) respectively. These muscles were individually packaged in bags of polyamide and polyethylene (SACOLIVA® permeability: 2.6 g H_20/m^2/day at 23 ºC/85%RH) and were horizontally placed in trays in a room at 2±2 ºC for 45 days. Thereafter nine samples from each muscle (N=324) were shaped as a parallelepiped (4x2x2 cm). The rest of the muscles were ground and packaged in metallic bags (SACOLIVA® permeability: < 1 mg H_20/m2/day, to 23 ºC/85%HR), and stored at 2±2 ºC until its posterior physicochemical analysis. Each parallelepiped was weighed in an analytical balance of 0.01 mg of precision (Mettler PE 300) and was dried in a drying tunnel at 3±2 ºC, 57.5±2.5% RH and 1m/s air speed, until desired levels of drying were reached. Levels of drying used corresponded to the range of water content 28,5- 59.7%. The dried samples were individually packed in plastic bags (20 µm polyamide / 70 µm polyethylene; water vapor permeability: 2.0 g/m^2/24h; SACOLIVA S.L.®, Castellar del Vallès, Barcelona) with a 70% nitrogen atmosphere and kept at 15±2ºC for a minimum of 30 days to allow homogenization of NaCl and water throughout the sample.

A texture Analyzer (Universal MTS Alliance model. RT/5, SEM, Barcelona, Spain) was used to determine hardness. Before carrying out texture analyses nitrogen-packed samples were kept for one hour at room temperature. The cores of the dry-cured loin slices were accurately carved with a scalpel into 10 x 10 x 10 mm (length x width x height) and triplicates were obtained. The samples were compressed to 50% of their original height. Force-time curves were recorded at crosshead speed of 1 mm/s. Hardness was defined by maximum peak force during the compression cycle.

The parallelepiped samples used for texture analysis were immediately cut up and water content was determined by drying at 103±2ºC until reaching constant weight (AOAC, 1990). Water content of the samples was expressed on a dry mater basis (DM) (X = kg H_2O / kg DM).
Sodium chloride content was determined by the Volhard method (ISO 1841-1: 1996), water content (AOAC, 1990) and pH (measured with a xerolyt penetration electrode Crison) were determined in the rest of the ground muscle.
Statistical analysis: All the statistical analyses were carried out with the SAS statistical package (SAS Institute, 1999). The relationships between hardness and water content on a dry mater basis (X) were studied through a non-linear regression analysis (PROC NLIN). The following model was used:

$$Y = aX^b \quad \textbf{(1)}$$

Where Y is the predicted hardness, a and b are the model parameters and X= water content on a dry mater basis.

Results and discussion

Figure 1 shows the relationship between hardness and water content (X) for all dry-cured samples. Experimental data indicate that for a range of X between 0.8 and 1.3 the hardness remains practically unchanged while for X<0.6 the hardness increases substantially. This substantial increase occurs at different values of X according to the product, i.e.: 0.8 in dry-cured loin (Ruiz-Ramírez, submitted) and 0.6 in dry-cured ham (Ruiz-Ramírez *et al*., 2003). In the present study, the substantial increase of the hardness is similar to the one observed in dry-cured ham. The different anatomical origin of the samples can explain the difference between ham and loin. Figure 2 (a) shows the predicted hardness by muscle. The BF and SM muscles presented similar hardness at each water content. Figure 2 (b) shows the predicted hardness according to pH measured in the muscle SM (pH_{SM}). The samples from hams with low pH_{SM} presented greater hardness than those from hams with high pH at X values from 0.6 to 1.3. But when X diminished below 0.6 the differences between samples from hams with different pH_{SM} were not significant (P>0.05). Nevertheless, at X>0.7, which corresponds to the inner part of the dry-cured ham, the samples from hams with low pH_{SM} presented greater hardness. The lowering of the meat pH closer to the isoelectric point of myosin, increases intermolecular linkages between negatively and positively charged groups, which would explain the greater hardness in the muscles with low pH. This result agrees with those reported by Arnau *et al*. (1998) and Guerrero *et al.* (1999), who found a higher hardness for low pH hams than for high pH hams at X values around 1.7.
The NaCl content also influenced the hardness of the muscles (Figure 3a and 3b). The muscles with higher NaCl content presented higher hardness for samples from hams with low pH_{SM}. These results are probably due to the fact that when high NaCl contents are used there is a compaction of myofibrillar structure (Shomer *et al*., 1987), which would increase the hardness. Similarly, Iseya (1998) found a higher hardness when the NaCl content was increased in dried fish products. The NaCl effect was different depending on the pH_{SM}. The NaCl effect was more evident in samples from hams with low pH_{SM} than in those from hams with high pH_{SM}, which presented similar hardness for NaCl content of 2% and 5% and higher for 8% in the range of X between 0.6 and 1.3. But when X diminished below 0.6 the significant differences between samples from hams with different NaCl content disappeared.
The behavior observed for the NaCl content was similar to that observed for pH, in the sense that when the X values diminished below 0.6, the differences among NaCl contents were lower. These results show that at X values below 0.6, which were achieved in the surface layer of dry-cured meats products, the hardness is more influenced by the water content than by NaCl or pH.
The estimates of parameters, the residual standard deviation (RMSE) and coefficient of determination (r^2) of the equation (1) obtained from the non-linear regression analysis for all dry-cured samples (general model), by muscle, pH_{SM} and NaCl content are showed in Table 1. The lowest RMSE for the model was for samples from hams with high pH_{SM} and 5% of NaCl and for samples from hams with low pH_{SM} and 2% NaCl content.

Conclusions

An exponential model was found appropriate for describing the relationship between hardness and water content (X). The hardness of the muscles was affected by the pH_{SM} and the NaCl content. As the NaCl content increases the hardness increases, especially at pH_{SM}<5.7. At X values lower than 0.6 the hardness is more influenced by water content than by NaCl content or pH.

Acknowledgements

This research was financed by the Spanish Ministry of Science and Technology (AGL2000-0517-P4-04 project). The authors gratefully acknowledge the National Funds of Science, Technology and Innovation FONACIT of Venezuela for co-financing the doctorate studies of Ruiz-Ramírez and CONDES-LUZ for financing the congress attendance.

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Table 1. Estimates of parameters from the non-linear regression of hardness versus water content on dry mater basis (X).

Dry-cured muscles		*a*	*b*	r^2	RMSE
General model		5.5119	-2.0985	0.82	3.70
pH	pH<5.7	6.0903	-1.9879	0.82	3.89
	pH>6.2	4.9980	-2.2022	0.84	3.41
NaCl pH_{SM}< 5.7	2%	4.4071	-2.1529	0.83	2.16
	5%	6.1547	-1.9141	0.86	3.29
	8%	8.7051	-1.5938	0.79	4.28
NaCl pH_{SM} > 6.2	2%	4.5340	-2.3245	0.73	4.08
	5%	4.3105	-2.4846	0.91	2.35
	8%	5.8779	-1.9775	0.87	3.52

a and *b* parameters of the model; r^2, coefficient of determination; RMSE, residual standard deviation.

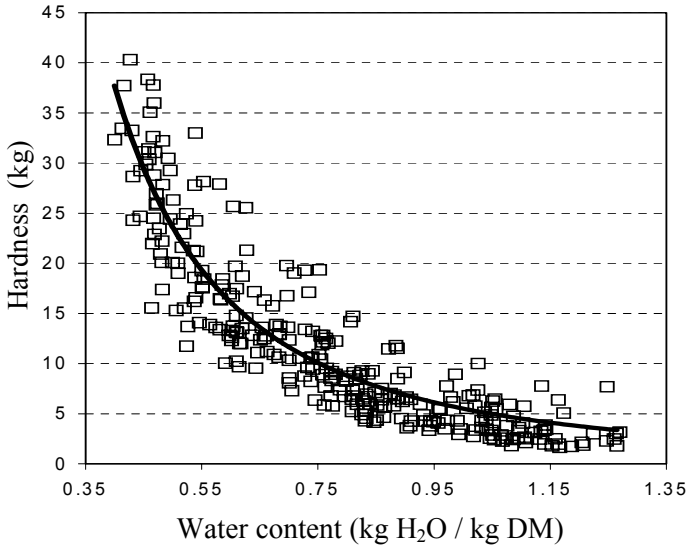

Figure 1. Hardness versus water content for overall samples. □ experimental hardness; — predicted hardness ($Y = aX^b$).

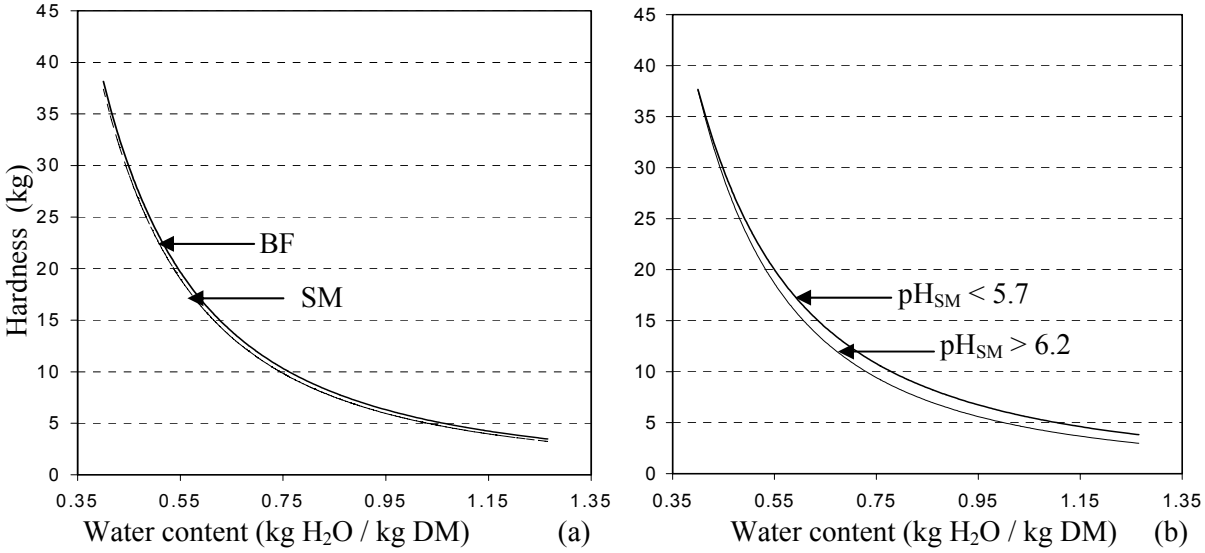

Figure 2. Predicted hardness versus water content by muscle (a) and by pH_{SM} (b) in dry-cured muscles.

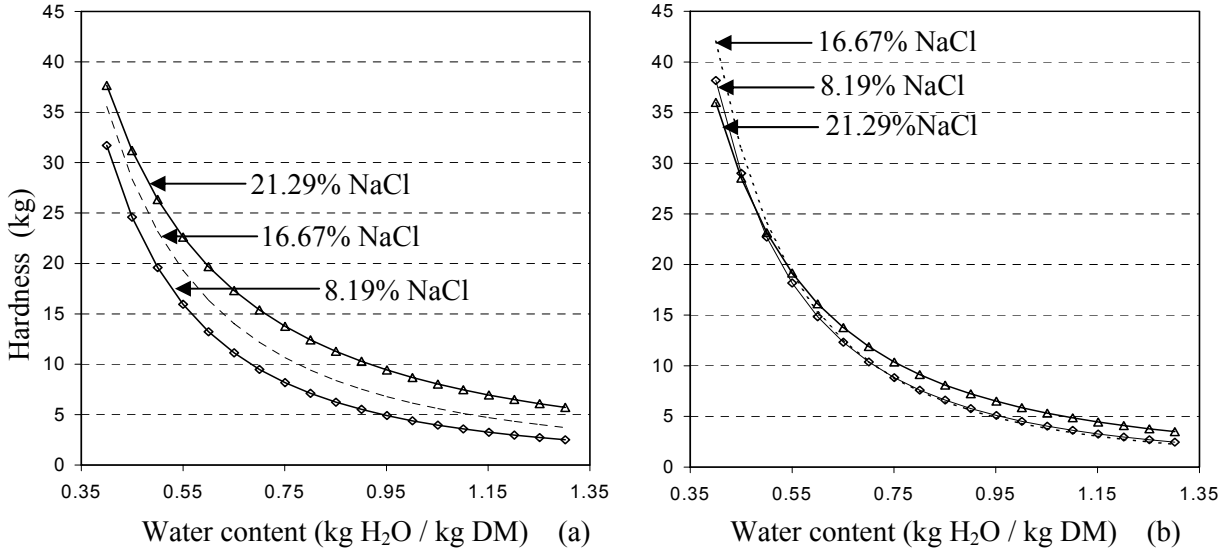

Figure 3. Predicted hardness versus water content by NaCl content in dry-cured muscles with $pH_{SM} < 5.7$ (a) and $pH_{SM} > 6.2$ (b).

ANTIOXIDANT ACTIVITY OF BORAGE (*BORAGO OFFICINALIS*) IN FRESH AND COOKED BEEF PATTIES

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Background

Lipid oxidation causes a rancid off-flavour and off-odour in meat. Numerous factors affect lipid oxidation including light, oxygen concentration, temperature, presence of anti- and pro-oxidants, degree of unsaturation of the fatty acids and the presence of enzymes (Skibsted *et al*., 1998). Lipid oxidation is normally not considered to be a limiting factor for shelf life of aerobic packed chill stored meat, as lipid oxidation occurs at a slower rate than discoloration or microbial growth (Zhao *et al*., 1994).

Meat colour is the primary attribute of fresh meat that affects consumer purchase decision. Consumers expect a uniform appearance within a group of similar ground beef products (i.e. patties with the same fat percentage) and relate any colour differences within similar products to deficiencies in product quality (Faustman & Cassens, 1990; Hood, 1990). Thus, any process that negatively affects the colour of fresh ground beef can lead to lower consumer appeal and marketability.

For cooked meat, thermal processes can promote lipid oxidation by disrupting cell membranes and releasing pro-oxidants, thereby inducing ‘‘warmed-over flavour’’ (WOF) during refrigerated storage and subsequent reheating (Sato and Hegarty, 1971). With the rapidly increasing consumer demand for precooked convenience meat items, a proper control of oxidation is of particular importance. One of the ways to minimize lipid oxidation and WOF in cooked meats is to use antioxidants.

There has been in the last years an increasing interest in borage (*Borago officinalis* L.), especially of its seed oil, by medical and nutritional research groups, due to the presence of high levels of γ-linolenic acid. Wettasinghe and Shahidi (1999) showed that borage meal exerted a concentration-dependent antioxidant activity in a meat model system. Borage extracts have demonstrated excellent antioxidant properties; the ability to retard lipid oxidation was attributable to the ability of its phenolic constituents to quench reactive oxygen species. Wettansinghe *et al*. (2001) reported that rosmarinic, syringic and sinapic acids are the major phenolic compounds present in the ethanolic extract of borage meal. It has been demonstrated that natural antioxidants rosemary extract, oregano extract and borage meal were highly effective in inhibiting lipid oxidation (TBARS formation) in beef patties packaged in modified atmosphere and stored in the dark at 2±1°C; in fact, borage meal suppressed totally TBARS formation (Sanchez-Escalante *et al*., 2003). The antioxidant activity of borage meal has been not studied in cooked meat products.

Objective

The purpose of this study is to determine antioxidant capacity of borage meal in fresh and cooked beef patties formulated with different content of fat and packaged in modified atmosphere and over-wrapped, respectively.

Materials and methods

Preparation of borage meal. Borage seeds were obtained from Spain. Seeds were cleaned, washed and dried, and after were stored vacuum packaged in polyethylene/polyamide pouches at 2-4°C until used. Borage seeds were ground in a mortar. The meal was defatted by shaking ground seeds with hexane (1:5 w/v, 5 min, three times). Dried and defatted borage meal was sifted with sieves of decreasing sizes (l.0, 0.5 and 0.2 mm) to eliminate the remaining husk and reduce the particle size of the meal.

Fresh patties and atmospheres. Meat was obtained fresh (3 day post-mortem) with a local producer, was excised from 2 beef carcasses, and minced using a conventional mincer through a plate with 4 mm holes. All two minced muscles were thoroughly mixed together in a single batch. Portions of uniform weight of the minced muscle (about 90 g) were mixed with salt (2%), borage (1%), and fat (10% for fresh patties and 10% and 20% for cooked patties). Also controls (no borage meal) were prepared with different fat levels. Fresh

beef patties were formed. One group was placed on styrofoam trays, for the study of fresh patties. Each tray with the round beef patty was introduced in a pouch made of a nylon and polyethylene laminate of water vapour and oxygen permeability 0.6 g/110 sq inch/24 hr and 3.5 cc / 100 sq inch / 24 hr 5-7 g/m^2/24 h at 23°C, respectively. The pouches were filled with a gas mixture of 80% O_2 + 20% CO_2, and sealed. Fresh patties were stored for 12 days at 2±1°C in the dark. Four packs were opened for subsequent analysis for each formulation every 3 days of storage; two of them were used for microbial sampling alone, while the 2 other were used firstly for colour instrumental analysis and thereafter for the determination of pH and TBARS.

Cooking and packaging. Meat patties were cooked on an open electric broiler by flipping every 3 min until the final internal temperature of 70 ºC (measured with a thermocouple) was reached. Samples were weighed before and after cooking to measure cooking losses. After cooling down to room temperature, meat patties were placed in styrene foam trays, over-wrapped in an oxygen permeable (6000–8000 cm^3/m^2/24 h at STP) cling film (Cryovac USA) and stored in a 2±1 ºC dark room for up to 12 days. Also a group of cooked meat patties were storage under light (1000 lux) at 2±1 ºC.

Colour measurement. Colour changes in the surface of fresh meat samples during storage were monitored by recording the CIE L*, a* and b* values using a Hunter Lab Colorimeter (D25 model). Measurement was achieved 30 min after package opening.

Lipid oxidation. Lipid oxidation in stored meat patties was also measured as thiobarbituric acid-reactive substances (TBARS) as described by Pfalzgraf *et al*. (1995).

Microbial analysis. Counts of aerobic psychrotrophic flora were determined in fresh and cooked patties in Plate Count Agar (Merck; Darmstadt, Germany) after incubation at 10°C for 7 days (Elliott *et al*., 1983). Counts were expressed as log cfu/g.

Statistical analysis. The significance of differences among samples at each day of storage was determined by analysis of variance (ANOVA) using the Least Square Difference method of the General Linear Model procedure of SPSS (SPSS 1995). Differences were considered significant at the $p<0.05$ level.

Results and discussion

Colour Measurement. Figure 1 shows that fresh beef patties with borage meal had ($p<0.05$) higher a* values, than those of controls with 10% of fat and without fat. The value of a* was influenced by the days of storage in each one of the treatments. The red colour diminished progressively along the storage in all the hamburgers, without caring the treatment neither the quantity of added fat. The control treatments, independently of the quantity of applied fat, showed significantly lower values ($p<0.05$) than the treatments with borage meal until day 9 of storage. It indicates that the borage meal is able to maintain high values of a* for more time. Red colour of borage samples was very intense instead to presence of meat pigments. These results were in agreement with Sánchez-Escalante *et al*. (2003) who reported that borage was effective in maintaining red colour for the first 10-12 days.

Lipid Oxidation. Figure 2 shows the changes in TBA value for fresh patties. Lipid oxidation increased rapidly with increasing time ($p<0.05$) in control samples. However, TBA values were kept to a minimum in samples containing borage meal. These results agree with the results of Wettasinghe and Shahidi (1999) and with Sánchez-Escalante *et al*. (2003), who found that borage meal possess antioxidant properties. With respect to cooked patties stored under darkness (Figure 3), our results show that the addition of borage meal is effective still after heating at 70ºC, since it delays the formation of TBARS; while control samples show an intense oxidation. Fat level did not affect antioxidant activity of borage meal; lipid oxidation was not increased with increasing time ($p<0.05$). Figure 4 show that lighting not increased lipid oxidation, thus borage meal was effective delaying TBARS formation, while controls developed an important lipid oxidation. The type of lighting significantly affected the shelf life of meat when is displayed for retail sale. The use of a lamp emitting radiation in the UV range (conventional supermarket fluorescent tube) is severely detrimental for the retail life of meat. TBARS values reveal that the retail life of beef meat is significantly more stable in the absence of UV light (Djenane *et al*., 2001).

Microbial Analysis. The results of counts of psychrotrophic aerobes (data not shown) in fresh and cooked patties show that the borage meal addition did not present an inhibitory effect on the growth of psychrothophic flora. Same behavior was already reported for Sánchez-Escalante *et al*. (2003) who observed that the borage meal has important antioxidant properties, but it does not have any antimicrobial effect in fresh beef patties.

Conclusions

Borage was very effective in preventing lipid oxidation, without dependence on the level of fat; thus the addition of borage meal resulted in a significant antioxidant activity in fresh and cooked beef patties, while it inhibited only partially metmyoglobin formation in fresh meat. All effects contributed to extending the shelf life of fresh and cooked beef patties. It is also important to consider that the smallest amount of UV radiation should be avoided in lighting devices for the retail display of meat.

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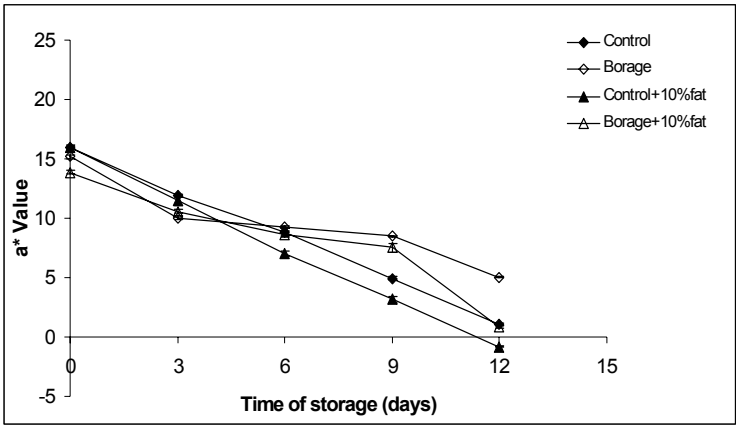

Figure 1. Effect of storage time at 2°C under darkness on CIE a* values of fresh beef patties.

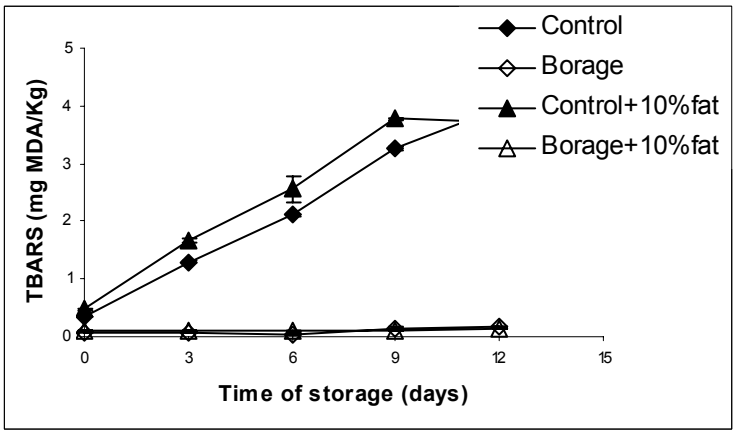

Figure 2. Effect of storage time at 2°C under darkness on TBARS of fresh beef patties.

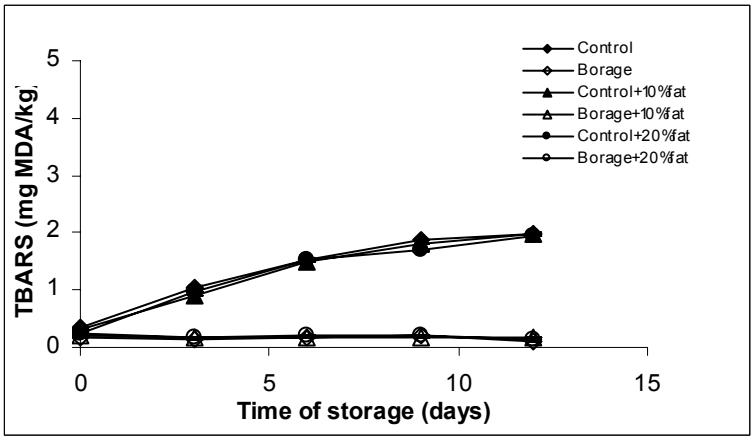

Figure 3. Effect of storage time at 2°C under darkness on TBARS of cooked beef patties.

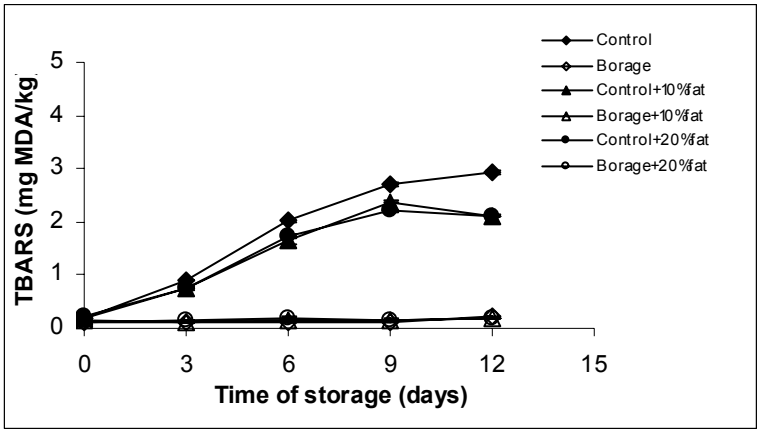

Figure 4. Effect of storage time at 2°C under lighting on TBARS of cooked beef patties.

PROTEOMIC CHARACTERISATION OF NORMAL AND PASTY SPANISH DRY-CURED HAMS

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Background

Tenderness is an important quality trait of dry-cured ham for which changes in the solubility and integrity of the muscle structural proteins are considered to be the main mechanisms responsible for the tenderization of hams during dry-curing. Monin *et al*. (1997) pointed out that the hardness and chewiness of hams increase during the initial stages of processing due to a decrease in protein solubility and water content and subsequently which decreases as proteolysis progresses. However, intense proteolysis can induce textural defects that may reduce the acceptability to consumers.
The relationship between excessive proteolysis and defective texture was first reported in Italian dry-cured hams (Parolari *et al*. 1994, Virgili *et al.* 1995). However, although pastiness and softness have been related to increased proteolysis measured as amino acid and peptide contents (Parolari *et al.* 1988, Careri *et al*. 1993, Virgili *et al.* 1999), but the proteins implicated have not been identified. The endogenous lysosomal cathepsins of muscle are thought to be the enzymes mainly responsible in this process (Parolari *et al*. 1988, Schivazappa *et al.* 2002). However, several factors have been reported to affect proteolysis: genetic type, sex, age, meat pH and processing (salting, temperature and time) (Buscailhon *et al*. 1994, Arnau *et al*. 1998, Garcia-Garrido *et al*. 1999, Tabilo *et al*. 1999).

The development of new separation techniques, such as 2-D electrophoresis, enables the study of those mechanisms related to meat and meat products quality.

Objectives

The study was carried out to compare protein 2D electrophoretic patterns of pasty and soft dry-cured ham to those from normal hams to find markers related to textural defects. To achieve this, low molecular ionic strength extracts were used to find markers related to the defective texture of dry-cured hams.

Material and methods

The normal-texture dry-cured hams (control) processed with different processing times: short (6 months), medium (8-12 months) or long (18 months); were selected at a Spanish company. The defectiveness samples (3) were selected according to their textural characteristics: soft (1), pasty (1) and very pasty (2). The analyses were performed on the *Biceps femoris* muscle.

Protein extraction/fractionation

150 mg of muscle were added to 1.5 ml of extraction buffer (150 mM NaCl, 25 mM KCl, 3 mM $MgCl_2$ and 4 mM EDTA) in an Eppendorf tube containing a glass bead. Homogenisation was performed using a Retsch MM2 agitator (Retsch, Haan, Germany) for 30 min at 4°C. Homogenates were centrifuged at 10,000g for 10 min at 10°C. The supernatant was collected and protein content was measured using Bio-Rad Bradford Protein Assay kit.

2D electrophoresis

2D electrophoresis was performed on both normal and defective quality samples. Immobilised pH gradient (IPG) isoelectric focusing (IEF) was carried out in a Protean IEF cell (BioRad), using BioRad strip, 17 cm pH 4-7. 90 µg of soluble proteins were loaded onto the strips for analytical gels. Protein loading on strips, IEF and SDS-PAGE were performed according to Morzel *et al*. (2004). Gels, in duplicate, were silver stainned following the protocol of Yan *et al*. (2000). Gel images were acquired using GS-800 densitometer and analysed using the PDQuest software (Bio-Rad).

Results and discussion

Different 2DE patterns were detected between dry-cured ham quality, although the profiles were also clearly influenced by the total processing time.

Actually, differences in profile evolution of the spot intensity were detected between processing times. However, a noticable number of spots were characteristic for middle (n=21 spots) or long (n= 11 spots) processing times (Table 1). Moreover, the intensity of a significant number of spots was also higher in both the long and the middle processing times. Such evolution could be explained by a higher protein solubility development as the processing time increased. And the relative number of characteristic spots to the higher intensity spots, for both the long and medium processes, is probably related to the different solubilities and degradation patterns of those proteins implicated. A high protein solubility would increase the intensity and/or the number of spots, while more degradation would produce progressively a decrease of protein molecular weight. This is consistent with the different spot pattern observed in gels between the processing times. For the long processing time, spots were located either at the upper zone of the gel or at the lower zone and at a pH area about 5 or 6 (Fig 1). In contrast, for medium processing, the characteristic spots were above 30-40 kDa.

Furthermore, the lower protein solubility from the short processing time was also consistent with an absence of any characteristic spot. But we observed numerous spots having lower intensity, mainly distributed between around pH 6 – 6.5, showing a decrease in solubility of the molecules having a pI close the dry-cured ham pH.

	Processing time		
Process	Long	Medium	Short
Characteristic	11	21	-
Higher Intensity	24	7	4
Lower Intensity	1	11	15

Table 1. Number of selected spots

The characteristic spots of the softness profile were mainly detected in the middle (2104-2201--5112-5113-5210) and bottom (2013-7019) parts of the gel. However, the soft hams had a very similar spot pattern to the normal quality ham pattern. Also several spots were detected in both normal and soft dry-cured hams but were more intense from soft hams (5707-5715-5717-5718-6712-6713-6714-6716-6717-7716).

Both softness and pastiness patterns had common spots at the upper zone of the gels, on the anode (3624-4517) side. The softness and pastiness pattern did not show the several low molecular weight spots characteristic of normal hams (Fig 2). These results are in agreement with the intense proteolysis previously reported (Parolari *et al*. 1988), assuming that these spots (protein or fragment) result from proteolysis.

In the upper gel zone, several characteristic spots (3621-3622-3625-3626-3733-3734-4213-4624-4735-4736-4824-4825-5616) were apparent of the gel from the pastiness ham. Other spots (3735-3736-4627-4628) were present in the pasty ham while in the very pasty hams, they were less intense or absent. In the middle part of the gels, the 5205 spot exhibited a lower intensity in high pasty hams which could be due to a more intense proteolysis in very pasty samples. However, the intensity of the 4116 spot increased as pastiness increased and seemed to be related to the extent of defectiveness.

Conclusions

Different 2DE patterns were detected between dry-cured ham quality, although the profiles were also clearly influenced by the total processing time. The protein solubility increased as the processing time increased.
The decrease in the molecular weight of proteins depended to the processing time.
The pasty and soft texture of dry-cured hams had more spots with high molecular weight.
2-D electrophoresis was effective in characterizing the quality of dry-cured hams. In further work , spots of interest (either proteins or fragments) will be studied by Maldi-tof spectrometry.

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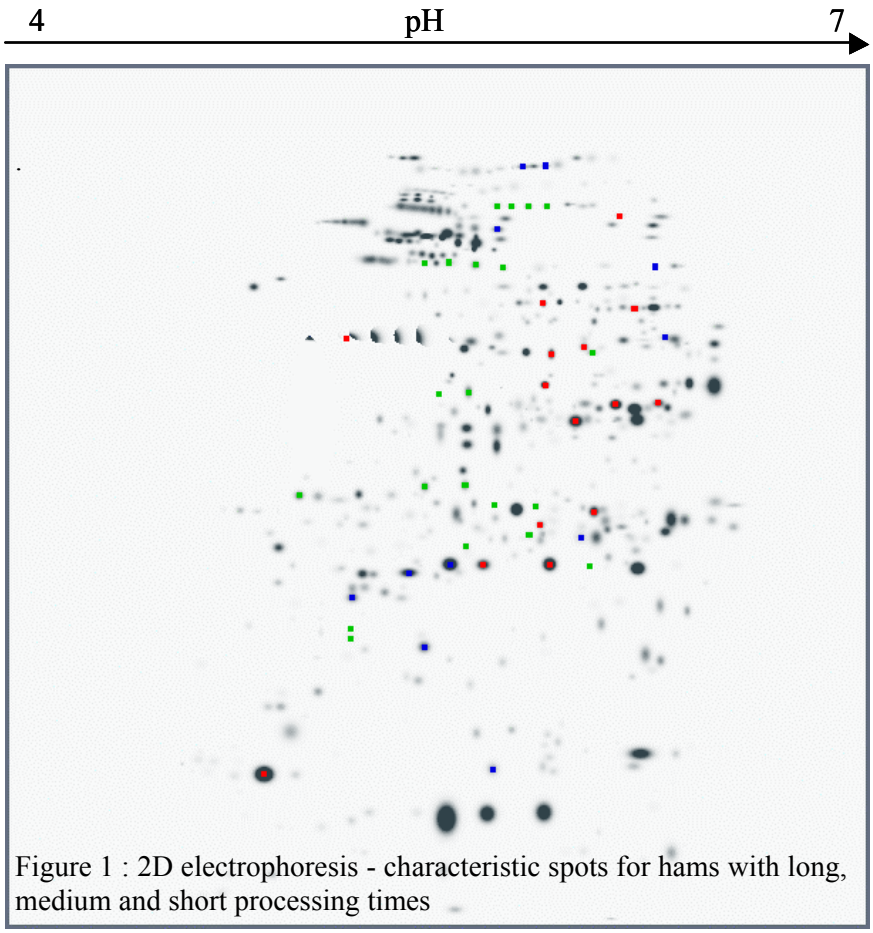

Figure 1 : 2D electrophoresis - characteristic spots for hams with long, medium and short processing times

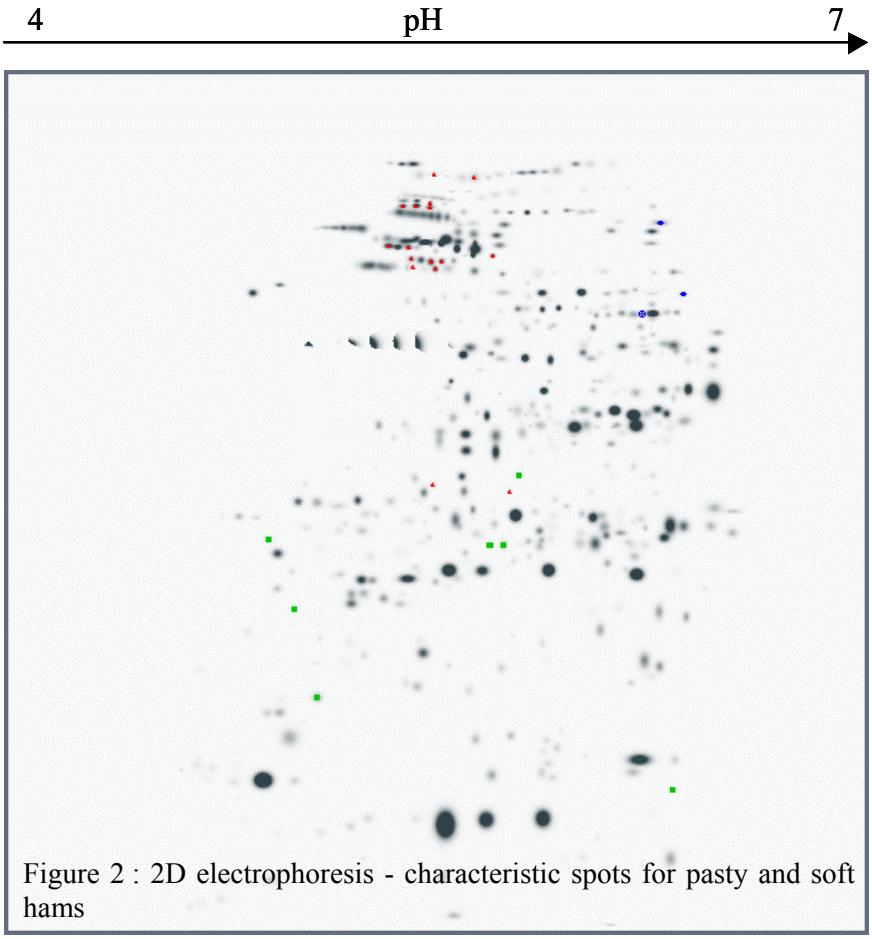

Figure 2 : 2D electrophoresis - characteristic spots for pasty and soft hams

PREDICTION OF INTER-MUSCULAR FAT IN THE FRESH PORK LEG

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Background

Consumers continue to demand home-cooked taste as they purchase more ready-to-eat foods. Ham is a key raw material utilized for many of these foods because of ease of preparation and high flavor. The demand for center cut slices and spiral sliced hams has increased, however, one of the drawbacks of these products is the presence of inter-muscular fat commercially recognized as "Star fat" and seam fat. Thus, the pork industry has identified a need to measure and predict star fat to aid in selecting hams more suitable for center cut slices.

Objectives

The objective of this experiment was to quantify the relationship of linear carcass measurements to star fat in hams and to predict the amount of star fat in hams.

Material and methods

A total of 90 carcasses, 45 gilts (G) and 45 barrows (B), of the same genetic background were selected based on hot carcass weight (HCW) at ranges of 70.8 - 83.6 kg, 84.4 - 90.4 kg, and 90.9 - 98.9 kg (L, M and H groups, respectively). Linear carcass measurements were taken at 24 h post-mortem. Paired fresh pork legs (n = 178) were utilized in which the left side was processed into hams for center cut slice (Figure 1) area measurements and processing yields. Four to six center cut slices were removed per ham, and total area of lean, star fat, and bone was measured per slice by three evaluators. Right side fresh pork legs were utilized for fresh ham composition and cut yields. Three subcutaneous fat thickness points were measured on the right fresh pork leg. The initial fat depth measurement was evaluated perpendicular to the midpoint of the dorsal-ventral line of the ham, while subsequent measurements were taken at 45° angles dorsal and ventral) from the midpoint. Individual muscles or muscle groups were excised and weighed. Data were analyzed with the MIXED procedure of SAS (2000) with the model accounting for the effects of weight group and sex with significance determined at the level of $P < 0.05$. Regression analysis was conducted with the REG procedure (SAS, 2000) utilizing star fat weight as the independent variable whereas cubic (C), quadratic (Q), and linear (L) models were compared with the F test for lack of fit (Neter et al., 1990).

Results and discussion

Selection based on HCW resulted in a wide range of fat and lean measurements. Mean ham weights ranged from 8.09 - 12.07 kg, and last rib fat ranged from 0.76 - 3.05 cm. Ham weights also varied for the left side of 11.26, 10.64, and 9.82 kg and for the right side of 11.19, 10.53, and 9.66 kg (H, M, and L, respectively). Also, last rib fat differed for B (2.49 cm) and G (2.16 cm; $P < 0.05$). Star fat weight increased with the weight groups (0.08, 0.10 and 0.11 kg, respectively for L, M and H; $P < 0.05$). Linear fat measurements did not consistently predict star fat weight. Star fat area measurements had a correlation to seam fat weight (0.29). The three subcutaneous fat depth measurements taken at the midpoint, ventral and dorsal sites had moderate correlations of (0.17, 0.19 and 0.19, respectively) to star fat weight. However, these midpoint, ventral and dorsal fat depth measurements had higher correlations to seam fat (0.35, 0.29 and 0.28, respectively) and subcutaneous fat (0.76, 0.72 and 0.71, respectively). Both, seam and subcutaneous fat can explain a portion of the variation in star fat weight using cubic ($R^2 = 0.27$) and linear regressions ($R^2 = 0.30$). In addition, HCW can explain a portion of the variation in star fat weight using cubic regression ($R^2 = 0.27$).

Conclusions

By utilizing a few key variables, like HCW or ham weight, star fat weight could be minimized in ham production. HCW was the single best predictor of star fat. Selecting lower weight carcasses having less backfat would decrease seam fat occurrence in hams, thus making them more suitable for bone-in products. Future research will focus on better understanding of all carcass variables impacting the development of inter-muscular fat in the fresh pork leg according to the key genetic background/commercial lines of the pigs.

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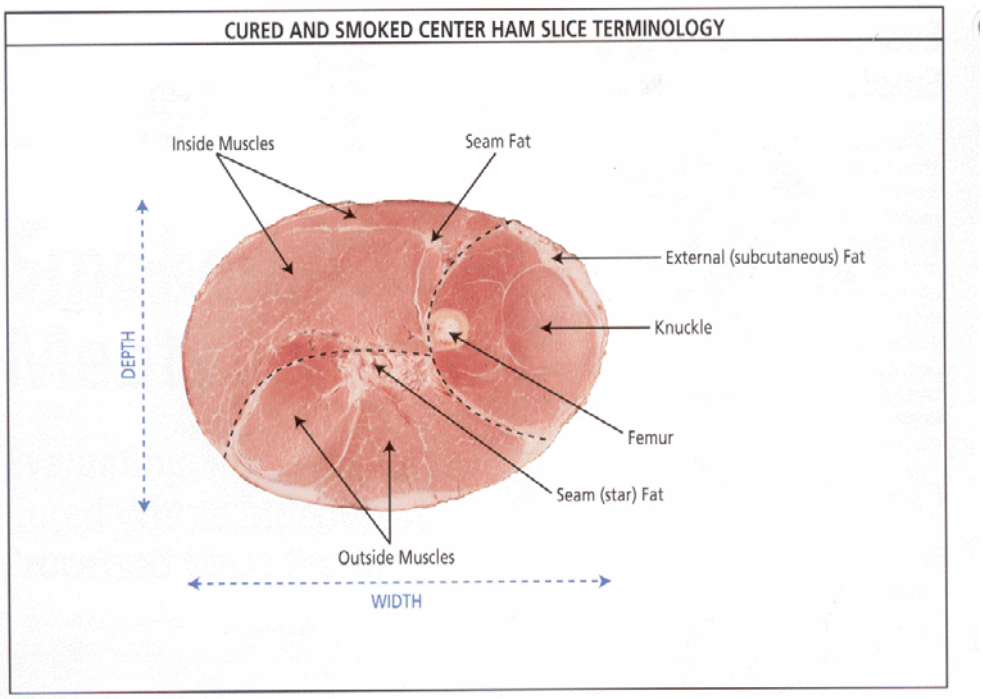
Figure 1. Location of "star fat" in the pork leg



WATER LOSS FROM PORK DURING COOKING

– DOES PH DURING COOKING HAVE AN IMPACT?

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Background

Eating quality of pork is a combination of appearance, flavour, tenderness and juiciness. The cooking procedure (centre temperature, heating time/temperature and heating method) has an impact on juiciness, and an increase of centre temperatures will decrease the juiciness (Bejerholm & Aaslyng, 2003). In a recent study (Aaslyng et al., 2003) as much as 50% of the variation in juiciness was explained by the variation in cooking loss in *Longissimus dorsi*. Both juiciness and cooking loss are affected by the pH_u and waterholding capacity (WHC) of the raw meat.

Aaslyng et al. (2003) also showed – in 10 different raw meat qualities – that while there was a large variation in cooking loss when measured at 60°C and 70°C, this difference was gone when the same samples were heated to 80°C. In addition, higher final cooking loss was registered in samples with low WHC and low pH_u. On the other hand, there was no difference in cooking loss when WHC and pH_u of the meat was medium or high. This indicated the presence of a threshold value for pH_u and/or WHC above which cooking loss is influenced neither by WHC nor pH_u.

Based on the above, it was speculated whether the observed differences in cooking loss were influenced by different pH courses in the meat during cooking. Hence, an experiment was carried out that followed the pH course and cooking loss during cooking of *L. dorsi* and *Biceps femoris* samples with high, normal and low pH_u.

Objectives

The objective of this study was to investigate a possible relation between the pH course and cooking loss during cooking of pork samples with high, normal and low pH_u.

Materials and methods

A total of 12 *L. dorsi* samples - four high, four normal and four low pH_u samples - and eight *B. femoris* samples – four high and four normal pH_u samples – were included in the experiment. The selection of samples was based exclusively on pH_u in commercial slaughter pigs. The samples were stored frozen and were defrosted for 20-24 hr at 4°C prior to cooking. Cooking procedure: Whole roasts in oven (convection oven) at 90°C. When the centre temperature in the samples reached 70°C, the oven temperature was increased to 105°C. The centre temperature, weight and pH were measured prior to cooking and at regular intervals during the cooking until a centre temperature of 90°C was reached. pH was measured using a Knick Portamess 910 pH meter with a Mettler Toledo Lot 406-m&-S7/25 until centre temperatures of approx. 55°C were reached, as the pH meter was unreliable above this temperature. The pH electrode was calibrated prior

Table 1 pH_u (measured prior to cooking) and final cooking loss (measured when the centre temperature had reached 90°C) in *L. dorsi* and *B. femoris* samples with high, normal or low pH_u[a].

	L. dorsi			*B. femoris*	
pH-group	high	normal	low	high	normal
pH_u	5.84 ± 0.04[a]	5.58 ± 0.04[b]	5.40 ± 0.03[c]	5.99 ± 0.03[a]	5.62 ± 0.03[b]
final cooking loss	36.7 ± 1.7[a]	39.5 ± 1.4[a]	47.8 ± 1.4[b]	43.9 ± 1.1[a]	45.6 ± 1.1[a]

[a] Least squares means and SEM are shown. Different letters for pHu and final cooking loss within muscle indicate significant differences ($p < 0.05$) between the high, normal and low pH-groups.

to each measurement in buffers equilibrated to the centre temperature ± 5°C of the sample at the specific measurement. Cooking loss was calculated on the basis of the measured sample weights.

The statistical analysis was carried out with the Statistical Analysis System, version 8.2 (SAS Institute, Cary, NC, USA). The MIXED procedure was applied when calculating the least square means and standard error of all the variables. A model including the fixed effects of pH group (high, normal, low) and centre temperature as well as their interaction, the repeated effect of centre temperature with sample as subject was applied for pH and accumulated cooking loss.

Results and discussion

pH_u (measured prior to cooking) of the three *L. dorsi* and the two *B. femoris* pH groups is listed in Table 1, showing that the pH values of the different pH groups were significantly different when the experiment was initiated.

The pH course during cooking is shown in Figure 1 for *L. dorsi* and in Figure 2 for *B. femoris*. During cooking, the pH courses of the high and normal pH_u samples were almost identical although taking place at different levels. In *L. dorsi,* pH fell approximately 0.3 pH units from 0°C until centre temperatures of 35-45°C were reached. When the centre temperature increased further, the pH slowly increased as well – approximately 0.2 pH units. In contrast, the pH fell less than 0.2 pH units in the low pH *L. dorsi* group, and the minimum pH was reached before the centre temperature reached 20°C. In *B. femoris*, pH fell approximately 0.2 pH units from 0°C until the centre temperatures reached 35-45°C. When the temperature increased further, pH in both pH groups increased again, with the highest increase in the normal pH_u group.

The final cooking loss (measured when the centre temperature had reached 90°C) is shown in Table 1. In *L. dorsi*, the final cooking loss in the low pH group was significantly higher than in the high and normal pH groups. There was no significant difference in final cooking loss between the high and normal pH group in either *L. dorsi* or *B. femoris*.

The accumulated cooking loss is shown in Figure 1 for *L. dorsi* and in Figure 2 for *B. femoris*, respectively. In *L. dorsi*, cooking loss

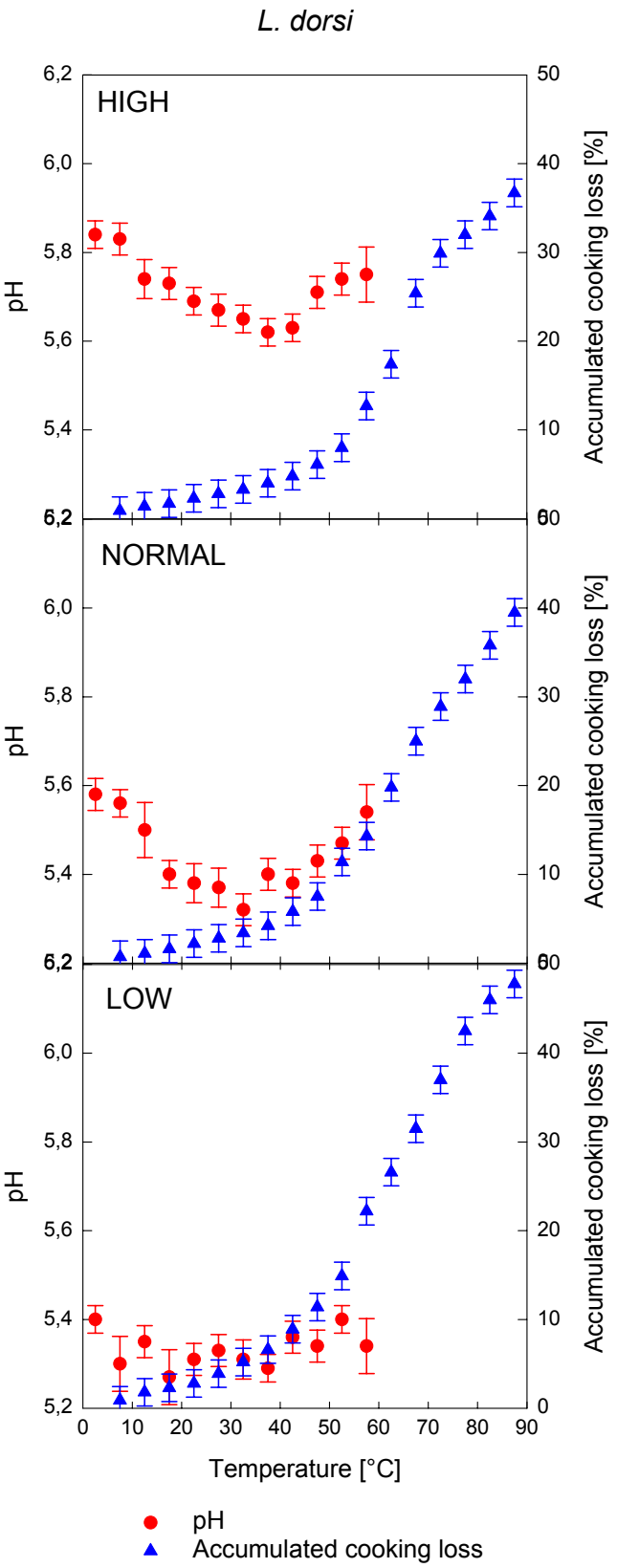

Figure 1 pH and accumulated cooking loss measured at centre temperature intervals of 5°C during cooking of *L. dorsi* samples of high, normal and low pH_u.

of the low pH group exceeded 1% cooking loss per 5°C temperature increase already at 30°C, and the highest cooking loss per 5°C temperature increase was observed in the temperature interval of 55-60°C. In contrast, the centre temperature in the normal and high pH groups was above 40°C and 45°C, respectively, before the cooking loss exceeded 1% per 5°C temperature increase and the highest cooking loss was observed in the temperature interval of 65-70°C for both groups. Thus, the water was lost at a lower temperature in the low pH group and the total cooking loss was higher.

The final cooking loss was higher in *B. femoris* than in *L. dorsi*, but below 40°C the cooking loss pattern observed in *B. femoris* was almost identical to that observed in *L. dorsi*, as the temperature was above 40°C before the cooking loss exceeded 1% per 5°C temperature increase and the highest cooking loss was observed in the interval of 65-70°C. However, in the temperature interval between 40°C and 65°C significantly more water was lost from the normal pH group compared to the high pH group and compared to the high and normal group of *L. dorsi*. From the high pH group water was lost at higher temperatures why the final cooking loss was not significantly different in the two groups.

The aim of this experiment was to study whether the pH course during cooking had an influence on when cooking loss takes place. As discussed above pH in the low pH group hardly changed during cooking while water was lost already at low temperatures. In the normal and high pH group, pH decreased when cooking was initiated and increased again when temperatures got above approximately 45°C. However, water was not lost to any considerable extent until the temperature got above approximately 40°C i.e. when pH had started to increase again. Hence, the pH course does not seem to have any direct influence on when water is lost during cooking.

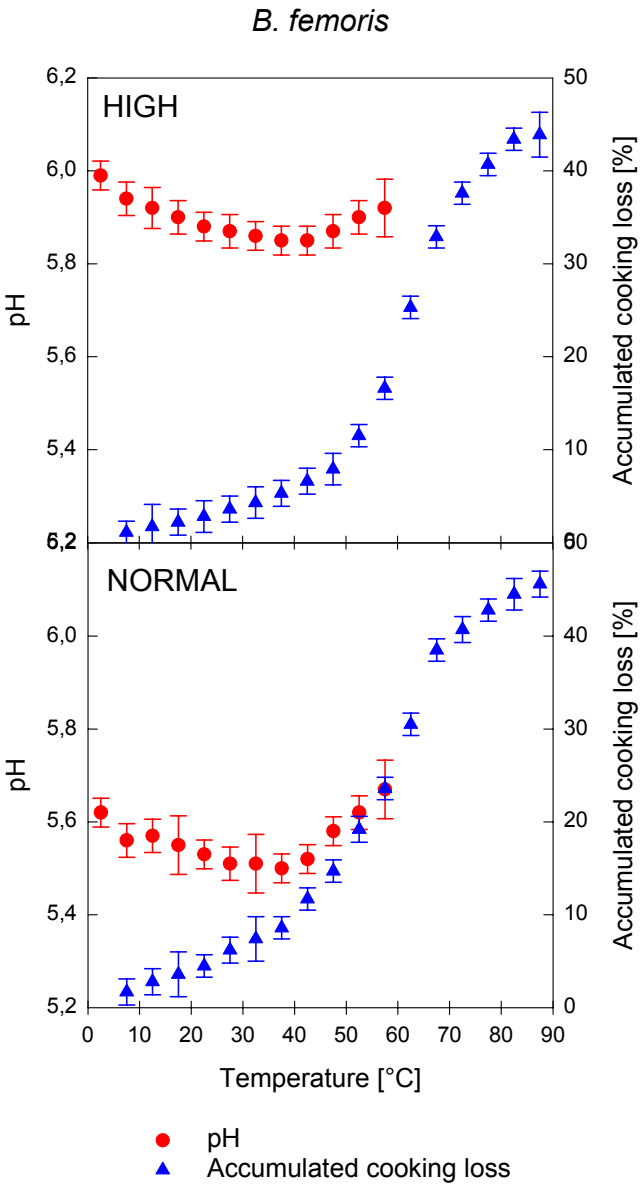

Figure 2, pH and accumulated cooking loss measured at centre temperature intervals of 5°C during cooking of *B. femoris* samples of high and normal pH_u.

The existence of a threshold value above which cooking loss is not influenced by WHC or pH has been suggested (Aaslyng et al., 2003). The final cooking loss was identical in the normal and high pH groups in *B. femoris*, but the water was lost at different temperatures in this muscle. In *L. dorsi* the water loss was almost identical in the two groups. This discrepancy between *L. dorsi* and *B. femoris* could be due to muscle differences, e.g. the final cooking loss being higher in *B. femoris* than in *L. dorsi*. However, the discrepancy may also be due to larger differences in pH_u between the two different pH groups in *B. femoris* compared to *L. dorsi* as well as considerably higher pH_u in the high pH group in *B. femoris* compared to the high pH group in *L. dorsi* (Table 1). These results indicate that pH_u does influence the water loss during cooking even at normal and high pH_u - although to a smaller extent than at low pH_u.

Conclusions

Final cooking loss in *L. dorsi* was higher in low pH_u samples compared with high and normal pH_u samples. In *B. femoris* there were no differences in final cooking loss between high and normal pH_u samples. However, there was no relationship between water loss during cooking and the changes in pH.

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THE INFLUENCE OF PHYSICAL AND CHEMICAL PROPERTIES OF PORK FAT ON THE QUALITY OF HEAT TREATED MEAT PRODUCTS

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Background

The influence of the fatty acid profile on the quality of fresh meat is well described (Eggert *et al.* 2001;Fiedler *et al.* 2003;Nguyen *et al.* 2003). However, knowledge about the influence of physical and chemical properties of fat in heat treated meat products is insufficient. Fat undergo solid-to-liquid phase transitions between ambient and body temperature. This reversible phase transition is highly desirable when fat is used as a functional ingredient in food providing properties such as structure, mouth feel, flavour delivery, and barriers to moisture migration. Vegetable and animal fat are polymorph, meaning they have several solid phases. Each solid phase has a certain melting point and consistency. This phenomenon has in detail been studied in cocoa butter where control of the crystallization process is essential for the eating quality of the chocolate (Schlichter-Aronhime and Garti 1988). All triglycerides have individually polymorphic properties depending on their fatty acid distribution and composition (Sato 2001). The triglycerid composition is different depending on the origin of the pork fat; leaf fat contains more saturated fatty acids than lard fat. Pork fat crystallization properties during production of heat treated meat products have only sparsely been studied and never systematically correlated to processing parameters and the sensory quality of the final meat products. This paper reports results from a fundamental study of lard/leaf fat crystallization and melting using Differential Scanning Calorimetry (DSC).

Objectives

The objectives of this study were to describe the physical properties of pork lard and leaf fat in pure form and as an ingredient in liver pâté.

Materials and methods

Two sources of pork fat were used in this study – lard and leaf fat. The physical and chemical properties of the fat were investigated in 3 different products: Pure fat extracted using chloroform by the method described by Folch *et al.* (1957) with minor modifications and raw unextracted fat as an ingredient in liver pâté. The ingredients in the liver pâté are listed in Table 1. The melting points for each product were determined with a DSC 820, Mettler Toledo (Schwerzenbach, Switzerland) based on the heat flux principle and cooled with liquid nitrogen. Experimental conditions were identical for all products: The samples were held at 80 °C for 30 minutes, cooled to (-20) °C with different cooling rates (0.5 °C/min, 1.0 °C/min, 5.0 °C/min, and 10 °C/min), and subsequently heated to 80 °C (heating rate: 1.0 °C/min and 5.0 °C/min).

Table 1 Liver pâté, list of ingredients.

Ingredients	Quantity	Ingredients	Quantity
Pork fat	37.04 %	Salt	0.93 %
Lard	30.35 %	Dried onions	0.56 %
Soup[1]	25.20 %	Glucose	0.19 %
Wheat fluor	3.09 %	White pepper	0.15 %
Dried milk	2.47 %	Thyme	0.03 %

[1] Soup is the designation of lard boiled in water.

Results and discussion

The melting curves of extracted lard and extracted leaf fat are not identical (Figure 1). This is probably due to differences in the fatty acid profile of the two fat sources. Leaf fat has the highest melting point at 48 °C while lard has the highest melting point at 39 °C, indicating that leaf fat is more saturated than lard fat. The number of melting points is almost similar for the two kinds of fat – 5 or 6 melting points were recorded depending on the cooling rate. In a study of particle size of lard fat, it was also found that lard fat have 6 crystal fractions at a cooling rate of 0.5 ºC/min (Wang and Lin 1995). From Figure 1 it can be seen that different cooling rates leads to different appearances of the curves. Consequently the cooling rate has an effect on fat crystallization in pork fat. The cooling rate was found to have an effect on both the lard and leaf fat. The peaks are more separated for slowly cooled fat than for fast cooled. This effect may be due to the fact that triglycerides have more time to embed in more stable crystal forms during slow cooling.

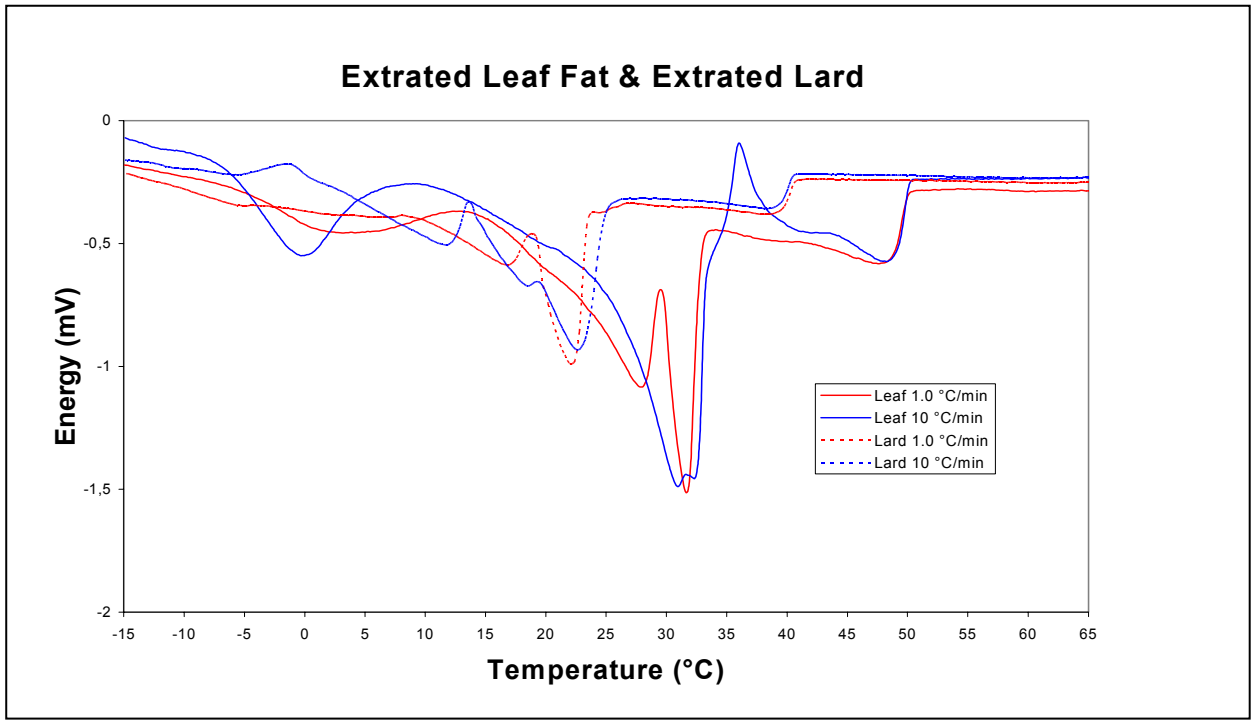

Figure 1 DSC melting curves of extracted lard and leaf fat. Heating rate: 1.0 °C/min. Inserted box indicates cooling rates.

The thermogram of the liver pâté in Figure 2 has the largest peak around 0 °C, due to water melting. The size of the peak indicates that liver pâté contains a significant amount of water. Due to the size of the water melting peak, central information about fat melting peaks might be hidden. Figure 2B is an enlarged version of the marked area in Figure 2A. From Figure 2B it is possible to see that the melting curves have several peaks. The slowest cooled sample has five exothermic peaks and the fastest cooled sample has four exothermic peaks indicating melting of fat crystals. This means that the cooling rate also has an effect on the melting properties of the liver pâté. It may thus be possible to produce liver pâtés with different lipid crystals, leading to varying consistence of the liver pâtés depending at the cooling rate. Future experiments will show if it is possible, by means of sensory experiments, to distinguish any differences in the mouth feel of differently cooled liver pâté.

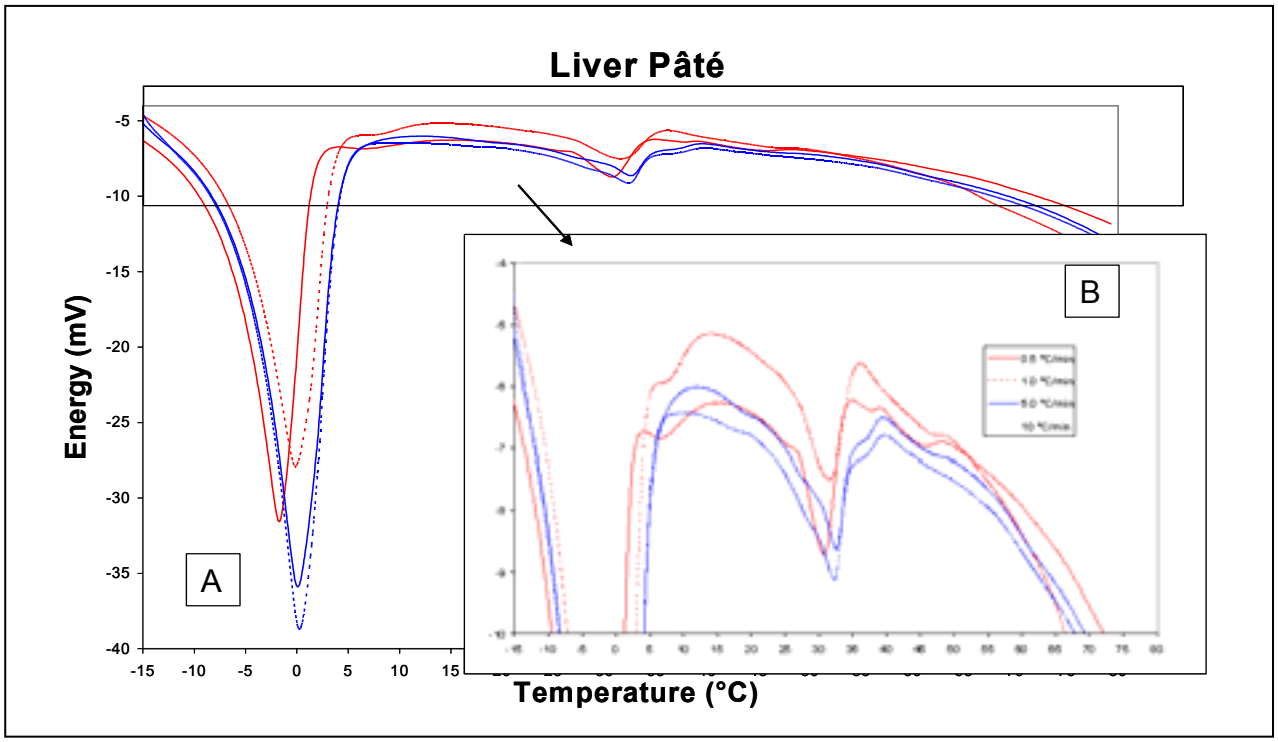

Figure 2 DSC melting curves of Liver pâté made of lard fat - heating rate 1.0 °C/min. B is an enlarged version of the marked area in A.

Conclusions

The cooling rate has a significant effect on the crystallization of both lard and leaf fat. The two sources of fat have 5-6 melting points depending on the cooling rate. The fat crystallization in liver pâté is also effected by the cooling rate. Consequently the cooling rate can most probably chance the physical properties of the liver pâté – i.e. it is possible to change the texture of the liver pâté by controlling the cooling rate after the liver pâté has been baked.

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PHYSICAL AND SENSORY CHARACTERISTICS OF MARINATED CHICKEN DRUMSTICKS TREATED WITH THE LACTOPEROXIDASE SYSTEM AND THERMAL TREATMENT

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Background

Marination is a procedure of treating meat with an aqueous mixture of vinegar, salt and spices before cooking. Marination of poultry is practiced to improve product's physical and sensory attributes (Hashim *et al*., 1999; Lemos *et al*., 1999; Xiong and Kupski, 1999; Zheng *et al*., 2000). Thermal or heat treatment, which is one of the most common physical methods to reduce the load of microorganisms, has been widely applied to preserve foods for years. In addition, heating is also involved in many food processes, such as cooking, scalding, pasteurizing, drying, and etc. The lactoperoxidase system (LPS), which consists of lactoperoxidase (LP), thiocyanate (SCN^-), and hydrogen peroxide (H_2O_2), is an inhibitory system that is present naturally in bovine milk, and has been shown to be inhibitory against some microorganisms (Kamau *et al*., 1990; Earnshaw *et al*., 1990; Zapico *et al*., 1998). Even though LPS has been reported to have little effect on the sensory and physical characteristics of the treated milk and dairy products (Zapico *et al*., 1998; Martinez *et al*., 1988), limited information on the contribution of marination, thermal treatment, and LPS to the physical and sensory characteristics of treated poultry products is available.

Objectives

The objective of this study was to investigate the effects of adding LPS and thermal treatment on some physical and sensory characteristics of the marinated chicken drumsticks.

Materials and methods

A marinade that contains acetic acid (1%) and salt (3%) was developed as a standardized marinade. LPS consisted of 1 μg/ml of LP, 5.9 mM of KSCN and 2.5 mM of H_2O_2 (30%) and was added to the marinade for the LPS-added treatment. The pH of the marinade was then adjusted to 4. For the sensory evaluation, flavoring agents (0.3% black pepper and 0.15% garlic powder) were added into the standardized marinade solutions for the marinated treatments 1 through 4; whereas no flavoring agents were added to the treatments 5 and 6 samples. No flavoring agents were added in the marinade for all 6 treatments for the physical evaluation. For the samples without thermal treatment (treatments 1 and 3), drumsticks were marinated in plastic containers with the marinade solution so that all the drumsticks were covered completely by the marinade and stored at 4°C for 18 hr. Treatment 5 control samples were submerged in distilled water. No water or marinade solution was added to treatment 6 samples. For those samples with thermal-marinating treatment (treatments 2 and 4), drumsticks were placed inside a plastic bag with the heated marinade solutions, which was previously heated in a water bath. After holding at 58°C for 2 min, the marinade-drumstick mixes in the bags were cooled by immersing the bags in running tap water. When the marination mix was cooled to 25°C (approximately 10 min), the mixes were moved into plastic containers and then refrigerated at 4°C for 18 hours. Physical characteristics, including the pH values of the marinate solutions and drumsticks, marinade absorption, L*a*b* values of the skin and muscles, cooking loss, and yield were evaluated. The sensory panel evaluated the sensory characteristics of the marinated products. Descriptive analysis was conducted to evaluate the intensities of sensory characteristics of the raw and cooked samples. Attributes of skin color, muscle color, marinated chicken aroma, and off-aroma were evaluated for the raw samples. After cooking, the samples were cooled to room temperature and served. Attributes for skin color, muscle color, marinated chicken flavor, off-flavor, juiciness, and tenderness were evaluated for the cooked samples. The sensory evaluation was conducted using a 1 to 9 scale, with 1 representing the lowest intensity and 9 the highest intensity for all attributes except for color (1 = light color; 9 = dark color), juiciness (1 = not juicy; 9 = very juicy), and tenderness (1 = not tender; 9 = very tender). Three trials were conducted. Data were analyzed using SAS GLM with a 5% level of significance. Means were separated using Duncan's multiple range test.

Results and discussion

In this study, treatment 1 had no LPS added and no thermal treatment; treatment 2 had no LPS added but with thermal treatment; treatment 3 had LPS added but without thermal treatment; treatment 4 had both LPS added and thermal treatment; treatment 5 had distilled water (instead of the marinade solution) added; treatment 6 had no water and no marinade solution added. After marinating or cooking, there were no significant ($p>0.05$) differences of muscle pH detected among the 1 through 4 marinated treatments. After marinating, the marinade solution pH values for the marinated treatments 1 through 4 were significant ($p<0.05$) lower than the solution pH of the control with addition of distilled water samples (treatment 5) as expected. There was no significant ($p>0.05$) difference of marinade absorption, cooking loss, and yield for the marinated treatments 1 through 4 (Table 1).

Table 2 illustrates the color evaluation of marinade chicken drumsticks with or without thermal treatment and/or addition of LPS. After marinating, all the skin L* values of treatments 1 through 5 increased, whereas the control without addition of distilled water (treatment 6) remained approximately the same, which was significant ($p<0.05$) lower. After cooking, all the skin L* values of the samples for treatments 1 through 6 decreased without significant ($p>0.05$) differences. After marinating, treatment 3 and treatment 4 (LPS added), had significantly ($p<0.05$) lower skin a* values than treatment 1 and 2 (no LPS added). Similarly, after cooking, the skin a* values of treatment 3 and treatment 4 were significantly ($p<0.05$) lower than the values of treatment 1 and 2. This significant reduction of a* values of the LPS treated samples was probably due to the addition of hydrogen peroxide, which is one of the components of the LPS treatment, and is a strong oxidizing agent that is occasionally used as a bleaching agent in the food industry. After marinating, the skin b* values were without any significant differences ($p>0.05$) among all 6 treatments. After cooking, the skin b* increased without any significant differences ($p>0.05$). The significant changes of skin a* values of the LPS treated samples (treatments 3 and 4) in the current study did not agree with the report by Wolfson (1992). Wolfson (1992) investigated the LPS effect on some physical characteristics of LPS treated poultry. In that study, treated chicken legs and thighs were immersed in a 50°C bath containing the LPS (1μg/ml LP, 5.9 mM KSCN, and 2.5 mM H_2O_2) for 5 min, and then stored at 4°C, and no LPS was added for the control samples. Wolfson (1992) reported that there was no significant ($p<0.01$) difference for the Hunterlab color values (L, a, b) between the LPS treated chicken thigh skin and controls after 24 and 48 hours storage at 4°C. The possible reason of this disagreement between the two studies is probably that the LPS-treated time (18 hours in the current study) was much longer than the time of 5 min in Wolfson's study. After marinating, all the muscle L* values of the marinated treatments 1 through 4 increased and were significant ($p<0.05$) higher than the values of the two control samples (treatments 5 and 6). After marinating, treatments 3 and 4 (LPS added) had significantly ($p<0.05$) lower muscle a* values than the values of treatments 1 and 2 (no LPS added). Similarly, after cooking, the muscle a* values of treatments 3 and 4 (LPS added) were significantly ($p<0.05$) lower than the values of treatments 1 and 2 (no LPS added). After marinating, the muscle b* values of treatments 1 through 6 increased without significance ($p>0.05$). After cooking, there was no significant ($p>0.05$) difference for the muscle b* values for marinated treatments 1 through 4.

In this study, total color difference (ΔE^*_{ab}) is calculated as: Total color difference (ΔE^*_{ab}) = square root of $[(\Delta L^*)^2 + (\Delta a^*)^2 + [(\Delta b^*)^2]$, where L* = light and dark, a* = red and green, b* = yellow and blue, and Δ representing the difference between the two processing steps, and it is commonly applied to evaluate the color changes of samples at the two different processing steps in food science studies. There was no significant ($p>0.05$) difference for total color difference of skin between before and after marination for the marinated treatments 1 through 4 samples, but the control samples without distilled water added (treatment 6) had a significantly ($p<0.05$) lower total color differences for skin color. There was no significant ($p>0.05$) difference for total color difference for skin color between before and after cooking for the all six treatment samples. Similarly, no significant ($p>0.05$) difference for total color difference for skin between before marination and after cooking for the all 6 treatment samples was obtained. In the current study, the control samples with or without distilled water added (treatments 5 and 6) had significant ($p<0.05$) lower total color difference in muscle color, when compared with the samples of the marinated treatments 1 through 3. There was no significant ($p>0.05$) total color difference in muscle between before and after cooking, and between before marination and after cooking for all 6 treatment samples (Table 3).

Sensory evaluation of marinated chicken drumsticks with or without addition of LPS and/or thermal treatment is shown in Table 4. Based on a 1-9 scale, the marinated treatments 1 through 4 samples in this

study had lower sensory raw skin and muscle color scores without any significant differences ($p>0.05$) among the marinated treatments. The lightness of the skin and muscle colors for marinated treatments 1 through 4 when comparing to the samples of the control groups was probably due to the addition of acid.

The marinated treatments 1 through 4 samples had significant ($p<0.05$) higher marinated chicken aroma scores, when compared with the controls probably due to lack of flavoring agents added. Low (1.3-1.6 based on a 1-9 scale) and without significant ($p>0.05$) difference for the sensory off-aroma scores for the raw samples for all treatments 1 through 6 were obtained in this study (Table 3). After cooking, the sensory skin and muscle color scores of the marinated treatments 1 through 4 samples increased. Also, there was no significant ($p>0.05$) difference for the sensory cooked skin and muscle color scores of all treatments 1 through 6. The marinated treatments 1 through 4 samples had significant ($p<0.05$) higher marinated chicken flavor scores. Low (1.1-1.9 based on a 1-9 scale) and without significant ($p>0.05$) difference values were obtained for the sensory off-flavor scores of the cooked samples for all treatments. There was no significant ($p>0.05$) difference for the sensory juiciness score of all treatments 1 through 6. In addition, no significant ($p>0.05$) difference for the sensory tenderness scores of marinated treatments 1 through 4 was observed.

Conclusions

In conclusion, the treatment consisting of addition of LPS (1 µg/ml of LP, 5.9 mM of KSCN, and 2.5 mM of H_2O_2) and thermal treatment (58°C for 2 min) did not impair the physical and sensory characteristics for the marinated chicken drumsticks.

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Table 1. Physical evaluations of marinated chicken drumsticks with or without thermal treatment and/or lactoperoxidase system (LPS) added

Parameter / Treatment No.	1	2	3	4	5	6
LPS addition	–	–	+	+	Control	Control
Thermal treatment (58°C, 2 min)	–	+	–	+	(water added)	(no water added)
Raw drumstick pH (before marinating)	6.83	6.81	6.91	6.79	6.88	6.85
Raw drumstick pH (after marinating)	5.60[a]	5.59[a]	5.53[a]	5.54[a]	6.76[b]	6.87[b]
Cooked drumstick pH	5.93[a]	5.72[a]	5.82[a]	5.64[a]	6.91[b]	6.97[b]
Marinade solution pH (after marinating)	4.37[a]	4.39[ab]	4.42[b]	4.40[ab]	7.22[c]	ND
Marinade absorption (%)	1.88[a]	1.72[a]	1.62[a]	2.48[a]	6.19[b]	ND
Cooking loss (%)	23.1[a]	22.7[a]	22.70	22.56[a]	19.76[ab]	16.34[b]
Yield (%)	78.33[a]	78.37[a]	78.56[a]	79.36[ab]	85.21[c]	82.65[bc]

[a, b]Means within a row without the same superscript are significantly different ($P<0.05$). ND: not determined.
[1]Lactoperoxidase system (LPS) = lactoperoxidase (LP, 1 µg/ml), KSCN (5.9 mM) and H_2O_2 (2.5 mM).

Table 2. Skin color evaluation[1] results of marinated chicken drumsticks with or without thermal treatment and/or addition of lactoperoxidase system (LPS).

Parameter		Treatment No.	1	2	3	4	5	6
	LPS addition[2]		–	–	+	+	Control (water added)	Control (no water added)
	Thermal treatment (58°C, 2 min)		–	+	–	+		
Skin	L* value	Before marinating	71.19	72.44	71.92	74.03	72.57	74.50
		After marinating	79.69^{ab}	84.53^{c}	83.10^{bc}	82.73^{bc}	77.93^{a}	72.06^{d}
		After cooking	63.45	69.29	63.26	62.33	62.73	65.02
	a* value	Before marinating	5.87	6.17	5.80	6.59	5.86	4.89
		After marinating	1.55^{a}	1.03^{a}	-1.73^{b}	-1.44^{b}	3.60^{c}	5.81^{d}
		After cooking	2.06^{a}	1.96^{ab}	0.44^{c}	0.62^{bc}	2.95^{a}	2.39^{a}
	b* value	Before marinating	6.67	7.29	6.84	4.59	8.21	8.68
		After marinating	5.96	7.88	7.40	6.20	7.80	8.05
		After cooking	19.70	22.30	18.51	19.80	21.72	23.69
Muscle	L* value	Before marinating	60.02	63.26	63.39	61.58	63.36	65.23
		After marinating	84.65^{a}	91.21^{ab}	98.10^{b}	85.11^{a}	69.81^{c}	63.13^{c}
		After cooking	66.44^{a}	71.75^{ab}	73.40^{b}	71.69^{ab}	73.47^{b}	69.32^{ab}
	a* value	Before marinating	13.93^{a}	13.02^{ab}	12.48^{ab}	13.26^{ab}	12.70^{ab}	11.69^{b}
		After marinating	3.00^{a}	3.36^{a}	-2.02^{b}	-1.64^{b}	10.83^{c}	12.16^{d}
		After cooking	3.63^{a}	2.73^{ab}	0.59^{c}	0.99^{bc}	7.33^{d}	5.71^{d}
	b* value	Before marinating	8.96^{a}	11.97^{ab}	13.33^{b}	9.21^{a}	9.61^{a}	11.48^{ab}
		After marinating	9.70	11.80	13.18	12.55	10.36	13.13
		After cooking	13.90^{a}	15.57^{a}	16.80^{ab}	16.48^{ab}	18.84^{b}	26.25^{c}

$^{a, b, c}$Means within a row without the same superscript are significantly different (P<0.05).
[1]Color evaluation: L* = light and dark, a* = red and green, and b* = yellow and blue
[2]Lactoperoxidase system (LPS) = lactoperoxidase (LP, 1 µg/ml), KSCN (5.9 mM) and H_2O_2 (2.5 mM).

Table 3. Total color differences[1] of marinated chicken drumsticks with or without thermal treatment and lactoperoxidase (LPS) added

Parameter	Treatment No.	1	2	3	4	5	6
	LPS addition[2]	–	–	+	+	Control (water added)	Control (no water added)
	Thermal treatment (58°C, 2 min)	–	+	–	+		
Skin							
Comparison of before and after marination		9.88^{ab}	13.20^{a}	13.57^{a}	11.95^{a}	5.85^{bc}	2.80^{c}
Comparison of before and after cooking		21.37	21.07	22.87	24.76	20.66	17.53
Comparison of before marination and after cooking		16.01	16.09	15.92	20.16	17.31	17.96
Muscle							
Comparison of before and after marination		27.17^{ab}	29.78^{ab}	36.12^{a}	18.02^{bc}	7.00^{c}	3.25^{c}
Comparison of before and after cooking		18.29	20.33	25.07	14.80	12.11	17.94
Comparison of before marination and after cooking		13.12	13.85	16.24	17.50	14.16	16.71

$^{a, b}$Means within a row without the same superscript are significantly different (p<0.05).
[1]Total color difference (ΔE^*_{ab}) = square root of $[(\Delta L^*)^2 + (\Delta a^*)^2 + [(\Delta b^*)^2]$, where L* = light and dark, a* = red and green, b* = yellow and blue, and Δ representing the difference between the two processing steps.
[2]LPS = lactoperoxidase (LP, 1 µg/ml), KSCN (5.9 mM) and H_2O_2 (2.5 mM).

Table 4. Sensory characteristic and intensities[1] of marinated chicken drumsticks with or without addition of lactoperoxidase system (LPS) and/or thermal treatment

Parameter	Treatment No.	1	2	3	4	5	6
LPS addition (1µg/ml LP, 5.9mM KSCN, 2.5mM H_2O_2)		–	–	+	+	Control (water added)	Control (no water added)
Thermal treatment (58°C, 2 min)		–	+	–	+		
Raw samples	Skin color	2.7^{a}	2.6^{a}	2.6^{a}	2.1^{a}	5.1^{b}	6.1^{b}
	Muscle color	2.3^{a}	2.6^{a}	2.7^{a}	2.3^{a}	5.0^{b}	6.4^{b}
	Marinated chicken aroma	4.3^{a}	4.9^{a}	4.9^{a}	5.1^{a}	1.6^{b}	1.3^{b}
	Off-aroma	1.3	1.3	1.1	1.0	1.0	1.0
Cooked samples	Skin color	5.6	5.3	5.3	4.7	5.4	5.6
	Muscle color	4.7^{ab}	3.9^{a}	4.0^{a}	3.4^{a}	5.7^{bc}	6.4^{c}
	Marinated chicken flavor	4.6^{a}	6.0^{a}	5.3^{a}	5.1^{a}	1.6^{b}	2.0^{b}
	Off-flavor	1.1	1.9	1.3	1.3	1.1	1.3
	Juiciness	3.4	3.3	3.6	3.4	3.3	4.4
	Tenderness	4.1^{ab}	3.3^{a}	3.7^{ab}	4.0^{ab}	4.3^{ab}	5.0^{b}

$^{a, b, c}$Means within a row without the same superscript are significantly different (P<0.05).
[1]1 to 9 scale (1 = the lowest intensity and 9 = the highest intensity)

PHYSICAL PROPERTIES AND FOLDING TEST OF COMMERCIAL MEAT BALLS IN THAILAND

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Background

Meat ball is quite popular for one-dish serving meal in Thailand. There are several ways of cooking meat ball i.e. deep fry, yum (spicy salad), roasting and even to be in recipe with noodle. If we look at the amount of produced meat balls in Thailand from year 2000 to 2003, the amount of pork balls was 843.36-1339.14 ton, chicken balls was 6923.72-4962.87 ton. There are basic three types of meat balls, i.e. pork ball, chicken ball and fish ball. Most of Thai people prefer to have meat ball with a high springiness and softness. To know the good quality of those meat balls, only the highly experienced person can tell. This is one restriction in processing of meat ball especially in SMEs (Small Medium Enterprises) since there is an instrumental limitation for checking and measuring the products. For a big manufacturer, the problem seems smaller since they can afford having a good quality control. Besides the taste of product, the texture of product is quite important factor to be judged for a good quality (e.g. no sponginess, smoothness, not too hard, etc.). This study wants to investigate the properties of some meat balls producing from some big and small factories by using the texture analyzer which is available in our laboratory and also using a simple method, e.g. folding test, to investigate those meat balls. We expect to find the correlation among those properties in order to encourage small factory to implement the simple method for checking the quality of meat ball.

Objectives

The purpose of this study is to investigate the physical properties of commercial meat balls (pork ball, chicken ball and fish ball) by using texture analyser and folding test and to look for their correlations.

Materials and methods

The materials are ten items of each type of meat balls (pork, chicken and fish) in the market of Thailand as shown in Table 1. We pick up some of the most popular and non popular meat balls in order to having a wide range of samples. The high price meat balls will be numbered from 1 to 4 while numbers 5-7 will be the medium price and numbers 8-10 will be the cheap ones. The texture analyzer model TA.TX2i was used to measure the physical properties. The conditions are as follows: TPA (Texture Profile Analysis), 75 mm of probe size, compression 30 % deformation, the pre-test speed and test speed at 1.0 mm/sec, post-test speed at 10.0 mm/sec. The properties such as hardness, fracturability, springiness and so on will be obtained from this instrument. To investigate the folding test, meat ball was sliced having thickness around 2-3 mm and then checked the cracking after folding one-half and one-fourth. The ranking of folding test score was 5 to 1 as shown in Table 2. Score 5 means meat ball having more softness and score 1 means meat ball having more hardness.

Results and discussion

When the texture analyzer (TPA) is used, several information can be obtained, i.e. hardness (g-force), fracturability (g-force), adhesiveness (g.sec), springiness, cohesiveness, gumminess and chewiness. Figure 1(a)-(c) shows the hardness and folding test scores of pork ball, chicken ball and fish ball, respectively.
Figure 1(a) shows pork ball having the hardness mainly range from 8000 -11000 g-force, and the folding test scores range from 3 to 5. We observe that the products having high scores (score 5) of folding test reveals the hardness from 8000 – 10000 g-force except two products, i.e., item 5 and item 9. This result may have occurred if the texture of that product was not smooth enough. When the hardness spot of product was touched by the probe, the high value of hardness can be found. We can say that folding test score 5 will be

found in the group of high price. Whereas, the score 3 and 4 will be found in the group of medium and low price which is implied that raw material for producing is in different quality.
Figure 1(b) shows the hardness and folding test score of chicken balls. They have the value of hardness around 5000 -8000 g-force and have the folding test scores around 3-5. There are some products having quite high hardness, i.e., items 1, 2 and 7. This may happen in the same manner with pork ball. However, we can conclude that the chicken ball at low and medium price have a low folding test score (score 3).
Figure 1(c) shows the hardness and folding test scores of fish balls. They are mainly around 2500-5000 g-force, and the folding test score around 4-5. The scores of folding test 3 will be not found here in fish ball. There is only item 4 which have a high value of hardness (around 8500 g-force). The other information can be plotted in the same manner, but they are not shown in here. For example, the springiness of pork ball, chicken ball and fish ball will be 0.761-0.850, 0.682-0.882, and 0.832-0.949, respectively. The cohesiveness of pork ball, chicken ball and fish ball will be around 0.344-0.472, 0.259-0.498 and 0.491-0.542, respectively. Figure 1(d) shows the correlation of hardness and folding test score of those meat balls. Since there is the fluctuation of data, the correlation was not fit so well (R^2 is quite low in three of meat balls). However, if we neglect the scattered data, we should have a better correlation. It is noticeable that there is no data at the low value of folding test score (1-2). This may imply that those commercial meat balls should not have such a low value. Since low score of folding test means too fragile and cracking can be found easily in the product. This kind of property should be avoided. Figures 2(a)-(d) show the result of other properties, i.e., fracturability, springiness, cohesiveness and gumminess versus the folding test score in order to look for the correlation among them. In Figure 2(a) shows fracturability and folding test score of three meat balls. The correlation of pork balls is quite fair since the value of R^2 is satisfactory ($R^2 = 0.81$), whereas correlation of chicken balls is poor ($R^2 = 0.54$) as well as of fish balls. As we can see the rest of all figures from (b)-(d), the correlation is quite poor. However, the correlation could not expand to the low range of folding test, i.e. score 1-2. Since in this range, when the product was fold one half, the cracking could be observed in the product which is undesirable property.

Conclusions

We can conclude that the folding test score of commercial pork balls is 3-5 (majority is 5), and hardness is 8184.95-15859.28 g-force. The springiness is 0.761-0.850. The folding test score of chicken ball is 3-5 (majority is 3), and hardness is 5217.65-12522.13 g-force, springiness is 0.682- 0.882. The folding test score of fish ball is 4-5 (majority is 5), hardness is 2401.88-8539.04 g-force, and springiness is 0.832-0.949. The correlation of folding test and their properties could not draw conclusion since R square value is quite poor.

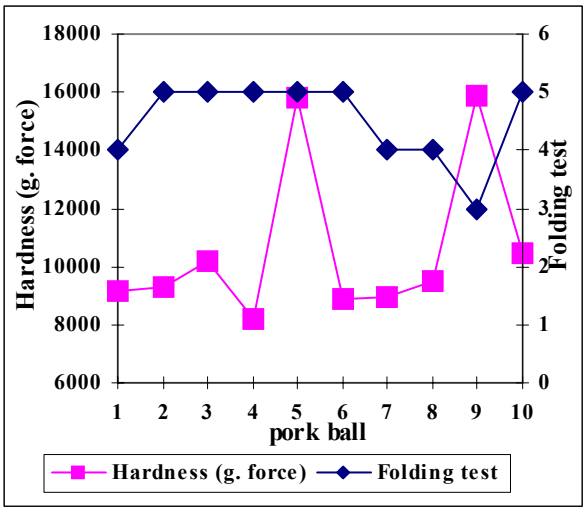
Table 1 Name and price of some commercial meat balls in Thailand

Pork ball		Chicken ball		Fish ball	
name	Price (Baht/kg)	name	Price (Baht/kg)	name	Price (Baht/kg)
1. Sechaun	150	1. C.P.	100	1. TaeJew	153.5
2. C.P.	135	2. Lotus	70	2. C.P.	110
3. Seanthai	125	3. Carefour	70	3. Tanachai	106
4. Moodee	90	4. BigC	69	4. Laojeung	103.5
5. Bualoi	75	5. Sahafarm	40	5. Lee Seafood	80
6. J.P.M.	72.50	6. B.K.P.	40	6. Heng Heng	70
7. Lotus	70	7. Golden B	35	7. Bangrak	63.5
8. Jitjaruen	70	8. A.P.B.	35	8. Jae Ju	55
9. Rodded	70	9. J.F.	30	9. See Praya	50
10. Top	64	10. P.P.	30	10. Ponjaruen	46

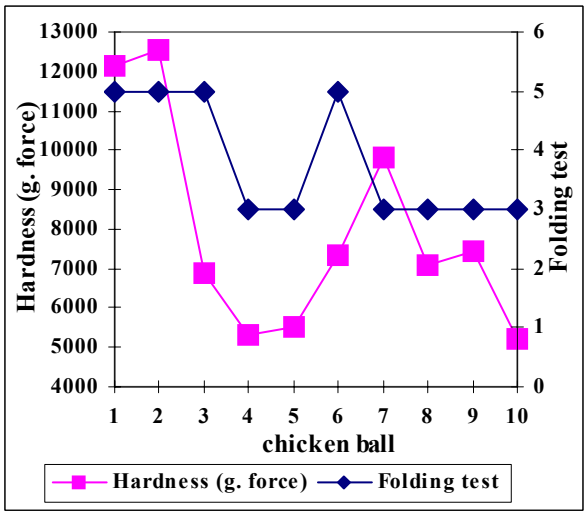
(48 Baht = 1 Euro)

Table 2 Ranking score of folding test

Ranking	Description
5	Folding one-fourth, cracking can not be observed
4	Folding one-fourth, cracking can be slightly observed
3	Folding one-fourth, cracking can be observed
2	Folding one-half, cracking can be slightly observed
1	Folding one-half, cracking can be observed



(a)



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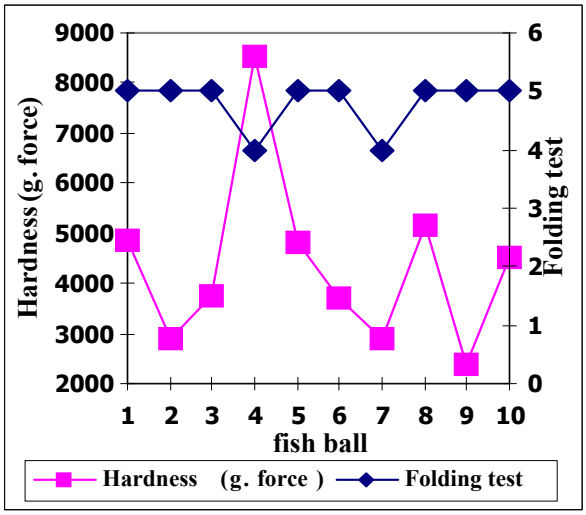

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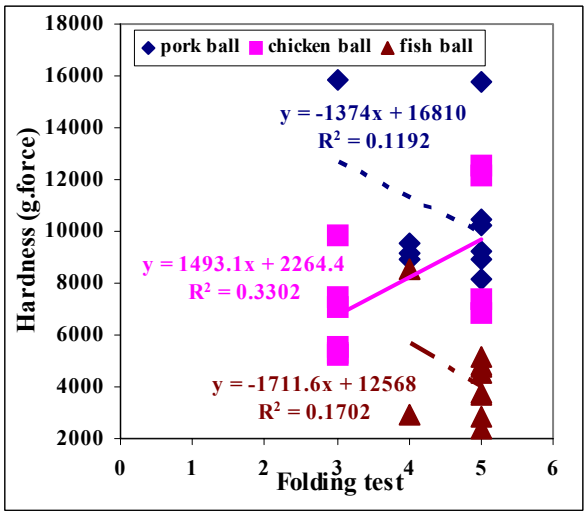

(d)

Figure 1 (a)-(c) Hardness and folding test of meat balls
(d) correlation of hardness and folding test

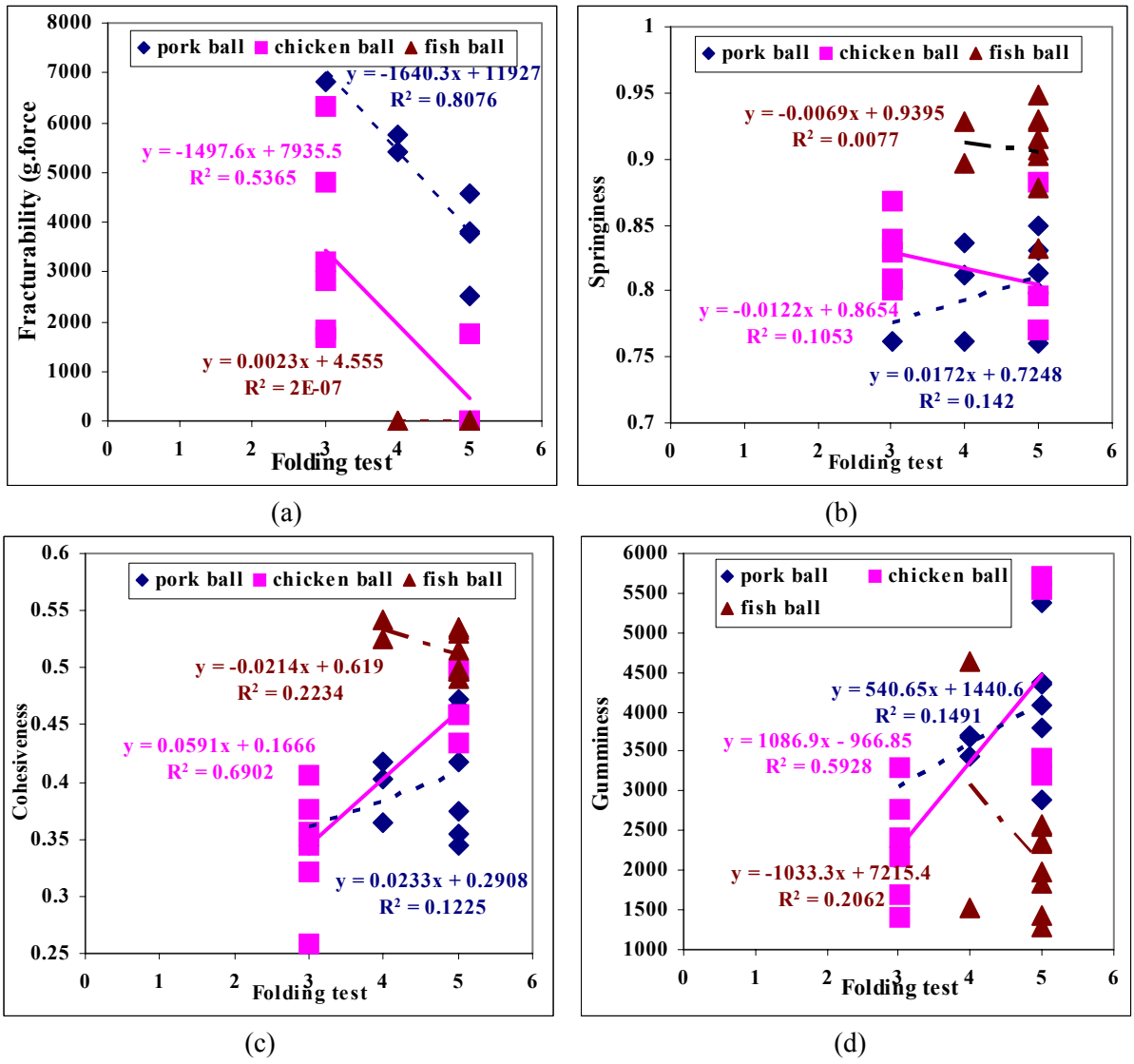

Figure 2 (a)-(d) Correlation of data from texture analyzer and folding test of meat balls

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Acknowledgement

This work was granted by the Fiscal year 2004, Faculty of Agricultural Industry, KMITL

VISIBLE-NIR SPECTROSCOPY: A NON-DESTRUCTIVE RAPID TECHNIQUE TO ASSESS END-POINT TEMPERATURE OF KAMABOKO GEL

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Background

Inadequate cooking of food products and use of improper holding times are common causes of food-borne disease outbreaks (Walton and McCarthy, 1999). Many of these outbreaks result from undercooking at food service and retail outlets where time and temperature of processing do not need to be adequately documented. Because of the high correlation of these outbreaks with consumption of undercooked food products, the elimination of these microorganisms from processed foods has become a prime concern for consumers, the meat industry, and the regulatory agencies. Heat treatment is given to meat to produce a palatable product, to improve the shelflife, and to minimize the risk of food-borne illness. Consequently, optimum cooking is necessary to keep the maximum food qualities but minimize the risk of heat-labile pathogens.

A large number of researches have been conducted to track the previous heat treatment (end-point temperature, EPT) of meat products (Townsend and Blankenship 1989; Miller *et al*, 2003), however, similar studies on marine products are almost negligible. Moreover, seafood are common vehicles of food-borne diseases can be contaminated with pathogens during several stages of processing. Therefore it became more important to achieve optimum heat treatment to seafood. We are conducting a series of study to assess EPT of processed marine products since they have an ever-growing role in human food source (Uddin *et al*, 2000; 2002a). Near infrared (NIR) spectroscopy, a non-destructive rapid technique, which has been widely used in the food industry, is based on the electromagnetic absorption of organic compounds. We successfully applied this NIR technique to investigate the heating adequacy of heated fish and shellfish meats (Uddin *et al*, 2002b).

Objectives

The purpose of this study was to assess EPT of kamaboko gels since surimi-based fish-meat gels have been gaining popularity in recent years for their protein quality, law fat content, and convenience in consumption. Surimi-based most common finished product kamaboko gel was used as a model of fish-meat gels. A potential useful application of NIR reflectance spectroscopy would be the reliable, rapid and non-destructive determination in these regards.

Materials and methods

Preparation of kamaboko gel

Two types of surimi were used to prepared kamaboko gel. Frozen SA grade walleye pollack (*Theragra chalcogramma*) surimi (Maruha Co., Tokyo, Japan) was thawed overnight at 5ºC then chopped in a kitchen-cutter (Yamada FP-1S, Mitsubishi Co., Ltd, Tokyo, Japan). Horse mackerel (*Trachurus japonicus*) surimi was prepared according to the procedure of Konno *et al*. (2000). The final moisture content was adjusted to 80% by adding cold distilled water prior to add NaCl equivalent to 2.5% of the surimi weight. Kamaboko gel was prepared by pressing the salt-ground surimi into a polyvinylidene chloride casing measuring 48 mm in circumference and approximately 150 mm in length. Both ends of each tube casing were tied with cotton thread, and all of the proceeding operations were accomplished at 5ºC. The filled casing samples were incubated at 10ºC intervals between 30 and 90ºC for 30 min in water bath incubators then cooled immediately in ice-cold water and kept at 4ºC overnight before NIR spectroscopic measurement.

Internal temperture of kamaboko gels were monitored using a recorder (Thermodac EF 5020A, Eto Denki Co., Tokyo, Japan) with connected thermocouples. Thermocouples (copper-constantan) were inserted through the side edge of separate samples in to the geometric center. Sixteen individual kamaboko samples

were prepared for each selected temperature therefore 112 samples were made for each walleye pollack and horse mackerel surimi respectively.

NIR spectroscopy

Visible-NIR spectra were recorded at 2 nm intervals using a NIRSystems 6500 scanning monochromator instrument (NIRSystems, Silver Spring, MD). Kamaboko spectra (n = 224) were collected using a surface interactance fibre optic accessory. Within a 4 cm square probe face, 7 quartz windows (1 x 20 mm) are fitted; windows are alternatively light exit (n = 4) and collection (n = 3) ports. Prior to spectral acquisition, the surface of the kamaboko was wiped with a paper tissue to remove excess moisture. Before spectra of the Kamaboko gels were measured, a background spectrum was collected. Spectra were collected between 650 and 1100 nm; above this range, the fibre optic probe becomes a significant absorber of radiation and were stored in optical density units log (1/R), where R represents the percent of energy reflected. Only a single spectrum was taken from an individual sample therefore 56 spectra were use to developed a calibration and remaining 56 spectra were used for validation set in both of walleye pollack and horse mackerel kamaboko gels. Two linear regression methods Partial Lest Squares (PLS) and Multiple Linear Regression (MLR) were used to develop calibration and validation set.

Data analysis was performed by the Vision spectral analysis software package (Version 2.11. NIRSystems, Silver Spring, MD) and The Unscrambler software (version 8.05. CAMO, USA).

Results and discussion

Figure 1 illustrates the spectra collected from kamaboko gel prepared by walleye pollack surimi heated at different temperatures. The higher curve is for the sample heated at lower temperature, whereas the lower curve applies to the higher temperature. Kamaboko made from horse mackerel surimi also showed similar fenomenon (data not shown). In every spectrum, the log (1/R) peaks at any wavelength decreased with increasing EPT. Water absorbs strongly in specific wavelengths which is expected and usually exhibits a broad band because of H-bonding interactions with itself and with other components in the meat. In visible-NIR spectroscopy, the regions from 740–760 nm and 960–980 nm are related to O–H bond of the water in the sample (Osborne *et al*, 1993; Murray and Williams, 1990). Since protein conformations, protein water interactions, or their combination might depend on the variation of water content, the NIR reflectance spectra of a heated sample could be affected separately with a different heating process. The kamaboko gels in this study were heat-treated in a stirred-water bath. The water content was constant before and after heat treatment which allowed us to minimize the differences in water contents between samples heat-treated at different temperatures. Therefore, these changes of NIR reflectance spectra upon heat treatment could be related to the heating temperature.

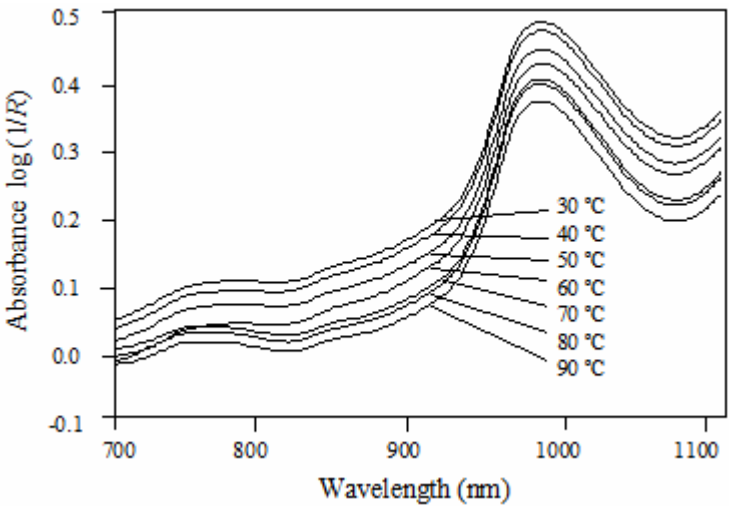

Fig. 1. Average reflectance spectra of kamaboko gels prepared from walleye pollack surimi.

Studies on thermal denaturation of muscle proteins indicate that the changes of meat proteins during cooking take place in a step-wise process (Findlay and Barbut, 1990). Moreover, food and feed substances of plant or animal origin are composed of constituents possessing functional groups such as C-H, O-H, N-H, S-H, and C=O which are selectively absorb NIR radiation. Therefore, if there is any linear relationship between NIR

spectral changes and heating temperature of meats, a calibration equation could be developed to track their previous heat treatment. It has been suggested that standard normal variance and de-trend (SNVD) transformation would remove multiplicative interference of scatter and particle size, baseline shift and curvilinearity. Consequently, derivative treatment reduces scattering effects and also increases resolution of spectrum peaks (Barnes *et al*, 1989; Ding and Xu, 1999). Therefore, to determine EPT of kamaboko gel, the spectra were subsequently scatter corrected using SNVD and 2nd-derivative treatments. Systematic differences in absorbance related to the heat treatment were observed at different wavelengths throughout the derivative treated spectra where the characteristic absorption peaks are more clearly separated. These differences are more visible between 800 and 930 nm region due to the main absorbance band of proteins (data not shown). Spectral changes upon heat treatment were related to the heating temperature which might be the reason for changes in the environment of the secondary structure due to the denaturation of proteins, and to changes in the state of water.

Table 1. Calibration and validation results obtained by MLR for assessing EPT of Kamaboko

Kamaboko sample	Wavelength selected (nm)				R	SEC (%)	SEP (%)	Bias (%)
	λ1	λ2	λ3	λ4				
Walleye pollack	910				0.81	5.07	5.41	-0.35
	910	848			0.94	3.22	3.95	-0.52
	910	848	822		0.97	1.88	1.73	-0.08
	910	848	822	938	0.98	1.76	1.71	-0.31
Horse mackerel	910				0.84	4.95	4.92	-0.26
	910	858			0.95	2.73	2.76	-0.06
	910	858	822		0.98	2.06	2.22	-0.28
	910	858	822	938	0.98	1.78	1.84	-0.07

R : Multiple correlation coefficient.
SEC: Standard error of calibration.
SEP: Bias corrected standard error of prediction.
Bias: The average of difference between actual value and NIR velue.

In order to predict EPT of kamaboko gels, the derivative-treated spectra were calculated. Spectral changes with heating temperatures were recorded by computer and analyzed by MLR and PLS regrassions. MLR analysis allowed us to select appropriate wavelengths related to specific known chemical bonds without interference. On the other hand, PLS regression has been widely applied recently because no selection of wavelengths is needed. However, even in PLS calibration, selection of the wavelength region was needed to make a good calibration equation, which was more complicated and time consuming. In MLR analysis, the wavelengths selected by a stepforward – stepreverse regression in this study to provide the calibration equations with the lowest standard error of calibration (SEC) and highest correlation coefficients of calibration (R) are given in Table 1. The first wavelength selection which most important and one of the major steps for MLR analysis 910 nm due to the absorbance of protein was selected by manually. The standard error of prediction (SEP) using 4 wavelengths were calculated to be less than 1.85%, suggesting that the calibrations developed are appropriate. Selected wavelengths for the prediction of EPT are extensively related to C-H, N-H and C=O groups (Murray and Williams, 1990; Osborne *et al*, 1993). It was suggested that the wavelengths selected by MLR could be used as a good indicator for selection of the wavelength region in PLS calibration (Saranwong *et al*, 2001). Therefore, the wavelength region selected by MLR was applied for PLS calibration which also gave better indication (Table 2).

Table 2. Calibration and validation results of PLS regression using whole spectrum and selected region obtained by MLR for assessing EPT

Kamaboko sample	Wavelength region	F	R	SEC (%)	SEP (%)	Bias (%)	RPD
Walleye pollack	650 - 1100	9	0.98	1.83	1.87	-0.21	2.63
	800 - 950	5	0.97	1.87	1.76	-0.07	3.07
Horse mackerel	650 - 1100	9	0.98	1.95	1.94	-0.13	2.25
	800 - 950	6	0.98	1.91	1.81	-0.11	2.49

F : The number of factor/variable used in the calibration equation.
RPD : The ratio of standard deviation of reference data in validation set to SEP.

Figure 2 depicts scatter plots of NIR predicted EPT against the actual heating temperatures obtained by MLR and PLS calculations of walleye pollack kamaboko gel. Similar results also observed from horse mackerel. A comparison of the NIR predicted EPT with known actual heating temperatures showed extreamely close detections. Both MLR and PLS could be used in making calibration equations with similar predictive eficiency. The R were better than 0.98 indicating a good model structure. Such a non-destructive, simple and reliable technique which is expected to assess EPT of processed products is essential in today's demand. In this study kamaboko was made from walleye pollack and horse mackerel surimi, however, it could be valid as a general technique for other species.

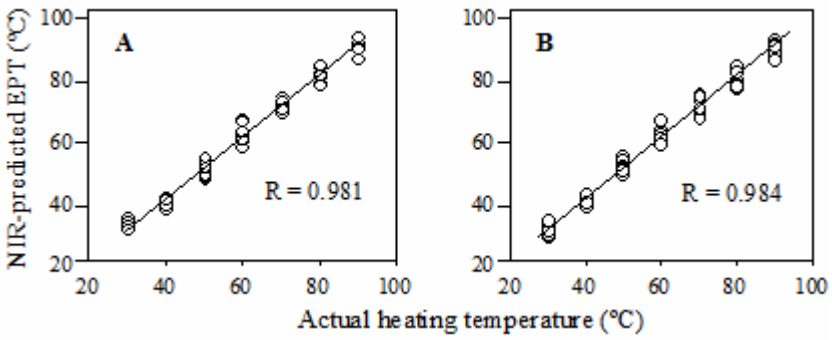

Fig. 2. NIR-predicted endpoint temperatures obtained by MLR (A) and PLS (B) of walleye pollack kamaboko gels plotted against the actual heating temperatures.

Conclusions

The results discussed above demonstrate the potential of visible-NIR reflectance spectroscopy for determining EPT of kamaboko gels in a rapid, reliable and non-destructive manner. Once perfected, this technique will have several advantages over other techniques, in that it will take the least time for analysis and will not require any consumables or supporting equipment.

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TEXTURAL AND SENSORY PROPERTIES OF OATMEAL ADDED LOW FAT SAUSAGE WITH DIFFERENT TYPES OF MEATS

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Background

Due to concerns with obesity and related diseases, consumers are looking for no fat or low fat meat products. With excessive fat reduction, however, the products desire bland and dry and texture can be hard, resulting in less accepted products by consumers. Fat substituents based on proteins and carbohydrates have been widely used in meat industry to overcome the problems. Oat and oat bran have shown promise for increasing yield and juice retention in meat. In addition, the constituents have been used in diets to control hypertension, diabetes, and health disease.

Objectives

The objectives of this research were to evaluate how the addition of oatmeal affects the properties and acceptability of low fat sausages and to compare the effectiveness of incorporated oatmeal on sausages prepared with different types of meats such as beef, pork, and chicken.

Materials and methods

Sausage preparation : Beef, pork, and chicken were purchased from a local market and trimmed to reduce fat content before grinding though a 3mm plate. The moisture contents of all types of meats were adjusted to 60% and oatmeal was hydrated to provide 60% moisture content. This was done to ensure that any differences observed for added oatmeal would not be biased by differing moisture content. 10% hydrated oatmeal was added to each meat in a basis of total weight. The low fat content sausages were also prepared without addition of oatmeal. For each batch, meat, hydrated, and ingredient were mixed throughly using mixer. Alter mixing, the mixture were stuffed into artificial cellulose casing with diameter of about 30mm using a stuffer. The sausages were then held for 24hrs at 4℃ to allow for ingredient equilibrium. The sausage samples were cooked 30min in a steam chamber (SAA10, Absury, Germany) until the center temperature of the sausage reached 70℃.

Proximate analysis and cooking yield : Moisture, fat, and protein content were determined in triplicate for raw and cooked products using AOAC methods (AOAC, 1990). The weight of each sausage was measured before and after cooking to determine cooking yield, which was defined as the cooked weight divided by uncooked weight then multiply by 100.

TPA (texture profile analysis) : Before analysis, cooked sausages were equilibrated at room temperature (20ºC) for l hr. Sausages were cored into a cylinder with 1 cm diameter and cut with 1.5 cm height using a sharp edged knife. TPA was performed by compressing the sample between parallel plates in a Universal Testing Machine (Model 3343) to 70% of the original height in two consecutive cycles at a crosshead speed 100mm/min. From the resulting twice/deformation curves, the textural parameter of hardness, cohesiveness, springiness, brittleness, gummness, and chewiness were calculated.

Sensory evaluation : A 10-member trained sensory panel evaluated the low fat sausages for color, aroma, flavor, off-flavor, juiciness, tenderness, and overall acceptability. Sausages were served in random order to panelists. They evaluated each sample on 9 point hedonic scale (1=extremely, 9=extremely strong).

Statistical analysis : The data were analyzed using statistical analysis systems (SAS. 1999). To evaluate the differences among treatments, data were analyzed by analysis of variance (ANOVA) and Duncun's multiple range test.

Results and discussion

The proximate composition of sausage samples with or without oatmeal is shown in table 1. Fat contents in different sausages ranged from 3.94 to 5.22% and protein content ranged from 20.40 to 26.47%. In general, fat and protein contents for beef, pork, and chicken were slightly reduced by the addition of oatmeal ($P<0.05$). There was no significant difference in cooking yield among samples. This indicates that neither meat types nor addition of oatmeal affect cooking yield. Table 2 shown TPA of the sausages with different types of meats and with/or without oatmeal. Generally, there was significant difference in texture attributes such as hardness, cohesiveness, among sausages sample with different meat type ($P<0.05$). For examples, sausages from beef showed highest values in hardness, cohesiveness, chewiness, while chicken did lowest ones. On the other hand, brittleness, hardness, gummness and chewiness for all sausages decreased by addition of oatmeal while cohesiveness increased. The textural properties the sausage of prepared with beef were most affected by addition of oatmeal. Table 3 shown sensory evaluations of the sausages with different types of meats and with/or without oatmeal. There was significant difference in color, aroma, tenderness and acceptability among sausages prepared with different types of meats ($P<0.05$). Tenderness value was decreased by addition of oatmeal. Consequently, the addition of oatmeal improved textural attributes such as juiciness and tenderness, leading to better acceptability, compared to low fat content sausages.

Conclusions

Fat and protein contents of trimmed low fat sausages were further reduced by the addition of oatmeal. The oatmeal-added sausages improved textural properties such as juiciness, tenderness compared to low fat content sausages. This leads to better acceptability to trained sensory panels. The low fat sausage prepared with beef was most affected by the addition of oatmeal.

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Table 1. Proximate analysis and cooking yield in low fat sausages with/without hydrated oatmeal

Treatments	Moisture (%)	Fat (%)	Protein (%)	Cooking yield (%)
*Beef	59.17^{BC}	4.66	26.47^{A}	97.32
*Pork	58.87^{C}	5.22	25.76^{A}	97.63
*Chicken	61.73^{A}	4.97	24.02^{B}	97.72
Beef + 10% hydrated oatmeal	58.33^{C}	4.94	20.87^{C}	97.10
Pork + 10% hydrated oatmeal	60.86^{AB}	4.83	20.40^{C}	96.89
Chicken + 10% hydrated oatmeal	61.70^{A}	3.94	20.55^{C}	97.64

• ^{ABC} : Means in the same column with identical letters are significantly different ($p<0.05$).
• * : Control; without hydrated oatmeal

Table 2. TPA (texture profile analysis) in low fat sausages with/without hydrated oatmeal

Treatments	Brittleness (kgf)	Hardness (kgf)	Cohesiveness (%)	Springiness (mm)	Gummness (kg)	Chewiness (kg*mm)
*Beef	0.46^{A}	0.63^{A}	56.79^{B}	14.00^{D}	35.64^{A}	498.66^{A}
*Pork	0.36^{B}	0.51^{B}	55.96^{B}	14.17^{C}	28.59^{B}	406.77^{B}
*Chicken	0.36^{B}	0.40^{C}	49.69^{C}	13.99^{D}	20.09^{C}	281.09^{C}
Beef + 10% hydrated oatmeal	0.19^{D}	0.27^{D}	61.77^{A}	14.52^{A}	14.52^{D}	233.04^{D}
Pork + 10% hydrated oatmeal	0.22^{CD}	0.28^{D}	57.77^{B}	14.41^{B}	16.12^{D}	232.16^{D}
Chicken + 10% hydrated oatmeal	0.26^{C}	0.31^{D}	49.33^{C}	14.10^{C}	15.74^{D}	221.76^{D}

• ^{ABCD} : Means in the same column with identical letters are significantly different ($p<0.05$).
• * : Control; without hydrated oatmeal

Table 3. Sensory evaluation in low fat sausages with/without hydrated oatmeal

Treatments	Color	Aroma	Flavor	Off-flavor	Juiciness	Tenderness	Acceptability
*Beef	8.65^{A}	6.78^{A}	6.35	0.58	3.93	4.68^{A}	3.95^{C}
*Pork	6.30^{B}	5.10^{AB}	4.65	0.50	4.63	4.45^{A}	4.60^{BC}
*Chicken	4.20^{CD}	4.10^{B}	4.38	0.80	5.38	4.78^{A}	5.28^{ABC}
Beef + 10% hydrated atmeal	7.13^{AB}	6.53^{A}	4.65	0.60	5.98	1.48^{B}	5.70^{AB}
Pork + 10% hydrated atmeal	5.50^{BC}	5.78^{AB}	4.18	0.43	5.60	1.95^{B}	5.80^{AB}
Chicken + 10% hydrated oatmeal	3.70^{D}	5.23^{AB}	3.20	0.26	5.58	2.15^{B}	6.63^{A}

• ^{ABCD} : Means in the same column with identical letters are significantly different ($p<0.05$).
• * : Control; without hydrated oatmeal
• Aroma (1-3 : weak, 4-6 : moderate, 7-9 : strong), flavor (1-3 : weak, 4-6 : moderate, 7-9 : strong), juiciness (1-3 : small, 4-6 : moderate, 7-9 : large), tenderness (1-3 : tough, 4-6 : moderate, 7-9 : tender), off-flavor (1-3 : weak, 4-6 : moderate, 7-9 : strong) and acceptability (1-3 : dislike, 4-6 : moderate, 7-9 : like).

EFFECTS OF PROTEIN CONCENTRATION, PH, IONIC STRENGTH ON HEAT-INDUCED GELATION PROPERTIES OF MYOSIN FROM RABBIT SKELETAL MUSCLES

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Background

The textural quality of reconstructed and comminuted meat depends on the functional properties of muscle proteins, especially the gelling, binding, emulsification of extracted proteins and water holding capacity (WHC) of the meat product (Boyer *et al* 1996b). Heat-induced gelation is the result of the nature and denatured muscle protein interactions, including inter-protein hydrogen, ionic linkage, hydrophobic interaction and so on. However, the equilibrium among protein-protein, protein-solvent and elements gravitation-repulsion are the most important factors in forming the fine three-dimensional gel matrix. Myosin accounts for about one-third of total muscle protein, 50%~55% of myofibril protein, morever, it has a lot of functional properties in determining the textural quality of meat products. Among these functional properties, wonderful gelling capacity of myosin has been aroused much attention from meat scientists (Siegel *et al* 1979, Boyer *et al* 1996a). Previous literatures indicated that a lot of factors such as protein concentration, pH, ionic strength, type of animal and muscle, extracting process of myosin, heating pattern, non-meat additives had significant effects on heat-induced gelation properties.

Objectives

The purpose of this study was to find out the effects of protein concentration, pH, ionic strength on the hardness, WHC and ultrastructure of heat-induced gelation of myosin from rabbit (New Jersey White rabbit) skeletal muscles (*Psoas major*, PM and *Semimenbranosus proprius*, SMp).

Materials and methods

Male rabbits with liveweight 2~3kg were obtained from Jiangsu Academy of Agriculture Science. After being fed with water only for about 18h, the rabbits were slaughtered and the PM and SMp muscles were used for extracting myosins. The extracting process referred to method of Nauss *et al* (1969), Hermansson *et al* (1986) and Wang *et al* (1994) with a little modification. Myosin concentration was measured by Biuret method (Gornall *et al* 1949) and the composition of each fraction was analyzed by SDS-PAGE on 10% slab gels according to Jun-yao Guo(2001). Myosin (15mg/ml) was held in 7ml plastic centrifugal tube (tubes were previously weighed as W_0 and the tube and protein weight was W_1), then was programmed heated from 20ºC to 65ºC at a rate of 1ºC/min and kept at 65ºC for 20min. Then gels were cooled to ambient temperature and held at 4ºC overnight .

Gel **hardness** (g) was measured with TA.XT2i Texture Analyzer (Stable Micro Systems Ltd, England). with P5 probe (5mm DIA CYLINDER STAINLESS). The test mode was TPA fracture.mac, and test speed was 0.5mm/sec. After centrifugation at 10,000×g, 4ºC, supernatant was decanted, the gel and tube was reweighed as W_2. The **WHC(**%) was calculated as $[(W_2-W_0)/(W_1-W_0)]\times100$. Each test was carried out with three repetitions. Duncan's Multiple-range test was used for ANOVA analysis with SAS8.2. For **SEM examination**, samples were prepared as described by Boyer *et al* (1996b) and observed with a 200 Hitachi Scanning Electron Microscopy (SEM, Hitachi Co Ltd, Japan) at an accelerating voltage of 20kv.

Results and discussion

Both the gel hardness and WHC linearly increased with the gradual increase of protein concentration (Figure 1A), and the differences were significant ($p<0.05$). Gels had maximum hardness of 15.18g for PM myosin , and 14.87g for SMp myosin at 15mmol/l. The maximum WHC was 66.85% for PM myosin and 51.89% for SMp myosin respectively. There was no difference in hardness between two types of myosin gels ($p>0.05$), however, when the myosin concentration was above 8mmol/l, PM myosin gels had higher WHC than SMp samples($p<0.05$).

pH played a significant role in the matrix formation and water retention of the myosin gels. Both the hardness and WHC varied with the increase of pH from 5.0 to 8.0 (Figure 1B). PM myosin gel had the highest hardness (20.79g) at pH6.0, while SMp myosin had a peak at pH5.5 (29.39g). WHC increased from pH 5.0, and reached maximum at pH6.0, and then decreased at higher pH. At the peak point, WHC was 82.99% for PM myosin gel which was higher than that of SMp myosin (67.27%)($p<0.05$).

Gel hardness decreased sharply when ionic strength increased from 0.2 to 0.6($p<0.05$) and decrased slowly at higher ionic strength (0.6~1.0) (Figure 1C). In gels at ionic strength 0.2, maximal hardness reached 45.60g and 44.61g for PM and SMp myosin respectively. In contrast, WHC decreased when ionic strength ranged from 0.2 to 0.4, and then increased gradually until ionic strength reached 1.0. PM myosin gel had maximal WHC (79.87%) at ionic strength 1.0, with no difference with that(74.56%) at ionic strength 0.2 ($p>0.05$). The similar profile in WHC occured for SMp myosin gel, which was 78.51% at ionic strength 1.0 compared to 76.69% at ionic stength 0.2. At all points, there was no difference between these two myosin gels.

Gel ultrastructure could be obtained by SEM only when myosin concentration was higher than 5mg/ml. PM myosin formed a looser network at concentration 5mg/ml than at 10,15 mg/ml (Figure 2B, Figure 3C). When heated, myosin molecules aggregated into cross-linkage with diameter 0.1~0.2μm(Figure 2A). The ultrastructure was a bit disorganized and pore size varied greatly from 0.2 to 1.3μm. Gel with 10mg/ml had a homogeneous granular structure exhibiting spherical corpuscles (diameter 0.1~0.2μm),uniform cavities(diameter~0.3μm), but the cross-linkages were shorter compared to those in 5mg/ml gel, which indicated more matrix formed in the course of heating.

Myosin formed a homogeneous network at 0.6mol/l KCl with spherical corpuscles exposed to the surface, short cross linkages, small and regular (diameter~0.3μm) cavities. But when the myosin was equilibrated in 0.2mol/l KCl by overnight dialysis (4□), another entirely different three dimension matrix formed and gel hardness and WHC increased significantly ($p<0.01$) compared to those at 0.6 mol/l KCl (Figure 1C). Gels at 0.2mol/l KCl formed a strand-type network (Figure 3-A, B, D, E). And PM gel was more homogeneous than SMp gel. Diameter of the strands is less than 0.1μm, and the length varied from 0.5 to 2.5μm. Most of the holes in PM gels were near circinal (most with diameter0.2~0.5μm). In some way, SMp myosin gave rise to denser strand than PM myosin. The strands tended to array in the same direction, so a kind of zonary or sandwich-like structure occured with small hole being in the strand region.

Ultrastructure of myosin gels at pH5.0 and pH5.5 showed serious protein-protein and protein-solvent interactions based on the charge distribution in myosin molecules(Figure4). When heated (pH5.0), molecules denatured and aggregated. Myosin conglomerations developed into column-like cross-linkages (diameter0.15~0.3μm, length 0.8~1.5μm). At pH5.0, the ultrastructure seemed to result from a "collapse" with long (0.6~1.2μm) and thin(~0.1μm) strand. Large cavities were left in these networks. Myosin formed consistently distributed three-dimension ultrastructure at pH6.0 and pH6.5 respectively. While molecules at pH6.5 aggregated into denser structure than pH6.0, a small sheet-like aggregate evenly distributed throughout the gel matrix. When pH increased to pH7.0, pH7.5 and pH8.0, gel structure became coarser with larger granular and holes in the network. In coincidence, the repulsion between molecules increased and coarser structure came into being as a result of more negative ionic in the protein.

Many studies have pointed out the difference between myosin gels of different muscle type, and generally concluded that white muscles (fast-twitch, like PM muscle) exhibited a higher gel-forming ability than those from red muscles (slow-twitch, like SMp muscle). (Egelandsdal *et al* 1995, Culioli *et al* 1993, Xiong, Y.L.1994). This, on the whole, resulted from the different cross-linking capacity of myosin heavy chain, and also the difference of surface hydrophobicity of denatured myosin and temperature discrepancy at which myosin denatured. The result of this experiment conducted was not the same with those represented in other literatures.

Salt and pH could affect the water balance and charge equilibrium in muscle and muscle product. Offer and Knight (1988) suggested that this balance was determined by (a) electrostatic repulsion, (b) restraining forces in structural protein, (c) chemical potential in the system. Water can be held in the gel by capillarity, electrostatic interaction at the protein surface and among the network. It has been known that myosin can only be soluble at high enough ionic strength (>0.3), but water holding capacity was not the same as the number of soluble protein in the solution. Heat-induced gelation could still have good WHC at ionic strength 0.2(Figure 1C). As demonstrated earlier (Boyer *et al* 1996a), myosin formed strand-type gel at ionic strength 0.2 and short cross-linkage gel with aspheric granular on the surface at ionic strength 0.6. Although there were large cavities in strand-type network, the thick strands were necessary for higher hardness and still could hold much water in the strand strips (Figure3A, B, C, D), While the granular structure lost much water when gel was under centrifugation at 10,000×g, 4ºC. Feng and Hultin(2001) also found that strong gels with

good WHC could be formed under low ionic strength conditions, and suggested that electrostatic repulsion of proteins is a major driving force behind gel formation and WHC.
Water loss decreased when pH increased from 5.0 to 6.0,and then increased above pH6.0, indicating that the iso-electricity point might be near pH6.0. This was the same as Kristinsson *et al* (2003). Morita, *et al* (1987) also found that PM muscle protein formed the hardest gel at pH6.0, while SMp gel was hardest at pH5.5. But at high ionic strength (0.6mol/l KCl) and high pH (>6.0), gel hardness and WHC were lower than at other conditions, which could result from electric screening behind this phenomenon, weakening the repulsion between neighboring matrix charges.

Conclusions

Myosin solution could form hard enough heat-induced gel with acceptable WHC only when concentration was above 5mg/ml. At ionic strength 0.2, gel had higher hardness and good WHC with strand-type ultrastructure, while myosin at ionic strength 0.6 aggregated into homogeneous network with aspheric granular on the surface and short cross-linkage throughout the gel matrix. Strong gel and good WHC could be formed at pH5.5 or pH6.0 at ionic strength 0.6. When pH raised above 6.5, myosin denatured and aggregated into coarse network. Gel hardness decreased with increase of ionic strength, but the maximal WHC was obtained at ionic strength 1.0. There was little difference between gelation properties of PM and SMp muscle myosin.

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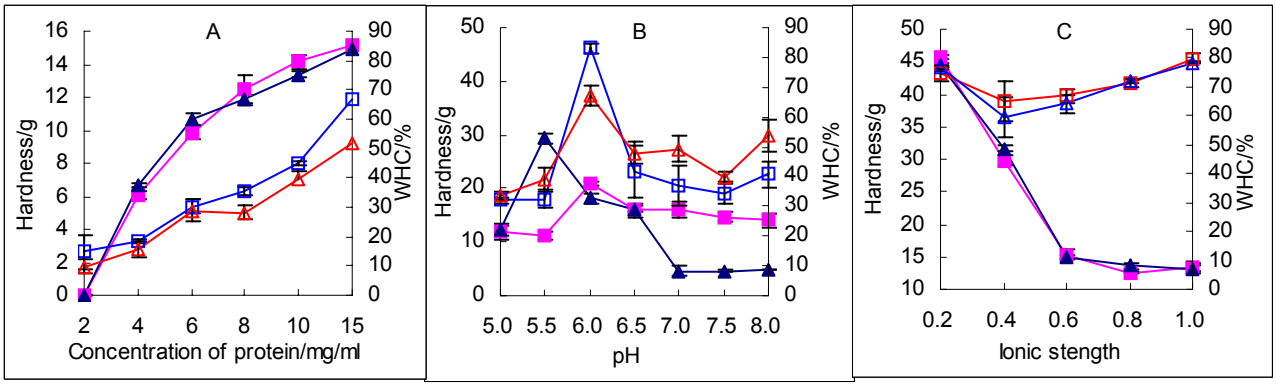


Hardness: ■-PM ▲-SMp WHC: □-PM △-SMp

Figure 1 Effect of concentration (A), pH (B) and ionic strength(C) on the gel hardness and WHC of myosin

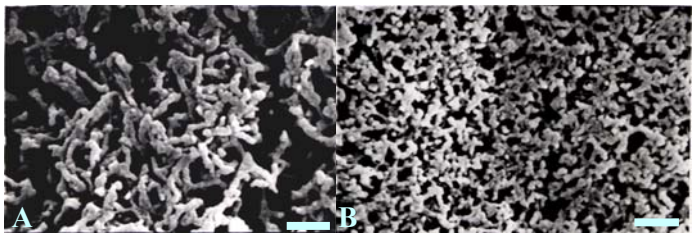


Figure2 Scanning Electron Micrographs of heat-induced PM myosin gels(pH6.0,0.6mol/l KCl) at different concentration PM (A 5mg/ml, B 10mg/ml), Bar length is 1 μ m

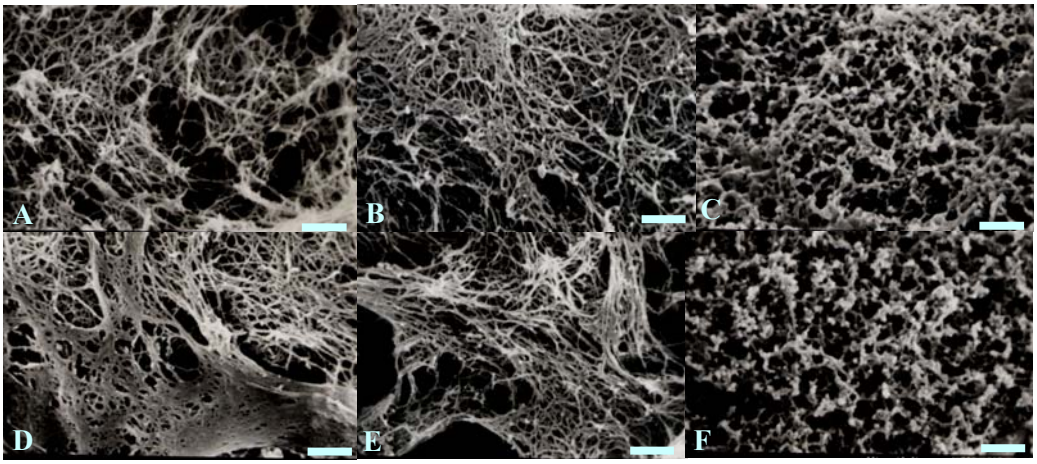


Figure 3 Scanning Electron Micrographs of heat-induced gels of myosin(pH6.0,15mg/ml) at different ionic strength A,B 0.2mol/l KCl PM;C, 0.6mol/l KCl PM; D E 0.2mol/l KCl SMp； F 0.6mol/l KCl SMp. Bar length is 1 μ m

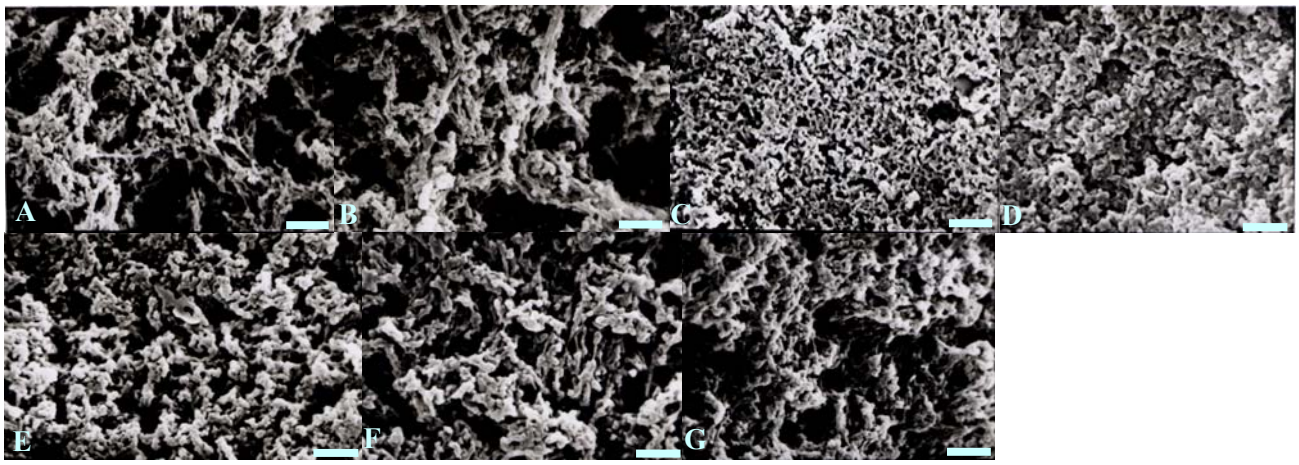


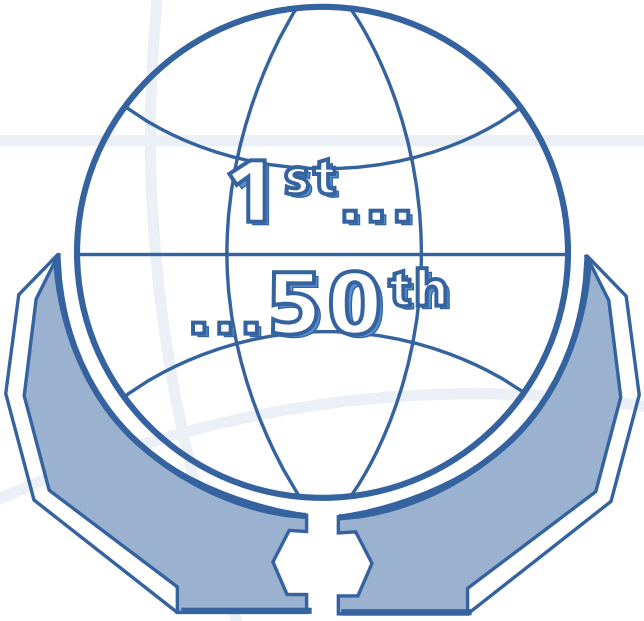
Figure 4 Scanning Electron Micrographs of PM myosin gels(0.6mol/l KCl,15mg/ml) at different pH PM (A pH5.0,B pH5.5,C pH6.0,D pH6.5,E pH7.0,F pH7.5,G pH8.0),Bar length is 1 μ m

50th International Congress of Meat Science and Technology

August 8th – 13th 2004

Helsinki, Finland

Session 5
Meat in nutrition and health



MEAT AS A COMPONENT OF A HEALTHY DIET – ARE THERE ANY RISKS OR BENEFITS IF MEAT IS AVOIDED IN THE DIET?

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Introduction

Meat is frequently associated with a "negative" health image due to a "high" fat content or in cases of red meat as a cancer promoting nutrient. Therefore a low meat intake, especially red meat is recommended to avoid risk for cancer, overweight and at least metabolic syndrome. However, this discussion overlooks the fact, that meat is an important source for a couple of micronutrients such as iron, selenium, vitamins A, B12 and Folic acid. These micronutrients are either not present in plant derived food or have a poor bioavailability. In addition, meat as a protein rich and carbohydrate "low" product contributes to a low glycemic index which is assumed to be "beneficial" with respect to overweight, diabetes development and cancer (insulin resistance hypothesis). Taken together meat is an important nutrient for human health and development. As an essential part of a mixed diet meat ensures adaequate delivery of essential micronutrients and amino acids and is involved in regulatory processes of energy metabolism. The following article will review the role of meat as a micronutrient source, especially for groups at risk for low intake or higher need and the importance of the single micronutrients for maintaining health.

Is there any benefit, if meat is avoided in human nutrition? Does meat promote or prevent cancer?

It is frequently argued that a diet which contains only "traces" of meat or excludes meat completely might be preventive with respect to colon cancer. Based on a couple of studies and with respect to the cancer preventive effects of selected nutrients present in meat, this hypothesis seems more and more unlikely.

Proteins

It has been assumed, based on per capita protein intake and colon cancer risk, that total protein, is related to colon cancer risk (Youngman and Cambell, 1998). However the majority of epidemiological cohort and case control studies could not confirm these assumptions. Only for red meat derived protein there is some evidence that risk increases if red meat is part of the diet twice a day and more (MacIntosh and Le Reu, 2001) or processed (cooked or fried). Whether the non-fat matrix of meat, e.g. the amino acid composition or the amount of heme iron plays a role in carcinogenesis is not really understood. McIntosh and co workers observed a non significant increase from 33% to 59% in incidence of mature rats with DMH-induced intestinal tumours when barbecued beef was substituted for whey protein concentrate against a high fat (20%) diet background (MacIntosh et al., 1998). Whether the concentration of protein in a diet determines the risk for cancer is controversly discussed (MacIntosh and Le Reu, 2001). In contrast the type of protein seems more important with respect to carcinogenesis. A high methionine diet determined by type of protein as well as quantity ingested has been reported to lead to increased circulating insulin which has been

assumed to contribute to colon carcinogenesis (McKeoween-Eyssen 1994). From epidemiological studies there is accumulating evidence that support the insulin resistance (IR) hypothesis to explain the risk for colon cancer. It was hypothesised that IR leads to increased initiation and promotion of colon cancer by an elevated serum insulin as a growth factor or raised glucose and triglycerides as fuels (McKeoween-Eyssen 1994). Bruce and co workers (Bruce et al., 2000) summarise different reasons which strengthen the hypothesis: Colon cancer patients frequently have evidence of glucose intolerance and insulin resistence (IR); cohorts of type 2 diabetes have been found to have an excess mortality due to colon cancer; cohort and case control studies have revealed a clear association of early colon cancer, colon cancer and colonic polyps with increased levels of fasting insulin, triglycerides, VLDL and abdominal obesity; subjects who developed colonic polyps consumed more carbohydrates with a higher glycemic index than controls; recent case control studies have also shown an association between plasma insulin like growth factor I (IGF-I), which is often increased in insulin resistant individuals, and IGF-binding protein (IGF-BP) levels with risk for colon cancer. From a couple of animal experiments there is evidence that IR leads to an increased colon cancer risk.

Diets with a high glycemic index are thought to be associated with or to favour insulin resistance (Frost et al., 1998). Red meat however, have a low glycemic index and may not contribute to the metabolic syndrome as long as its fat/energy content does not contribute primarily to the daily energy intake. Koohestani and co-workers (1997) showed that IR rats on a high fat diet promotes aberrant crypt formation (ACF) as an important step in colon carcinogenesis. Based on their results they conclude that: diets high in energy, saturated fat, and glycemic carbohydrate and low in w3-fatty acids could be deleterious affecting cell signalling in colonic cells in ways that lead to IR and colon cancer. Dietary intervention that reduce IR may also reduce colon cancer risk. So far low fat meat seems not to contribute to colon cancer.

Fat

Several epidemiological early case control and cohort studies suggested a positive correlation between fat intake and incidences of breast-, colon- and prostate cancer (Schottenfeld and Fraumeni, 1996; Potter et al., 1993). However more recent cohort, large case control and pooled analysis of 13 case control studies failed to detect an association between fat intake and colon cancer (Howe et al., 1997; Giovannucci et al., 1994). The relation between dietary fat intake and breast cancer has been examined in a couple of prospective studies. In a pooled analysis no overall association was seen for total fat intake over the range of 15% to > 45% of energy from fat (Hunter et al., 1996). In contrast, among the small number of women consuming less than 15% of energy from fat, breast cancer risk was elevated twofold. With respect to prostate cancer the few existing studies show a relative consistency in supporting an association between consumption of fat containing animal products and cancer incidence. However the majority of the studies were not adjusted for total energy intake. Studies adjusting for energy intake did not find an increased risk (Kolonel, 2001). Meat intake however, correlates more or less with prostate cancer risk. 16 out of 22 case control or cohort studies showed an increased risk above a risk ratio of 1.3. A recent prospective study (Michaud et al., 2001) documented an elevated risk for prostate cancer (RR 1.47) and red meat consumption (>5/week). Up to 2-4 times a week the RR was below 1.0 (0.96) demonstrating that moderate red meat consumption as usually recommended does not contribute to prostate cancer risk.

Inspite the long history of studies on fat and cancer, there remains some controversy. It is more or less generally suggested that animal fat rich in saturated fat is more closely related to cancerogenesis and plant derived mostly unsaturated fat (PUFA) is more protective. In animal models, the tumour promoting effect of fat intake have been observed primarily for PUFA (Hopkins and Carroll, 1979; Hopkins et al., 1981) A couple of studies show that polyunsaturated w6 fatty acids (linoleic acid) enhance cancer development in rodents (Carroll 1991; Fay et al., 1997). The prostaglandin E2 (PGE2) seems to be involved in colon carcinogenesis and is formed from w6 fatty acids (arachidonic acid). EP1 (PGE2-receptor) k.o. mice showed resistance to AOM induction of neoplastic colon lesions (Watanabe et al., 1999). w3 fatty acids (linolenic acid) however, suppress colon carcinogenesis by inhibiting the arachidonic pathway (Takahashi et al., 1997).

The suggestion that consumption of red meat as a source of dietary fat increases risk of colon cancer is based on the rather simple fat-colon cancer hypothesis, which is based on the premise that dietary fat promotes excretion of bile acids which can be converted to carcinogens (Reddy 1981). The controverse results from

different studies and the fact that meta analyses show that fat might be a rather minor component, if even any, in cancerogenesis of the colon or in other cancer sites might be explained in different ways. The fat content of red meat varies in a wide range and shows different patterns. So palmitic acid but not stearic acid present in different amounts in red meat has been shown to be a strong mitogen of adenoma cells in culture (Friedman et al., 1989). Fat, derived from red meat, might be less absorbed, due to either its composition (stearic acid) or due to matrix (muscle) interactions. Polymorphisms of genes involved in the expression of cleavage and reesterification of triglycerides may also play important roles regarding the individual susceptibility. Finally, components not belonging to lipids might contribute to carcinogenesis such as HCAs or at least the iron content of meat. Dietary iron enhances lipid peroxidation in the mouse colon (Younes et al., 1990) and increases the incidence of DMH-induced colorectal tumours in mice and rats (Siegers et al., 1988; Nelson et al., 1989). Indeed, studies in humans point on an relationship between body iron stores and the incidence of colon tumours (Stevens et al., 1988; Knekt et al., 1994a). Finally carcinogens and promotors e.g. HCAs are formed when meat is fried or cooked and may contribute more or less to the individual cancer risk, especially in colorectal, breast and prostate cancer.

Processed meat and genetic polymorphisms

HCAs are converted to their hydroxyamino derivatives by cytochrome P450s especially CYP 1A2 and further activated by esterification enzymes acetyltransferase and sulfotransferase. The reactive ultimate forms produce DNA adducts with guanines at their C8 position, resulting in base substitution and at least mutation. Similarly oxidative modification of the DNA via reactive oxygen species (ROS) results in the formation of 8-oxo-deoxy-guanin with subsequent base substitution and mutation.
Epidemiological studies revealed some positive (Zheng et al., 1998) and some negative (Augustsson et al., 1999) links between cancer risk and intake of welldone meat or fish. Studies that have examined intensity of cooking have tended to show positive associations with breast cancer (Knekt et al., 1994b), others not (Ambrosone et al., 1998). The latter investigated the role of genetic polymorphisms of enzymes involved in DNA adduct formation of HCAs, which could play at least a critical role in individual cancer susceptibility. HCAs require enzymatic activation to bind to DNA and to initiate carcinogenesis. N-acetyltransferase (NAT2) may play a role, its rate determined by a polymorphic gene. The results of Ambrosone and coworkers (Ambrosone et al., 1998) were recently confirmed by studies of Delfino and coworkers (2001) who did not find neither a correlation between NAT2 and breast cancer nor any association between red meat intake for any doneness and breast cancer.

Genetic polymorphisms including environmental aspects (gene environment interactions) may also play a critical role in colorectal cancer with respect to red meat intake. Le Marchand (1999) investigated the colorectal cancer rates in Japanes immigrants in Hawaii. The colorectal cancer incidence of these group (214.000 immigrants between 1886 and 1924) was intially very low and is now the highest in the world. The fast acetylator genotype (NAT2), without the polymorphism is present in 90% of Japanese compared with 45% of Caucasians; the frequency of CYP1A2 phenotype is similar in both groups. Consumption of a welldone meat together with a specific genotype of NAT2 and CYP1A2 may increase colorectal cancer risk substantially. Among the Japanese migrants who ate well done red meat, those without the polymorphisms in both NAT2 and CYP1A2 had a 3.6 times greater risk of developing colon cancer than those with the polymorphisms. Another family of genes might determine individual susceptibility: glutathione transferase M1 (GSTM1) and T1 (GSTT1). Both code for cytosolic enzyme glutathione S-transferase which are involved in phase 2 metabolism especially in polycyclic aromatic hydrocarbon metabolism. The results of the few studies dealing with genetic polymorphisms of GST are inconsistent. Two studies suggest increased colon cancer risk in subjects with high meat intake and GST nonnull genotype, contrary to the underlying hypothesis. One study suggests a strong inverse relation between colorectal adenomas and broccoli consumption, particularly in subjects who are GSTM1 null (review see Cotton et al., 2000). As long as genotypically defined cohorts are not studied with respect to their susceptibility against meat intake (in different forms) the risk of red meat cannot be clearly identified. White meat and fish seem to be without risk, red meat only in a form were cytotoxic by-products such as HCA are formed.

Formation of HCAs can be significantly reduced by inexpensive and practical measures like avoidance of exposure of meat surfaces to flames, usage of aluminium foil to wrap meat before oven roasting and the

employment of microwave cooking. Another protecting approach is the combination with protective bioactive constituents derived from plant food. For example diallyl sulfide an organosulfur compound in garlic, blocks HCA carcinogenesis (Hasegawa et al., 1995; Morie et al., 1999). Nevertheless, even there may be some induction or promotion factors in meat it may depend on the composition of the diet whether anticarcinogenic factors from plants neutralise any harmful factors in meat. In addition meat contains bioactive constituents known to be protective against cancer formation.

Protecting factors in meat with respect to cancer

Folate

Meat is an important source for methyl donors such as folate and vitamin B12 and transfer factors methionine and choline. Folate and methionine as methyl donors influencing methyl group availability may also been associated with colon cancer incidence. It is frequently argued that the increased risk for different types of cancer resulting from low intake of fruits and vegetables is a result of a folate deficient diet, because fruits and vegetables are important sources for folate. Indeed, this is true, however it has to be considered that the bioavailability of folate from meat and liver is much better than from fruits and vegetables. A few studies measured folate directly and it was claimed that a low folate intake has been related to an increased occurrence of colon adenomas (Giovannucci et al., 1993a; Benito et al., 1991) and cancer (Freudenheim et al., 1991; Lashner et al., 1989). Zhang and coworkers (1999) studied the effect of alcohol and folate on breast cancer. The increased cancer risk associated with alcohol consumption (>15g/day) was reduced in women who consumed at least 300µg folate/day. The major source of folate were supplements. Even an intake of 300µg/day does not reach the RDA (400µg/day) this study shows that increasing the intake might be beneficial with respect to cancer. Indeed, increasing folate intake via supplementation, a form which has a very good bioavailability compared t vegetable derived folic acid, decreases risk for colon cancer significantly (Giovannucci et al., 1993b). The decreased risk however was evident not before 15 years, documenting that protective factors, if they indeed exist as single bioactive constituents need to be present in the diet for a long time period. Vice versa there absence or a low intake might also contribute to an increase cancer risk after a long time period. In rodents, diets deficient of methyl donors or transfer factors (folate, B12, methionine, choline) induce tumours at different sites (Shivapurkar and Poirer, 1983; Wainfan et al., 1989; Cravo et al., 1992). A methyl deficient diet lowers the concentration of the methyl donor S-adenosylmethionine which leads to a reduction in methylation of DNA cytosine. Tumour supressor genes are inactivated by methylation of normally unmethylated sites. DNA hypomethylation due to a diet low in methyl donors (e.g. low in meat) may contribute to a loss of protooncogene expression (Nyce et al., 1983). Indeed, throughout the different stages of colonic neoplastic transformation genomic hypomethylation (Goelz et al., 1985) methylation of usually unmethylated sites (Makos et al., 1992) and abnormal elevated DNA methyl transferase activity (Issa et al., 1993) is described.

An additional aspect, also involved in methylation reactions, which might contribute to the individual colon cancer risk is a genetic polymorphism of a key enzyme of folate metabolism: the Methylenetetrahydrofolate-reductase (MTHFR). This encyme converts 5,10- methylenetetrahydrofolate to 5- methyltetrahydrofolate, the major circulatory form of folate in the body and primary methyl donor for the methylation of homocysteine to methionine. This pathway is a critical key in the methylation process of the DNA. As described above alterations in the methylation process can result in abnormal expression of oncogenes and tumour suppressor genes (Baylin et al., 1991). The polymorphism of the human MTHFR gene (alanine to valine substitution, coding for a thermolabile enzyme with reduced activity) results in elevated plasma homocysteine levels. Homozygous individuals have 30% normal enzyme activity, heterozygous 65%. Up to now there are controverse results in correlating this polymorphism with individual colon cancer risk. However, supplementation of folate or a diet rich in folate with optimum bioavailability lower homocystein and might therefore influence the individual risk (Bronstrup et al., 1998)

Vitamin A

The German society for nutrition recommends the increase in vitamin A intake for pregnant women by 40% and for breastfeeding women by 90%. Pregnant women or those who want to become pregnant are asked to avoid the intake of liver out of very unsure scientifically proved reasons, therefore the provitamin A-carotenoid ß-carotene stays the essential vitamin A source. The most important bearers of vitamin A are

orange and deep green vegetable followed by enriched juices, which represent between 20 and max. 40% of the daily supply. In Germany the intake in the mean is by about 1.5 and 2 mg of ß-carotene a day. If one supposes a conversion rate for ß-carotene for juices of 4:1, for fruit and vegetable of 12:1 up to 26:1, therefore this supply leads to a vitamin A sufficiency of 10 to 15%. Because the liver consumption of the population per head and per year amounts less than 500 g, ß-carotene is an important vitamin A source for young women and especially pregnant women and breastfeeding women.

Studies from Great Britain showed that in the middle and working class the vitamin A supply can be seen as insufficient. The American association for paediatrics called vitamin A as one of the most critical vitamins during pregnancy and the breastfeeding period, especially in terms of the lung function and its maturation. If the vitamin A supply of the mother is too low, the supply of the fetus is too low too and also the values of the mother's milk which can't be compensated by postnatal supplementation. At the same time one has to keep in mind that there is a relationship between folic acid, vitamin A and iron status and low birth weight. This applies especially to earlyborn's which show a direct correlation between the vitamin A supply and the turn up of complications like the respiratory arrest syndrom, as one of the most frequent and serious complications.

The major source of vitamin A is liver, which contributes to approximately 75% to the human vitamin A intake. Concerning a sufficient vitamin A supply, the provitamin A, ß-carotene is of minor importance. Its cleavage efficacy seems to be nearer to 1:12 and not 1:6 as frequently mentioned. Beside its well known effects in the visual cycle vitamin A plays an important role especially in mucous membranes.

Is a sufficient supply of vitamin A involved in the individual lung cancer risk? On the basis of a few reports it is assumed that a „local" vitamin-A-deficiency exists in meta- and dysplastic-areas. Measurements of vitamin-A concentrations in metaplastic areas of the respiratory epithelium and the cervix epithelium actually proved that vitamin A, in contrast to the surrounding healthy tissues, was no longer to be found.

At the moment it is difficult to distinguish between cause and effect. Studies carried-out by Edes and co-workers (Edes, 1991) hint to an induction of metaplasia caused by a vitamin-A-deficit. These studies showed that a depletion of vitamin-A-ester stores in different tissues (Edes, 1991) is caused by toxins, that are present in cigarette-smoke (predominantly polyhalogenated compounds).

Epidemiological evidence supports the assumption that the development of obstructive respiratory diseases plays an important role in the scope of cancer mortality of smokers. It was shown that the relative risk for smokers to be affected by lung cancer, when they suffered from obstructive ventilation disorder (FEV 1% < 60 (Melvyn et al., 1987) respectively 70) (Skillud et al., 1987), was significantly higher than that of comparative groups with normal lung-function-parameters.

A survey about the dietary habits within the scope of the „National Health and Nutritional Examination Survey" showed that an inverse correlation (Morabia et al., 1989) exists between obstructive respiratory diseases (COPD) and vitamin-A-supply as the only one of 12 examined dietary components. COPD however, increases lung cancer risk significantly. If a diminished supply of vitamin A increases the appearance of obstructive respiratory diseases, a marginal or local vitamin-A-deficit could be responsible for the observed changes of the respiratory mucosa. Such a deficit results in a loss of cilia, an increase of secreting cells and finally the formation of squamous metaplasia (Stofft et al., 1992; Chytil, 1985; Biesalski et al., 1985).

Such changes (decrease of ciliated cells with simultaneous increase of the secretion) are noted for smokers (Gouveia et al., 1982; Mathe et al., 1983) and cause a reduction of the mucociliary-clearance. This reduction of the mucociliary-clearance, associated with an increased adsorption of the respiratory syncytial virus (RSV) (Donelly, 1996), could explain the extraordinarily high morbidity and mortality for respiratory infections of children with vitamin-A-deficiency in developing countries (Sommer, 1993).

There is sound evidence from experimental studies that the alteration of the respiratory mucosa, caused by the vitamin-A-deficiency, can be redifferentiated into its functional original epithelium, in vivo as well as in vitro, following vitamin-A-supply (Biesalski et al., 1985; McDowell et al., 1984a,b, 1987a,b; Rutten et al.,

1988 a,b). Squamous metaplasia of the bronchial mucosa, which occurs in smokers in spite of a sufficient supply with vitamin A as an effect of inhalative noxae could also be reversed through systemic application of high retinoid-concentrations in vitro (Lasnitzky and Bollag, 1982; 1987) and in humans in vivo (Gouveia et al., 1982; Mathe et al., 1983).

Selenium

Selenium is found largely in grains fish and meats and enters the food chain through plants at geographically variable rates dependent on selenium concentration of the soil. The best known biochemical role for selenium is as part of the active site of the enzyme glutathione peroxidase (GPx). The metabolic function of this enzyme are vital for cells, as it is part of a mechanism responsible for the metabolism and detoxification of oxygen. It is assumed that GPx can protect the DNA from oxidative damage and consequently from mutation leading to neoplastic transformation of cells (Combs and Clark, 1985). At relatively high levels selenium protects against the action of certain carcinogens in various animal models (Halliwell and Gutteridge, 1989). In in vitro and in vivo studies, organic and inorganic selenium has been demonstrated to inhibit proliferation of normal and malignant cells and inhibit tumor growth (Griffin, 1982; Redman et al., 1997). Apoptosis may result from competition of selenium for s-adenosyl-methionine with ornithine decarboxylase (ODC). ODC acitivity is indeed critically involved in cancerogenesis. From geographical studies it is documented that in areas with sufficient selenium concentrations in the diet (depending on selenium concentrations of the soil) there is an inverse relationship between selenium status and cancer (Schrauzer et al., 1977; Clark et al., 1991). Epidemiological studies showed inverse associations of selenium intake or plasma levels and cancers of different sites (prostate, colon, skin etc.). In a recent double blind placebo controlled cancer prevention trial 200µg selenium (approx. 3 time the RDA) were given daily to patients with histories of basal and squamous skin carcinoma (Clark et al., 1996). Selenium supplementation did not influence the primary endpoint prevention of recurrent skin cancers, but was surprisingly inversely associated with the incidence of and mortality from total prostate, lung and colorectal cancers. Recently Yoshizawa and co workers (1998) reported a strong inverse association of toenail concentration of selenium and prostate cancer risk (65% reduced risk in the highest quintile). Toenail concentration reflect long term intake of selenium with the diet and is consequently influenced by bioavailability. From the intervention trial and from epidemiological studies there is now evidence indicating "that substantial increases in the consumption of selenium by men taking 80-90µg/day or more may have striking impact on prostate cancer rates" (Giovannucci, 1998). Recent surveys indicate that average intake of selenium may be as low as 30-40µg/day (Rayman, 1997). Intake data however do not really reflect the bioavailability. Consequently the diet has a strong influence on total selenium supply of tissues. Especially in areas with low soil selenium dietary sources containing substantial amounts of selenium with good bioavailability should be recommended. In the US Selenium is mainly supplied by cereals, breads, meats and meat products. Beef alone is estimated to contribute approximately 17% of the total selenium in the American diet. Two recent studies in humans showed that meat was as good a source of selenium, as is wheat (van der Torre et al., 1991) and that SeMet was absorbed more rapidly than selenite in selenium deficient men (Xia et al., 1992). In a recent study the bioavailability of selenium was estimated from various portions of fully cooked commercial cuts of beef, including liver, striploin, round, shoulder and brisket in rats (Shi and Spallholz, 1994). The bioavailability from the beef diets was compared with that of selenium as selenite or L-selenomethionine (SeMet). Liver GPx recovery (after depletion), muscle tissue depostion and plasma levels were taken as markers of bioavailability. Liver GPx-recovery was highest from SeMet > beef muscle > selenite = beef liver. Muscle deposition was highest from SeMet > beef muscle > selenite = beef liver. From this results the authors concluded that the bioavailability of selenium from beef is higher than or at least equal to that of selenite and slightly lower than that of SeMet. Taken together meat is an important source for bioavailable selenium.

Zinc

Zinc is a component of a couple of metalloenzymes and is important for cell growth and replication, osteogenesis and immunity. It may further contribute to the overall antioxidative defence. The primary dietary sources of zinc are red meat, sea food, poultry, grains, dairy, legumes and vegetables (Groff and Grooper, 2000). Lower zinc levels were described in cancer patients (without answering the question of cause or consequence) (Mellow et al., 1983; Rogers et al., 1993); others did not find this association (Kok et al., 1988; Kabuto et al., 1994). There is good evidence that zinc may critically contribute to prostate cancer incidence. Total zinc levels in the prostate are 10 times higher than in other soft tissues (Mawson and Fischer

1952). Uptake of zinc via a membrane transporter into prostatic epithelial cells is under the control of hormones (testosterone, prolactin) (Costello et al., 1999). Physiologic concentrations of zinc inhibit growth of androgen sensitive and androgen-independent prostate cancer cell lines via cell cycle arrest, apoptosis and necrosis (Iguchi et al., 1998; Liang et al., 1999). Epidemiological findings are not consistent and a few studies estimating the effect of supplementation on prostate cancer risk are more or less controversial (for review see Platz an Helzlsouer, 2001). One important reason for this inconsistency might be the high variability of zinc content of different sources, especially meat and sea food. Furthermore zinc has a much better bioavailability from meat than from vegetables (Groff and Groopper, 2000) and other factors, present in the diet may increase (citric acid, histidine, cystein) or decrease (phytate, oxalate) absorption of zinc (Groff and Groopper, 2000). Consequently, the use of food questionnaires might not be a sufficient approach to measure zinc intake. Adequate biomarkers, at present not available, may help to estimate the individual zinc status and consequently the individual risk. At present there is no clear cut evidence for a preventive effect of zinc on prostate cancer from epidemiological studies. Some small case control studies indicate low plasma zinc or low prostatic zinc levels in patients with prostate cancer compared with healthy controls (for review see Platz an Helzlsouer, 2001). An optimum zinc intake can be recommended not only with respect to prevention and intakes below RDA should be avoided. One factor contributing to low intake might be a decline in red meat consumption which has been reported in New Zealand (Laugesen and Swimburn, 2000), as well as in UK (Whitehead, 1995), USA (Popkin, 1989) and Canada (Zafiriou, 1985), concomitant with an increase in intakes of unrefined cereals, nuts and legumes. Read meat is a rich source of readily available zinc, whereas cereals contain different levels of phytic acid, a potent inhibitor of zinc absorption. Indeed, the recommendation to decrease or even avoid meat intake may result in a low zinc status as recently documented in women from New Zealand (Gibson et al., 2001). In a cross sectional study of 330 women the authors assessed the interrelationship among dietary intakes, biochemical zinc status and anthropometric indices. Changes in food selection patterns (reduction of red meat) were suggested to be responsible for a lower biochemical zinc nutriture. This study shows exemplary that a mixed and balanced diet including meat and meat products is the best way to ensure sufficient intake of all essential and potentially cancer preventive components.

Conclusion:
Meat consumption, especially red meat, is not per se carcinogenic, even it contains components which, based on epidemiological and animal experiments, are assumed to contribute to cancer formation. On the other hand lot of studies exist which demonstrate a reduced cancer risk in persons with a high intake of fruit and vegetables. As a basis for the preventive effect protecting factors such as carotenoids, flavonoids and further phytochemicals are discussed. Within these fruit and vegetables derived protecting factors folic acid, selenium, zinc and other components are claimed to be preventive candidates. Why should theses compounds less effective if they reach the body via meat as an important source? The balance of promoting and protecting factors within the diet is important for the protection against cancer. Furthermore the IR-hypothesis shows that a nutritional behaviour leading to a metabolic syndrome (high energy, high glycemic carbohydrates) might favour colon cancer or even cancers from other sites. The “goals for nutrition in the year 2000” (Willett, 2000) give a very good and comprehensive advice: “ Current nutritional recommendations for the prevention of cancer include increased consumption of fruits and vegetables; reduced consumption of red meat and animal fat; and avoidance of excessive alcohol. For many individuals a daily multivitamin that contains folic acid may also be part of a reasonable cancer prevention strategy.”
To improve the “preventive capacity” of an individual a balanced diet, rich in fruits and vegetables including meat and meat products in moderate quantities, normal body weight and a reasonable amount of exercise is the best choice.

The importance of meat as a source for micronutrients

The reason for the importance of meat as an essential source for a couple of micronutrients is due the the fact that meat is either the only source or has a much higher bioavailability.

Two important micronutrients occur only in meat: The vitamin A and B12. Both cannot be compensated by plant derived provitamins. A B12 provitamin does not exist and the provitamin A, ß-carotene, has to be taken in high amounts due to a poor conversion rate (1:12).

With respect to iron, iron has a higher bioavailability when derived from meat as hem iron than plant derived iron. Similarly folic acid has a nearly ten-fold higher bioavailability from meat (especially liver) and eggs than from vegetables. Consequently a low or near to cero intake of meat (including liver) is associated with a risk for deficiencies in selected micronutrients.

Who needs meat – or: are there groups at risk for a low intake of meat derived micronutrients?

Elderly people are generally considered at risk to develop vitamin and trace element deficiencies, especially regarding the vitamins A, D, E, and folate as well as iron and calcium (Martins, Dantas, Guimar, Amorin, 2002; Bates et al., 2002; Anderson, 2001; Viteri & Gonzalez, 2002). The multifactorial cause of this health hazard comprise quantitative and qualitative decreased food intake, reduced energy expenditure due to sedentary life style and loss of metabolic active body cell mass, and the development of chronic age-associated disorders.

It was generally assumed that impaired bioavailability of micronutrients was a common problem among elderly. However, the digestive and absorptive capacity of the digestive tract is well retained through ageing and if malabsorption of macronutrients occurs it is the result of a disease rather than ageing itself (Black, 2001). An exception to this rule is the impaired bioavailability of dietary iron due to gastric mucosal atrophy which occurrence is age-related and in many cases could be regarded as a disorder rather than a disease. Nevertheless, the frequently occurring atrophic gastritis in elderly, which also affects vitamin B12 absorption, should be one reason to recommend meat intake in this risk groups.

Adequate nutrition during pregnancy plays an important role in the well-being of mother and child and influences health of the offspring during childhood and adulthood. Nutritional requirements are increased during pregnancy, but will generally be met by dietary intake and adaptive physiological changes (Draper, Lewis, Malhotra & Wheeler, 1993). However, the micronutrient status of vitamin D, folic acid, iron, and zinc may become compromised without supplementation (Draper, Lewis, Malhotra & Wheeler, 1993; Fogelholm, 1999; Saletti, Lindgren, Johansson & Cederholm, 2000) especially when meat is avoided, which can be frequently seen in women in the child bearing age. Folic acid supplementation is generally recommended to decrease the risk of serious births defects. Especially in multifarious women the essential fatty acid status may become impaired and negatively affects the neurological and cognitive development of the offspring (Lowik, van den Berg, Schrijver, Odink, Wedel & Houten, 1992; Reynolds, 2002).

The micronutrient intake of vegans is easily imbalanced with respect to the recommended dietary allowances. Especially the intake of vitamin B12, riboflavin, and selenium often is inadequate. Even the use of dietary supplements often does not meet the recommended intake of vitamin B12 and selenium (Boelsma, Hendriks & Roza, 2001; Andersson et al., 1986).

Although it seems obvious that subjects on a weight reduction diet are at risk to develop micronutrient deficiencies, the scientific evidence for this is weak. Most reports include less than fifty subjects and deal with the combination of energy restriction and increase of physical activity, and hence are difficult to interpret (Philipsen-Geerling & Brummer, 2000). The micronutrients at risk appear to be iron, magnesium, zinc, fat soluble vitamins and essential fatty acids. Although a nutritionally adequate low-fat diet seems feasible in a motivated, free-living population (Gassull & Cabre, 2001), data exist which show that a high protein intake even when associated with a higher fat intake might favor weight loss.

A recent meta-analysis of studies dealing with dietary weight loss show that protein rich diets low in carbohydrates and a moderate or high fat content resulted in a better weight loss than diets low in fat and protein and rich in carbohydrates (Bravata et al., 2003). Several factors are discussed to contribute to this increased weight loss: better satiety, higher energy expenditure (thermogenesis), greater loss of fat cell mass.

Energy restricted diets and traditional cholesterol lowering diets are typically focussed on the reduction of total fat intake while simultaneously increasing the proportion of polyunsaturated fatty acids (PUFA's). However these PUFA's usually contain high concentrations of n-6 fatty acids and low amounts of n-3 fatty acids. This may have an adverse effect not only on reducing the risk for obesity-related metabolic disorders such as vascular disease and heart rhythm disturbances, but also on the regulation of the intermediary metabolism of brain monoamines as serotonin, which may lead to impaired mood status and depression (Alonso-Aperte & Varela-Moreiras, 2000).

It is well known that malnutrition is far more common among institutionalized and chronic hospitalized elderly compared to free-living subjects in the community and that the prevalence of malnutrition is associated with the severity of morbidity, functional impairments and mental state (Bates, Mansoor, van der

Pols, Prentice, Cole & Finch, 1997; Selhub, Jacques, Bostom, Wilsin & Rosenberg, 2000; Rasmussen, Ovesun, Bulow, Kudesn, Laurberg & Perrild, 2000; Koehler, Baumgarter, Garry, Allen Stabler & Rimm, 2001). The deficiency effects a broad spectrum of micronutrients, such as the B vitamins, especially B1, B6, folate and B12, vitamin C, vitamin D and E, essential fatty acids and selenium (Brubacher, Moser & Jordan, 2000; Bates, Prentice et al., 1999). Thiamine and folate status need special attention in this respect as a deficiency of these nutrients is associated with depression and impaired cognition and dementia (Block, Norkus, Hudes, Mandel & Helzlsouer, 2001; Report on Health and Social Subjects No. 49, 1998). Intervention trials with micronutrient supplementation consisting of zinc and selenium, vitamin C, beta-carotene and alpha-tocopherol were associated with a reduction of infectious events, probably due to the micronutrients administration rather than the supplementation of vitamins (Gey, Brubacher & Staehelin, 1987; Gey, 1993).

Chronic use of drugs may lead to micronutrient deficiency by decreasing food intake mainly due to impaired appetite or upper GI motility, by decreasing the bioavailability of micronutrients, for example cholestyramine which impaires the absorption of fatty acids and fat-soluble vitamins, or by interfering with metabolism (Gregory, Foster, Tyler & Wiseman, 1990). A number of micronutrients, such as zinc and magnesium, play a role in phase I oxidation reactions involved in drug metabolism. Typical examples are the increased folate requirements with chronic use of sulphasalazine, methotrexate, or valproic acid. However, a relevant interaction between drugs and specific micronutrients only occur in case of prolonged use of specific drugs in high doses in susceptible subjects.

Selected meat derived micronutrients

Meat is an excellence source for different micronutrients: Low fat pork meat contains 1.8mg iron, 2.6mg zinc, pig liver contains 360mg magnesium, 20mg iron and 60µg selenium per 100g. Meat and liver (100g/day) can cover up to 50% of the RDA for iron, zinc, selenium, Vitamins B12, B1, B2, B6 and 100% of vitamin A.

Vitamin A
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Studies from Great Britain showed that in the middle and working class the vitamin A supply can be seen as insufficient. The American association for paediatrics called vitamin A as one of the most critical vitamins during pregnancy and the breastfeeding period, especially in terms of the lung function and its maturation. If the vitamin A supply of the mother is too low, the supply of the fetus is too low too and also the values of the mother's milk which can't be compensated by postnatal supplementation. At the same time one has to keep in mind that there is a relationship between folic acid, vitamin A and iron status and low birth weight. This applies especially to earlyborn's which show a direct correlation between the vitamin A supply and the turn up of complications like the respiratory arrest syndrom, as one of the most frequent and serious complications.

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Vitamin A is essential for growth and development of cells and tissues. In its active form, retinoic acid, it controls the regular differentiation as a ligand for retinoic acid receptors (RAR, RXR) and is involved in the integration (gap junction formation) of cell formations (Kurokowa et al., 1994; Morree, 1992). Vitamin A plays a substantial role, especially in the respiratory epithelium and the lung. During moderate vitamin-A-deficiency, the incidence for diseases of the respiratory tract is considerably increased and repeated respiratory infections can be influenced therapeutically by a moderate vitamin-A-supplementation (Pinnock et al., 1986; Sommer, 1993; West et al., 1991). In addition to the importance of the vitamin for the lung function, vitamin A is also responsible for the development of many tissues and cells as well as for the embryonic lung development. Recent studies proved that the control occurs by different expressions of retinoid receptors as well as by time-dependent changes of the vitamin A-metabolism respectively via cellular vitamin A-binding proteins (CRBP: cytoplasmatic retinol binding protein; CRABP: cytoplasmatic retinoic acid binding protein).

The influence of vitamin A for the maturation and differentiation of the lung
The alveolar cells of type II are especially prepared to synthesize and secrete the surfactant (Zachman, 1989). Retinoic acid (RA) is able to stop, concentration-dependently (Metzler and Snyder, 1993) the expression of the surfactant-protein A (SP-A) in human fetal lung explants. Insulin, TGF-ß and high concentrations of glucocorticoids can also down-regulate the SP-A-mRNA-expression (Weaver and Whitsett, 1991) , but lower concentrations of glucocorticoids are stimulating the expression of these genes (Odom et al., 1988). In contrast, the SP-B-mRNA-expression is increased in human fetal lung explants both by hyperoxia (rats) (Metzler and Snyder, 1993) and by dexamethason (human fetal lung explant) . Consequently, the formation of some surfactant-proteins is regulated differently and selectively by RA together with glucocorticoids.

Prostaglandins of type PGE2 are able to increase the surfactant-synthesis. Under the influence of EGF (epidermal growth factor) the formation of prostaglandin rises, especially of PGE2. On the other hand, the expression of the EGF-receptor is increased by RA. EGF increases the proliferation of the lung tissues and this leads to an amplified formation of surfactant phospholipids (Sundell et al., 1980). RA as well as EGF are both leading to an increase (40%, 80%) of the PGE2-secretion in fetal lung cells of the rat in vitro (Haigh et al., 1989). The combination of RA and EGF though leads to a more than a six-fold increase of the PGE2-secretion. Consequently, RA can interfere in the lung development by its modulating effect on the EGF-expression and the subsequent PGE2-induced surfactant formation. A sufficient and continuous availability (either on the blood pathway or by local storage sides) is pivotal, especially for a time-dependent regulation of the lung-development and the related formation of the active metabolite retinoic acid.

Vitamin-A kinetic during fetal lung development
In fibroblast-like cells close to the alveolar cells, in type-II-cells as well as in the respiratory epithelium retinyl-esters, as local extrahepatic stores are present. The importance of these retinyl-esters as „acute reserve“ during the development of the lung becomes apparent during the late phase of gestation and the beginning of lung maturation. During this period a rapid emptying of the retinyl-ester storage’s in the lung of rat embryos occurs (Geevarghese and Chytil, 1994). This depletion is the result of an increased demand in the process of the lung development, because the retinoic acid is „instantly“ needed for the process of cellular differentiation (e.g. proximalization) and metabolic work (surfactant).

The prenatal lung development is also influenced by glucocorticoids. The steroid hormones have a similar effect on lung development as vitamin A resp. the two factors complement each other. This is not surprising, because the receptors for steroids and retinoids belong the same multireceptor-complex. The mode of action of glucocorticoids does not only come into action on the level of gene-expression, but seems to have an impact in a much earlier phase of the vitamin release. The application of dexamethason leads to an increase of the maternal and fetal retinol-binding protein. Thus, the vitamin A-supply is improved via the regular hepatic export pathway. Such an increase of the vitamin-A-concentration in the systemic circulation diminishes obviously the morbidity and mortality of prematures due to bronchopulmonal dysplasia (Shenai et al., 1987; 1990). Dexamethason respectively glucocorticoids are not only leading to an improvement of the total vitamin-A-supply through a change of the release from the liver, but they also influence, as recently described (Geevarghese and Chytil, 1994), the metabolization of the vitamin-A-esters, which are stored in the lung. After administration of dexamethason, as well as after administration of steroids, a significant reduction of retinyl-esters in the maturing lung can be detected, together with a moderate increase of retinol,

the hydrolyzation product of retinyl-ester. This observation may explain the therapeutical success with steroids respectively also their failures during the therapy of lung-distress-syndrome of prematures. As far as an insufficient supply is concerned, inappropriate retinyl-ester stores, caused by a shortage of supply of the fetal lung during the late pregnancy, the regulatory effect of glucocorticoids for the vitamin-A-metabolism of the lung cells cannot take place.

Very low plasma-vitamin-A-levels (Shenai et al., 1981) are recurrently found in prematures, especially in cases with lung-distress-syndrome. This can, amongst other things, be attributed to the relative immaturity of the liver for the synthesis of retinol-binding proteins. The neonate is almost exclusively dependent on the mother in its supply, this includes the lung retinyl-esters which are either absorbed by the cells directly (from chylomicrons) or by esterification of retinol after uptake into the cells. These lung retinyl-ester stores can only be sufficiently filled if the mother guarantees an appropriate vitamin-A supply especially during the late pregnancy.

The influence of an insufficient vitamin-A-supply on the post-natal development of the lung
A disease seen recurrently in connection with vitamin-A-supply is the bronchopulmonary dysplasia (BDP). The pathogenesis of BDP certainly depends on a multitude of factors. Some of the observed morphological changes are very similar to the ones seen in vitamin-A-deficiency of humans and animals. In particular, there is focal loss of ciliated cells with keratinizing metaplasia and necrosis of the bronchial mucosa as well as an increase of mucous secreting cells (Stahlmann, 1984; Stofft et al., 1992).

Especially focal keratinizing metaplasia, as it may occur after a vitamin-A-deficiency, is strengthening the assumption of an impairment of the differentiation on the level of the gene-expression. Since vitamin A regulates the expression of different cytokeratins and therefore influences the terminal differentiation, it seems obvious to suppose common mechanisms. Consequently, the premature but especially the neonate are dependent on a sufficient supply with vitamin A, to ensure the regulation of the cellular differentiation of the respiratory epithelium and lung epithelium. The earlier a child is born before due date, the lower its serum-retinol-levels are (Mupanemunda et al., 1994). Since a further decrease of the serum-retinol-level and RBP-level occur postnatally, the plasma value at the time of birth, is considered to be a critical parameter regarding the lung development.

Repeatedly it was shown that serum-retinol-level and RBP-level in prematures are significantly lower than in neonates (Shah and Rajalekshmi, 1984). In the liver of prematures significantly lower retinol levels can be found in comparison to neonates (Shenai et al., 1985). Plasma values lower than 20 µg/dl are not rare in this case and they should be taken as an indicator of a relative vitamin-A-deficit.

Reduced plasma levels during the first months of life have got a considerable influence on the overall development as well as on the susceptibility of infants to infections. With reduced retinol-plasma-levels repeated infections are more often described (Barretto et al., 1994; Filteau et al., 1993; Pinnock et al., 1986) and they are counted among the main complications of a poor vitamin-A-supply in developing countries. In addition, the serum vitamin-A level during infectious diseases, particularly of the respiratory tract, continues to drop (Neuzil et al., 1994). On the one hand, this can be explained with an increased metabolic demand and on the other hand with an increased renal elimination of retinol and of RBP during acute infections (Stephensen et al., 1994).

Conclusion:
The discussion whether liver as a component of a healthy diet should be avoided is primarily based on questionable contaminants suspected in the liver (e.g. hormones, xenobiotics, metals etc.). If ß-carotene arriving from vegetables would be the only source of vitamin A we must eat more than 500g mixed and ß-carotene rich vegetables per day to reach the recommendations of 1mg retinol. Concerning contaminants it has not been evaluated whether this amount of vegetables contains more contaminants than a portion of liver. A small portion of liver (100g) twice a month is neither toxic nor teratogenic and contributes to a sufficient supply of the body with vitamin A.

Iron

Iron supports oxidative metabolism. It is essential for gas exchange at the tissue and cellular levels through hemoglobin oxygenation in red cells and myoglobin in skeletal muscle (Beard, Dawson & Pinero, 1996). Moreover, iron-containing enzymes are involved in cellular energy metabolism and in host-defense responses (Beard, Dawson & Pinero, 1996; Griffiths, 1996). These various roles are due to iron's biological catalytic activity. Like many other transition elements, it posses unfilled atomic orbitals that allow it to co-ordinate electron donors and participate in redox processes (Griffiths, 1996; Fraga & Oteiz, 2002).

Iron is one of the most abundant elements in the Earth's crust, paradoxically, iron deficiency is the most common and widespread nutritional disorder in the world (DeMaeyer & Adiels-Tegman, 1985). Due to biological losses such as cyclical monthly bleeding of fertile-aged women, excessive infestation with blood-feeding parasites, or poor bioavailability of iron from plant-based diets, it is estimated that as many as 4-5 billion people, 66-80% of the world's population, may be iron deficient (DeMaeyer & Adiels-Tegman, 1985; World Health Organization, 1992). At any given time, 2 billion people – over 30% of the world's population – are anemic, mainly due to iron deficiency, and in developing countries, frequently exacerbated by malaria and worm infections (World Health Organization, 1992).

Iron deficiency is a particular risk for women and girls of child-bearing age, because of menstrual losses. In a recent Irish food consumption survey, almost half of women aged 18-50 year had inadequate iron intakes when compared with national average requirements [62]. In the British National Diet and Nutrition Survey, iron intakes were found to be low in girls (aged 7-18 years), with iron intakes decreasing with age. Adolescent females (15-18 year) were found to have extremely low intakes of iron when compared with UK dietary reference values. Dietary supplements made no difference to mean intakes and iron status was also low in these groups. Depending on the composition of the individual diet the bioavailability of iron can differ 5 to 10-fold (lit 8 und 9 aus fleisch und gesundheit). The different bioavailability depends on the presence or absence of different ligands (phytates from cereal products, tannins from coffee and tea and oxalates from vegetables) which form complexes with iron and zinc and block their absorption. A diet which is primarily composed out of vegetables, rice, beans and corn is associated with a poor iron bioavailability which at least explains the high incidence of anemia in developing countries. 100g pork meat added to the above described vegetarian diet increases the iron absorption 3.6 fold.

Folic acid

In European countries the average folate intake in adults was found to be remarkably similar, around 300 µg/day in adult males and 250 µg/day in adult women (De Bree, van Dusseldorp, Brouwer, van het Hof & Stegers-Theunissen, 1997). This is about the recommended intake level, but lower than recommended for pregnant women and women with pregnancy wish. For these groups an intake of >400 µg/day is considered protective against neural tube defects. More than 90% of women in the childbearing age have dietary folate intakes below this optimal level. However, it must be realised that it is difficult to assess dietary folate intake because databases often do not have complete and updated information for folate content of many foods.

The link between poor folic acid status and neural tube defects is well documented but poorly characterized. Poor status is also linked to raised plasma homocysteine, a risk indicator for cardiovascular diseases and poor status may also increase the risk of neurological disorders and cancers. Supplementation with folate (100% bioavailability) reduces risk for neural tube defects up to 70%. Regarding the bioavalability, liver might be also a good source.

Vitamin B12

Vitamin B12 is found only in animal products. In a recent UK study of 250 vegetarian and 250 vegan men, approximately one quarter of vegetarians and more than half of vegans had sub-optimal intakes of vitamin B12. Plasma vitamin B12 levels were low in the vegetarians and extremely low in the vegan group, with more than a quarter below the threshold level where neurological signs may develop (130 ng/L) (Lloyd-Wright, Allen, Key & Sanders, 2001). The elderly are also at risk of vitamin B12 deficiency, due to physiological changes resulting in reduced absorption. In the UK, vitamin B12 status in some people aged 65 and over was inadequate. There was no difference in mean levels between men and women. However, vitamin B12 intakes were adequate when compared with UK dietary reference values (Finch et al., 1998).

Selenium
Selenium is often considered as belonging to the group of antioxidant nutrients, since it is incorporated into the enzyme glutathion peroxidase, which acts as a cellular protector against free radical oxidative damage. A secondary end-point analysis of a randomised placebo-controlled skin cancer prevention trial suggested that supplemental selenium might reduce the incidence of and mortality from cancers at several sites (Clark et al., 1996). However, the efficacy of selenium as a cancer preventive agent should await the results of large on-going controlled studies. Selenium is, like many other nutrients, necessary for a well-functioning immune system, and has been pointed out as particularly efficacious in HIV and AIDS. A systematic review found no evidence for a clinical relevant function of selenium in that respect (Ozsoy & Ernst, 1999).

Although selenium is widely distributed in the environment, the selenium content of foods is greatly affected by soil on which crops grow or animals graze. Recent evidence suggests that selenium intakes in most parts of Europe are falling and are low when compared with recommended intakes (Rayman, 1997; Rayman, 2000). Declining intakes in the last three decades have been attributed mainly to a change in the source of wheat for bread and cereal products, from predominantly North American to European origin (from a high to low selenium content). These are reflected in decreasing plasma or serum selenium levels. Due to its antioxidant effects, selenium may be protective against chronic degenerative diseases. In the UK, selenium intakes were low in the majority of the elderly (aged 65 and over) in the British National Diet and Nutrition Survey when compared with UK dietary reference values (Thane & Bates, 2001). Selenium intakes decreased with increasing age in this population subgroup. Sufficient zinc intake is important for the proper function of the immune system.

Zinc
Zinc deficient individuals demonstrate slower wound healing and are more prone to infections. However, studies of the effect of zinc supplementation aimed at the healing rate of venous leg ulcers have been inconclusive. A Cochrane review concluded that oral zinc did not appear to aid the healing of leg ulcers, and that there was only weak evidence that zinc was of benefit in patients with venous leg ulcers and low serum zinc (Wilkinson & Hawke, 2002). Zinc has been found to inhibit rhinovirus replication in vitro. Some studies have demonstrated that zinc may beneficially affect cold symptoms; however a meta-analysis of randomised controlled trials concluded that the evidence for the effectiveness of zinc in reducing the duration of common cold symptoms is lacking (Jackson, Lesho & Peterson, 2000). Finally, in settings with high rates of stunting and low plasma zinc concentrations, zinc supplementation may improve children's growth (Brown, Peerson & Allen, 1998). So far studies with supplementation did not reveal consistent results. However, a low intake of zinc is associated with a weakened immune system. T-cell-count, T-cell-proliferation- and function and NK-cell activity are reduced. Especially in elderly a reduced zinc status is evident (Lukito et al., 2004). In elderly a higher protein intake (together with slight exercise) stops sarkopenia, a progressive loss of lean body mass. During pregnancy and lactation a higher need of zinc is documented as well as during chronic inflammatory diseases (Rink and Gabriel, 2000).

Conclusion:
Meat as a component of a mixed and healthy diet contains important and essential micronutrients. The adaequate intake ensures a normal function of the immune system, the mucous membranes and the general metabolism of substrates. At least a sufficient intake ensures that during time periods of higher need e.g. diseases, pregnancy, this need is adequately covered. Especially in risk groups (elderly, pregnant women, growing children) meat should be consequently recommended.

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MEAT FATS IN NUTRITION

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Abstract

This article reviews the fat content and fatty acid composition of meats in the human diet and discusses nutritional facts related to meat, meat products and other meat-containing foods as sources of dietary fats. Meat is an increasingly important source of high-value animal protein worldwide. Meat fat comprises mostly monounsaturated and saturated fatty acids, with oleic (C18:1), palmitic (C16:0), and stearic acid (C18:0) being the most ubiquitous. Meat and meat products are considerable sources of cholesterol in the diet. In most industrialized countries, a high meat intake contributes to a higher than recommended total and saturated fat and cholesterol intake. Another concern is that meat may replace sources of other important nutrients in the diet. Therefore, the advice to consumers is to prefer lean meats and low-fat meat products and use meat in moderation only.

Keywords: meat, meat products, fatty acid consumption patterns, food sources of fatty acids, cholesterol, dietary intake

Rising global meat consumption

Meat is an important source of high-value animal protein in many regions of the world. Around the globe, the diets of relatively more urbanised populations are characterised by a higher content of meat, poultry and other animal products than the less diversified diets of rural communities (WHO, 2003). In South Asia and in Sub-Saharan Africa (excluding South Africa) meat consumption was still very low in the late 1990s, at under 10 kg annually per person. However, meat consumption has increased by, on average, more than 10% worldwide since the beginning of 1960s. Average annual consumption per person has increased by more than 20 kg during that period in Latin America and the Caribbean, East Asia and the industrialised countries. The consumption of meat is highest, on average close to 90 kg per year, in North America and most other industrialised countries (Bruinsma, 2003). Meat per se comprises roughly 10-20% of energy intake in most meat consuming countries (FAO, 2002; WHO, 2003). The combined share of energy of meats, meat products and of composite foods containing meats is larger, because of other energy-rich ingredients used in the preparation of these foods. The purpose of this article is to review the fat content and fatty acid composition of meats in the human diet and discuss nutritional facts related to meat, meat products and other meat-containing foods as sources of dietary fats.

Fat and fatty acid content of meats

Meat and meat products vary greatly in their fat content according to the animal species, age of the animal and part of the carcass used ([Swedish]Livsmedelverket, 2004; [English] Ministry of Agriculture, Fisheries and Food, 1998; Ovaskainen, Reinivuo & Korhonen, 2001). The fat content and fat composition is also affected by animal feeding, a fact that is exploited for modification of the meat fatty acid composition, with the relatively best results in single-stomached pigs and poultry (Wood & Enser 1997; Wood, Enser, Fisher, Nute, Richardson, & Sheard, 1999; Bolte, Hess, Means, Moss, & Rule, 2002). Data on the average fat content and fatty acid composition of meats and meat products are published as part of food composition tables throughout the world (e.g. Livsmedelverket, 2004; Ministry of Agriculture, Fisheries and Food, 1998; Ovaskainen *et al,* 2001).

The average fat content and fatty acid contents of selected meat fats and meats are shown in Figure 1 (modified from Livsmedelverket 2004, Ovaskainen *et al,* 2001). Total fat content of meats and meat products varies around 3-25 g/100 g. Chicken skin has an even higher fat content, about 48 g fat/100g (Fig 1). While cured beef and other cold-cuts may have a fat content of below 5%, the fat content in reduced fat sausages is usually around 10%, in common sausages around 15-25%, and twice as high in salami (~40%) and pepperoni (50%) type of sausages (Ministry of Agriculture, Fisheries and Food, 1998, Livsmedelverket, 2004, Ovaskainen *et al,* 2001).

Meat fat comprises mostly monounsaturated fatty acids (MUFAs) and saturated fatty acids (SFAs). The most ubiquitous fatty acids are oleic (C18:1), palmitic (C16:0), and stearic (C18:0) acids. Poultry and pork contain somewhat more unsaturated fatty acids (~10-15% of total fatty acids) than beef and lamb, and also a notable amount of polyunsaturated fatty acids (PUFAs). Linoleic acid (C18:2) is the predominant PUFA (~0.5-7%) , followed by alpha-linolenic acid (up to 0.5%) (Livsmedelsverket, 2004, Ministry of Agriculture, Fisheries and Food, 1998, Ovaskainen *et al,* 2001, National Public Health Institute, 2001). Trans-fatty acids comprise about 1-2 % of total fatty acids across all types of meat; in ruminant meats they represent ~2-4 %. Conjugated linoleic acid (CLA), a group of polyunsaturated fatty acids that appear in dairy products and are thought to have beneficial effects on health, are also found at low mg-levels in meats, especially in beef and lamb (Belury, 2002).

In addition to fatty acids, cholesterol is a nutritionally important component of meats. The cholesterol content of meats varies between about 30 and 120 mg/100 g of food, being even higher in offals (Ministry of Agriculture, Fisheries and Food, 1998; Ovaskainen *et al,* 2001; [Finnish] National Public Health Institute, 2001).

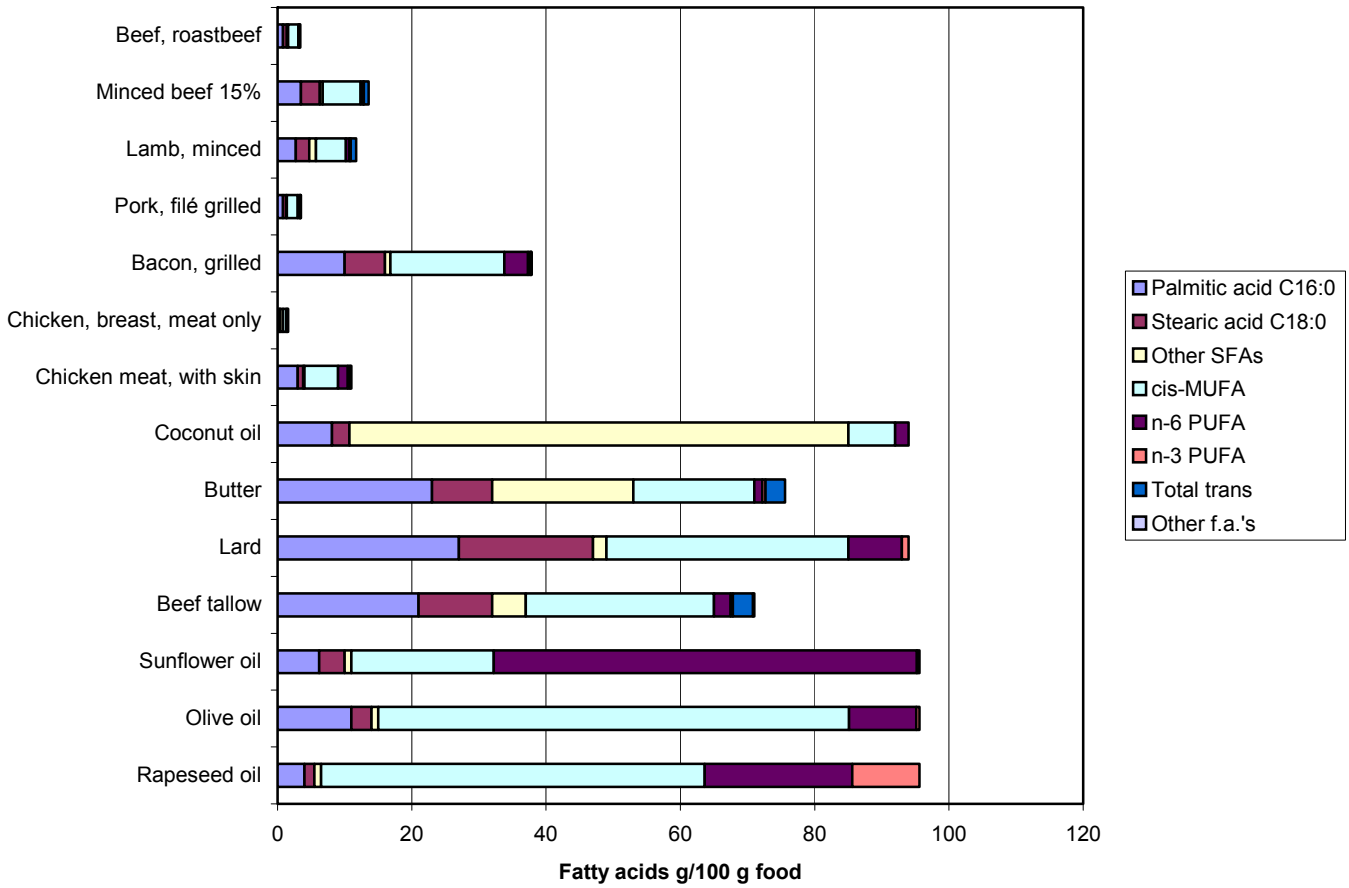

Figure 1. Common fat content and fatty acid composition of selected meats and fats (modified from Livsmedelsvärket, 2004, and Ovaskainen *et al,* 2001).

Fat contribution of meat in relation to recommended fat and cholesterol intake

The most recent nutrient intake goals published as a result of a joint WHO/FAO expert consultation (WHO, 2003) are based on the widespread consensus that a "balanced diet" has preventive effects on chronic non-deficiency diseases, e.g. obesity, type 2 diabetes, cancer and cardiovascular diseases. These guidelines include the following targets for fat intake: total dietary fat, 15-30% of energy (En%); saturated fatty acids (SFA), <10 En%; n-6 polyunsaturated fatty acids (n-6 PUFA), 5-8 En%; n-3 polyunsaturated fatty acids (n-3 PUFA), 1-2 En%; trans fatty acids, < 1 En%. The target for monounsaturated fatty acids (MUFA) is calculated as follows: MUFA = total fat – (SFA+PUFA+trans fatty acids). Saturated fatty acids are well known to raise total and low-density lipoprotein (LDL) cholesterol. The main causes of this effect are myristic and palmitic acids; stearic acid is converted to oleic acid in vivo and has not been shown to elevate blood cholesterol. Myristic and palmitic acids are common fatty acids in dairy products and meat.

When European patterns of food and nutrient intake were recently evaluated, it was shown that despite gastronomic diversity, in most EU countries where the available data allowed for analysis, a higher intake of total fat was associated with a higher intake of energy as well as, in most cases, of all main classes of fatty acids (De Henauw & De Becker, 1999; Haraldsdottir, 1999; Valsta, 1999; Hermann-Kunz & Thamm, 1999; Moschandrease & Kafatos, 1999; Löwik, Hulshof & Brussaard, 1999; Serra-Majem, Ribas & Ramon, 1999). Higher intake of saturated fat was mainly associated with higher intake of energy and MUFA (De Henauw & De Becker, 1999; Serra-Majem *et al,* 1999, Hermann-Kunz & Thamm 1999; Löwik *et al,*, 1999; Valsta 1999). The intake of cholesterol was shown to be higher in the high-fat as well as high-SFA intake groups in both Eastern and Western Germany (Hermann-Kunz & Thamm, 1999) and in the Netherlands (Löwik *et al,* 1999), but only in the high-SFA group in Greece (Moschandrease & Kafatos, 1999).

When in this European analysis the food intake in the high-fat quartile was evaluated, the higher intake of fat and/or saturated fatty acids was broadly associated with higher consumption of meat and/or meat products. In Belgium, the consumption of both fresh meat and processed meats was higher in the highest fat intake quartile of the adult population (De Henauw & De Becker, 1999). In a Danish analysis, it was shown that the high-fat diet consumers, defined as the top quartile of fat intake (% of energy), had a higher intake of fats (especially butter), meat and whole milk (Haraldsdottir, 1999). In an analysis of Finnish dietary intake data, the highest fat intake and highest SFA intake quartiles were both found to have a larger mean consumption of meats and to contain more consumers of pork meat and sausages. A similar pattern was seen in Spain for processed meat (Serra-Majem, Ribas & Ramon, 1999) and in Sweden for sausages (Becker, 1999). Interestingly enough, in the Finnish diet, the proportion of beef consumers was larger in the lowest fat intake quartile. Additionally, in the highest SFA intake quartile a larger mean consumption of poultry was found. By contrast, in Finland the lowest SFA intake quartile contained more poultry consumers than the high SFA intake quartile (Valsta, 1999). In Germany and the Netherlands the proportion of consumers of meat products in the overall population was close to 100 %, and the high-fat and high-SFA consumers ate more sausages and meat products compared to the low-fat or low-SFA consumers, respectively (Hermann-Kunz & Thamm, 1999; Löwik, Hulshof & Brussaard, 1999). In Greece, on the other hand, only the proportion of the population consuming pure meat was larger in the high-SFA quartile compared to the low-SFA quartile (Moschandrease & Kafatos, 1999). In the Irish population, those consumers with the fattier diets ate more red meat and sausages than those with a low-fat diet. The Irish pattern was based on a somewhat larger proportion of consumers of red meat and sausages in the high fat intake quartile and, in addition, a larger average portion size for red meat (Flynn & Kearney 1999). In Italy the association between high fat intake and meat consumption was less clear (Turrini, Leclercq & D'Amicis, 1999). These results indicate that like the consumption of dairy products, meat consumption is tied to higher saturated fat and total fat intake in most European countries.

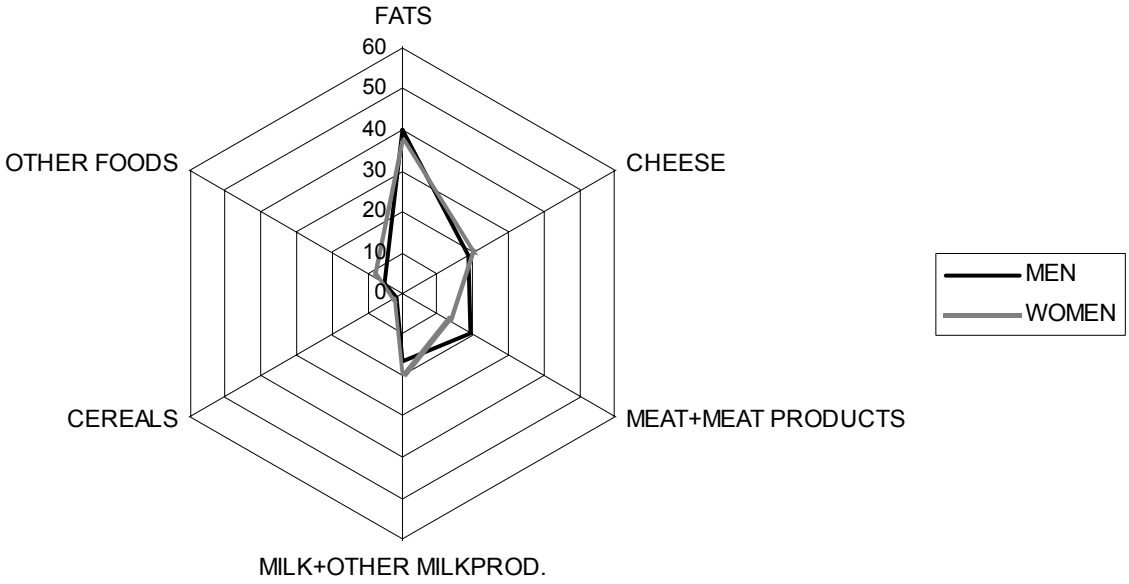

Figure 2a

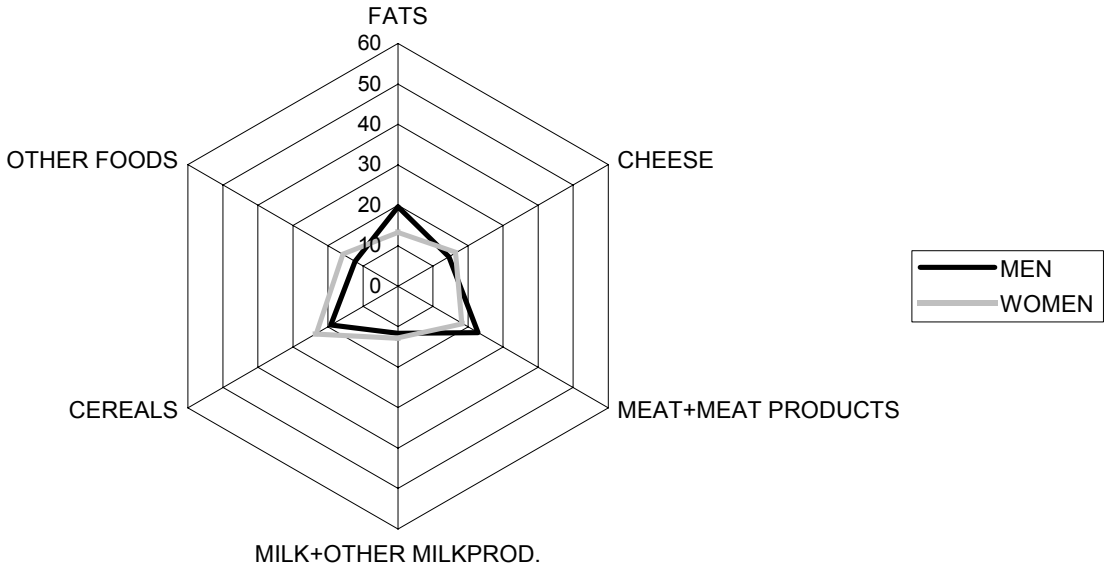

Figure 2 b

Figure 2 a and b. Sources of saturated fatty acids in the Finnish diet, a) at ingredient level, b) in prepared foods, (% of total intake) (Männistö, S., Ovaskainen, M-L. & Valsta, L., 2003).

In addition to the direct contribution of meat and meat products to dietary fatty acid composition, it is also nutritionally important to assess what components of diet are being replaced by growing meat consumption. In a large Swedish study, increasing total meat intake (expressed in quintiles and adjusted for energy, n=11648) was associated with decreasing intakes of poultry, fish, fruit, bread and cereals as well as cheese (Elmstahl, Holmqvist, Gullberg, Johansson and Berglund, 1999). Poultry and fish, bread and cereals are foods that decrease SFA intake when replacing red meat, while fruit decreases mainly the fat content and energy density of the diet when replacing meat.

Fresh meat, meat products and meat dishes as sources of fat and fatty acids

Approximately 60% of the SFAs in the U.S. diet are obtained from meat, poultry, fish and dairy products (Dupont, White & Feldman, 1991). In a more detailed analysis from the late 1980s of the fatty acid consumption pattern of Americans, it was shown that palmitic acid was the predominant SFA in the U.S. diet then, contributing 52-57% of SFA intake (Jonnalagadda, Egan, Himbach, Harris & Kris-Etherton, 1995). Of the SFAs, short chain fatty acids and lauric acid and myristic acid are obtained from dairy products, while the predominant sources of palmitic acid and stearic acid are meat, poultry, fish and blended foods. These same food categories were also the major source of monounsaturated fatty acids, especially oleic acid; among them, beef, pork and chicken meat were the main contributors (Jonnalagadda *et al,* 1995).

Another way of analysing the effect of meat and meat products on fat and fatty acid intake in diet is to compare their share of fatty acid intake at an ingredient level (fresh meat, plain meat products) with their share of fatty acid intake as prepared dishes. In Finland meat and meat products, as consumed, contributed to about one-fourth of the total fat recorded in the National FINDIET 2002 Survey (Männistö, Ovaskainen & Valsta, 2003). This study was carried out using the 48-h dietary recall method and the Finnish National Food Composition Database Fineli® (Ovaskainen, Reinivuo, and Korhonen, 2001) among 25-64-year-old men and women (n=2007). Accordingly, figures 2-4 show that in Finland the food group of fresh meat and meat products as consumed, contributes more saturated, monounsaturated and polyunsaturated fatty acids than do plain meat and meat products. This points to the fact that Finnish meat dishes are frequently prepared with fat-containing ingredients (cream, fat spreads, oils, etc.), which increase the often modest intrinsic fat content of meats.

Ways to modify the contribution of meats to fat intake and quality

Culinary traditions and recipes of meat dishes are an obvious focus if the share of fats from meat dishes and meat products is to be modified or reduced (Papadopoulos, Nowak, Miller, Cross, Savell, Brauchi & Scott, 1992). Fatty ingredients in meat dishes can be reduced in order to increase the nutritional quality of these foods. Methods of supplementation as well have been applied to this purpose in meat product development; for instance, it is possible to increase the proportion of unsaturated fats by processing vegetable oils into the meat product (Hammer, 1991). Manipulation of the fatty acid composition of animal feeds has successfully been used to improve the fatty acid profile of carcass fat in pigs (Morgan, Noble, Cocchi & McCartney, 1992, Vanoeckel & Boucque, 1992). Similarly, dietary fatty acid modification is considered a viable method of adding value to poultry products for the health conscious consumer (Hargis & Vanelswyk, 1993). In addition to both simple and more sophisticated techniques of this kind to modify the fat quality of meat products and meat-containing mixed dishes in order to follow nutritional recommendations, a commonly recommended approach is for consumers to moderate their intake of meat and meat dishes as well as other foods clearly contributing to dietary SFA content. (Dupont, White and Feldman, 1991; Smith-Schneider, Sigman-Grant & Kris-Etherton, 1992).

Conclusion

In conclusion, meat, meat products and meat dishes are sources of valuable, but replaceable, animal protein in the diet. From the point of view of fat content, the drawback of this protein source is that it is tied to a considerable level of saturated fatty acids and cholesterol. In several countries with a very low meat intake, a moderate increase in meat consumption could contribute to a nutritionally better diet. By contrast, in most industrialized countries, high meat intake contributes to a higher than recommended total and saturated fat and cholesterol intake and may replace sources of other important nutrients in the diet. Thus, as a part of a balanced diet in these countries, lean meats and low-fat meat products are preferred, and meat is recommended to be used "sparingly" (Willet, 2001) or at most in moderation today (National Nutrition Council, 1998, USDA 2000, Health Canada 2001).

Acknowledgements

The authors wish to thank Christine Bartels, Ph.D. for skilful language editing of the manuscript.

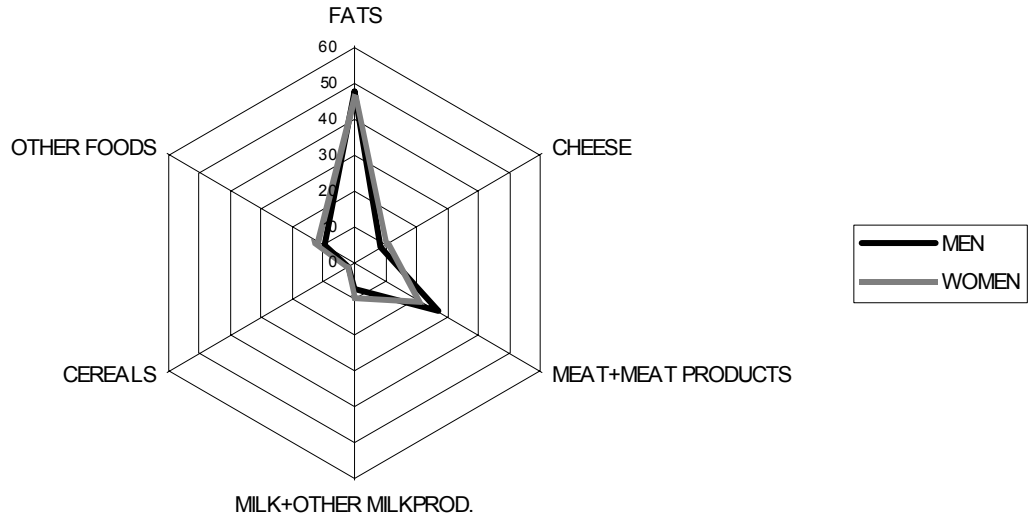

Figure 3 a

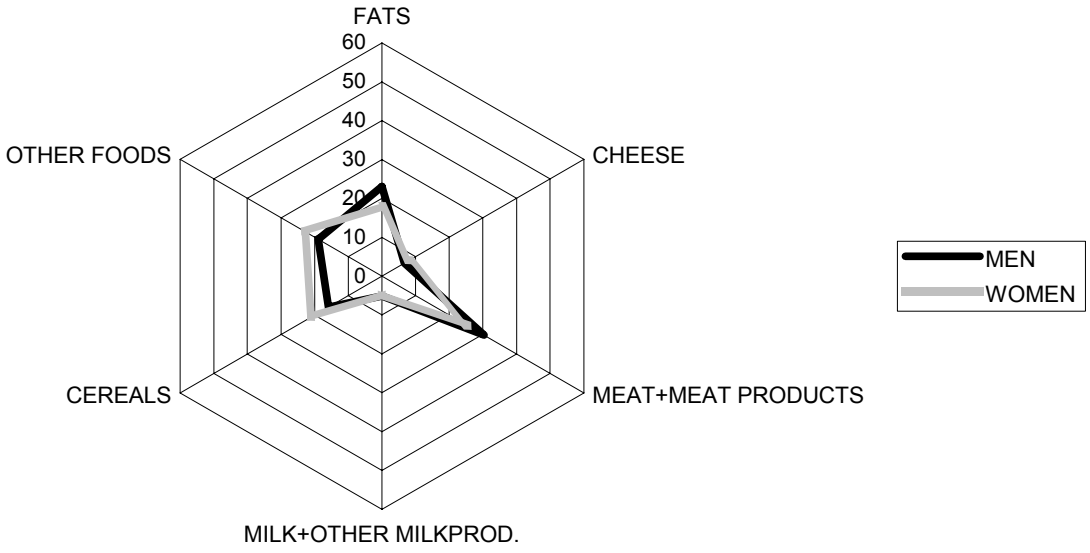

Figure 3 b

Figure 3 a and b. Sources of monounsaturated fatty acids in the Finnish diet, a) at ingredient level, b) in prepared foods, (% of total intake) (Männistö, S., Ovaskainen, M-L. & Valsta, L., 2003).

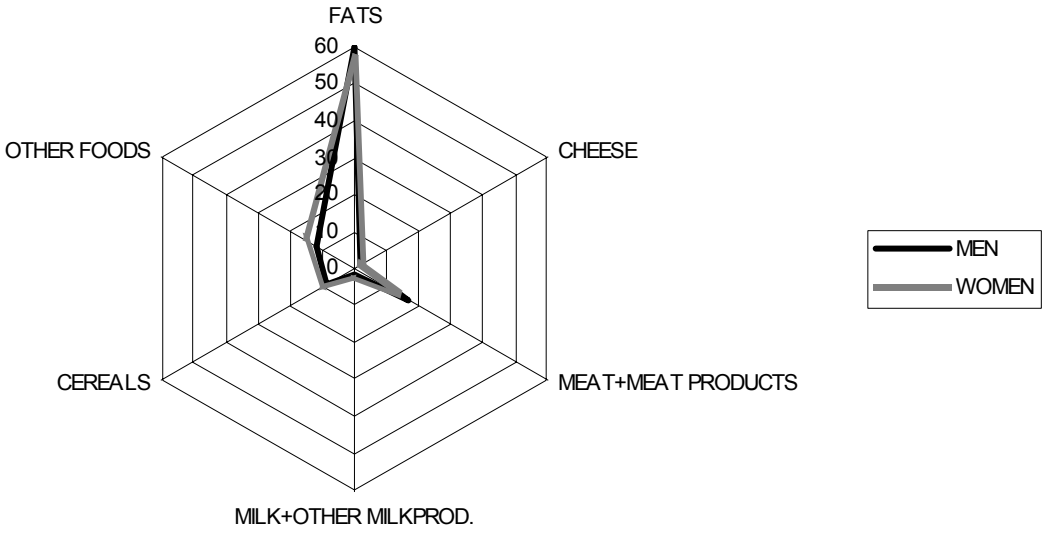

Figure 4 a.

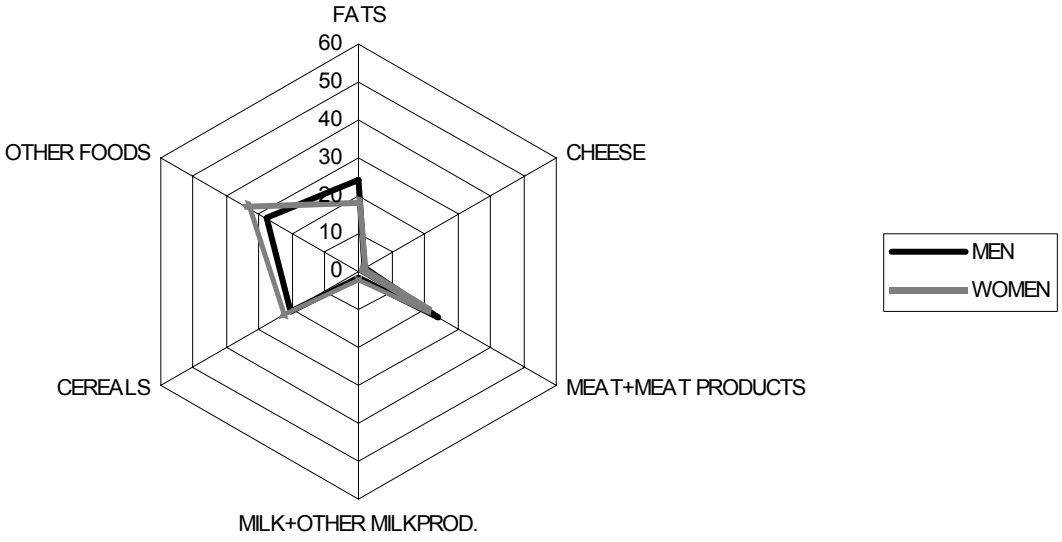

Figure 4 b.

Figure 4 Sources of polyunsaturated fatty acids in the Finnish diet, a) at ingredient level, b) in prepared foods, (% of total intake) (Männistö, S., Ovaskainen, M-L. & Valsta, L., 2003).

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SODIUM IN MEAT PRODUCTS

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Introduction

Sodium intake exceeds the nutritional recommendations in several industrialized countries. Excessive intake of sodium has been firmly linked to hypertension (Dahl, 1972; Fries, 1976; Law, Frost & Wald, 1991a,b,c). High blood pressure may in turn increase the risk of stroke and premature death from cardiovascular diseases. Tuomilehto *et al.* (2001) found that high sodium intake correlated with mortality and risk of coronary heart disease, independent of other cardiovascular risk factors, including blood pressure. These results provide direct evidence of the harmful effects of high sodium chloride (NaCl) intake in the adult population. Sofos & Raharjo (1994) have also presented a review on the health aspects of dietary sodium chloride and other salts.

The main source of sodium in diet is sodium chloride. On a population basis, it has been established that the consumption of more than 6 g NaCl/day/person is associated with an age-increase in blood pressure. Therefore, it has been recommended that the total amount of dietary salt will be maintained at about 5-6 g/day (Aho *et al*., 1980, WHO, 1990). Such recommendations are addressed to the general public. It is, however, recognized that genetically salt susceptible individuals and hypertensives will particularly benefit from low-sodium diets, the salt content of which should range between 1 and 3g/day.

Sodium chloride is one of the most frequently used ingredients in meat processing. Sodium chloride affects flavour, texture and shelf life of meat products. Besides the perceived saltiness, the NaCl brings out the characteristics taste of the meat product enhancing the flavour (Gillette, 1985). Sodium chloride also has an important role in the texture of meat products. It improves the water and fat binding properties of meat products resulting in the formation of a desirable gel texture upon cooking (Terrell, 1983). The preservative effect of NaCl is primarily due to its ability to lower water activity (Marsh, 1983; Sofos, 1984)

Most sodium chloride in the diet comes from processed foods. The NaCl contents of processed meat products should be reduced. To control the dietary intake of sodium from these products is difficult because of the different levels of sodium found in the same type of product. For example, in 30 liver sausages from different manufacturers the sodium content varied from 0.5 to 1.0 g/100 g (Greubel, Kluthe & Zuercher, 1997). Because of the large variation in NaCl content of the same type meat products, one easy way for reducing the average sodium intake is to reduce the NaCl content particularly of those products in which NaCl content is higher than the average.

Potential sodium chloride reduction depends on aspects connected with the type of the product, its composition, the type of processing required and the preparation conditions. These factors determine the type of product that can be modified and the technological limitations of salt reduction.

This paper reviews possibilities to reduce the sodium (NaCl) content in meat products. Mainly sensory and technological aspects will be covered.

Sodium intake and recommendations

Total dietary sodium intake in Europe has been estimated to range between 3.5 and 5 g/day (9 to 12 g NaCl) (Intersalt Cooperative Research Group, 1988). Finnish men consume on average 9.9 and women 6.8 g NaCl/d, respectively. The sodium intake from meat dishes is 22% for women and 24% for men (the National FINDIET 2002 Study, 2003).

The UK Department of Health has estimated the UK intake of sodium chloride at 8.2 g/person/day. Others (e.g. Godlee, 1996) refer to 9 g/person/day, calculated from data for urinary excretion of sodium. In populations with high sodium consumption there is usually no difference in sodium intake between normotensive and hypertensive individuals (Liu *et al.*, 1979; Morgan *et al.*, 1978; Schlierf *et al.*, 1980; Swaye, Gifford & Berrettoni, 1972; Tuomilehto, Karppanen, Tanskanen, Tikkanen & Vuori, 1980).

Sources of sodium from meat products

Meat itself contains sodium but the amount is less than 100 mg Na per 100 g. The main source of sodium in meat products is sodium chloride which is added during processing. Sodium chloride contains 39.3% sodium. Sodium is a part of also many other additives added when preparing meat products, e.g. in monosodium glutamate which is a flavour enhancer, sodium phosphates, sodium citrate and sometimes also sodium lactate. The amount of sodium from other additives is, however, much lower compared to the amount of sodium from sodium chloride. Practical experience has shown that when reducing NaCl content, meat industry may start to use sodium lactate to improve the shelf life and perceived saltiness. The amount usually added is 1.2% sodium lactate containing 0.24% Na. This amount of sodium equals to 0.6% NaCl.

The sodium chloride content of Finnish cooked sausages ranges from 1.6 to 2.2%, and that of cooked hams from 1.9 to 2.7%. This is equivalent to 0.6-0.9 g Na/100 g in cooked sausages and to 0.8 – 1.2 g Na/100 g in cooked hams. In other countries, meat products contain more NaCl. The sodium chloride content of meat products has been reduced, e.g. in Finland NaCl content of cooked sausages was in 1973 on average 2.4%, and in 1995 1.7% (Karanko & Puolanne, 1996). In the USA, the sodium content of 100 studied food products was shown to have reduced by 10-15% between 1985 and 1997. (Center for Science and Public Health).

Sodium reduction in meat products

Based on the scientific information, meat industry and consumers have become more aware of the relationship between sodium and hypertension and, therefore, in many countries, the demand for a variety of low salt meat products has increased. Food processors are developing numerous low-salt products to meet the demands of consumers. Developing low-salt meat products is, however, not straightforward. Sodium chloride plays such an important role in meat products. A particular problem with low-salt meat products is that not only the perceived saltiness, but also the intensity of the characteristic flavour decreases, when salt is reduced.

Meat product manufacturers have marketed low-salt alternatives, or have progressively reduced salt content over the years, where technological and microbiological considerations have made this possible. In most cases, when low-salt meat products are developed, the benchmark for the low-salt meat product is the normal-salt product. Consequently, the same quality characteristics must apply to low-salt meat products as to the correspondent normal-salt meat products. This causes problems because the properties of low-salt products are almost always different. The most important difference is the weaker characteristic flavour.

Before a satisfactory seasoning substitute to sodium chloride is found, the best way to reduce the intake of sodium would be for the food industry to gradually reduce the sodium chloride content in products. This demands that the formulations and the manufacture procedures of low-salt meat products must also be modified. Olson (1982) reported that a 25% reduction in NaCl is probably the most that can be achieved

without detrimentally affecting product characteristics (flavour, texture, shelf-life). Of course it is easier to reduce the higher salt contents than the lower salt contents without any detrimental effect. According to Ruusunen, Särkkä-Tirkkonen and Puolanne (1999), the NaCl content of cooked bologna sausages made with added phosphates may be reduced to 1.4% added NaCl without loss of flavour pleasantness. In cooked hams, based on saltiness evaluations it is possible to reduce the salt content of cooked ham to 1.7% NaCl (Ruusunen, Särkkä-Tirkkonen & Puolanne, 2001). The level of NaCl in the diet of a population determines the acceptable level to which NaCl content in meat products can be reduced. Consequently, same NaCl levels do not necessarily apply to every population.

There are several approaches for reducing the sodium content in processed meats: 1) lowering the level of sodium chloride (NaCl) added; 2) replacing all or part of the NaCl with other chloride salts (KCl, $CaCl_2$, and $MgCl_2$); 3) replacing part of the NaCl with non-chloride salts, such as phosphates, or with new processing techniques or process modifications; and 4) combinations of any of the above approaches (Terrell 1983; Sofos 1984, 1986, 1989).

Terrell and Olson (1981), Hand, Terrell and Smith (1982a,b) as well as Puolanne, Saarela and Ruusunen (1988) have studied meat products where NaCl has been substituted with other salts (KCl, $MgCl_2$). Replacement of sodium chloride by potassium chloride or magnesium chloride can lead to bitterness (Terrell and Olson, 1981), though some success has been achieved with a blend of sodium and potassium chloride.

The use of mineral salt mixtures is, however, a good way to reduce the sodium content in meat products. The same perceived saltiness can be achieved with salt mixtures at a lower sodium content (Puolanne *et al.*, 1988; Wettasinghe and Shahidi, 1997). In Finland, there are cooked sausages made with salt mixtures on the market where the salt content based on sodium chloride content is 1.2% or lower. These sausages are allowed to be labelled as low-sodium sausages.

There is, however, a disadvantage when using salt mixtures: the consumers will not get used to weaker perceived saltiness of low-salt meat products when salt mixtures are used, because of characteristic flavour still remains strong.

Perceived saltiness and flavour intensity

The perceived saltiness of NaCl in meat products is mainly due to the Na^+ cation with Cl^- anion modifying the perception (Miller & Barthoshuk 1991). Sodium chloride is also a flavour enhancer increasing the characteristic flavour of meat products (Gillette, 1985; Matulis, McKeith & Brewer, 1994; Ruusunen, Simolin & Puolanne, 2001; Ruusunen, Särkkä-Tirkkonen & Puolanne, 1999). Both the perceived saltiness and the flavour intensity depend on salt content in meat products (Matulis, McKeith, Sutherland & Brewer, 1995, Ruusunen *et al.*, 1999, Crehan, Troy & Buckley, 2000). The authors of this review stress that a certain amount of salt has to be added to food products before it can even be discerned. A small amount of sodium chloride might taste sweet (Beauchamp, Bertino & Moran, 1982) which is not appropriate in meat products. The sweet taste is probably due to stimulation of the receptors that mediate sweet perception rather than to stimulation of receptors mediating sodium chloride perception (Barthoshuk, Murphy & Cleveland, 1978).

Sensitivity may be defined as the ability to detect or recognize gustatory stimuli. Detection and recognition thresholds are extensively used measures of this taste attribute. All thresholds, however, are forms of discrimination relative to a background medium. Thus, the conditions under which a threshold is determined can greatly influence the resulting value. In addition, the sensitivity of individuals varies due to the influence of numerous endogenous and exogenous variables. With respect to the salty taste, one of the most important of these factors is the salivary sodium concentration. The taste receptors are bathed in saliva, and adapt to the sodium level of this fluid. To elicit a salty sensation, this level must be exceeded by a given amount in an individual. The perception of many flavour characteristics depends greatly on the nature of the food matrix. The food matrix plays an important role in controlling flavour release at each step of food product preparation and consumption. (Mattes, 1984, 1997).

Sensitivity to sodium chloride has been the most extensively studied parameter with respect to hypertension. Fallis, Lasagna and Tetreault (1962) were the first group to assess the NaCl detection and recognition thresholds of normotensives and hypertensives (diastolic blood pressure (DBP) >100 mmHg). While no differences in NaCl detection thresholds were observed, the hypertensives displayed elevated recognition thresholds.

Although many people add sodium chloride to enhance the taste of foods, their preference may change because of the pleasantness of NaCl is influenced by customary dietary levels. When someone follows a low-salt diet from a few weeks to a few months, they get used to the mild taste of low-salt products (Bertino, Beauchamp & Engelman, K., 1982; Blais *et al.*, 1986). There are various existing hypothesis about why diets with different salt contents changes one's preference to salt, for instance by changing the sodium content in saliva. An individual's partiality to salt can therefore be changed, but it is not necessarily easy, as a lower salt content in food does not initially meet one's expectations, customs and preferences. People get used to the taste of salty products faster than they do that of low-salt products (Bertino, Beauchamp & Engelman, 1986).

The manner in which sodium chloride is added effects how strong is the perceived saltiness. Sodium chloride has a more concentrated taste if it is sprinkled on a cooked meat instead of on raw meat before cooking. If sodium chloride is sprinkled on a cooked steak, only half as much NaCl to achieve the same level of perceived saltiness will be needed (Ruusunen, 1985).

The effect of fat and lean meat content on perceived saltiness

Fat and sodium chloride together contribute to many of the sensory properties that are characteristic of cooked sausage. When NaCl level rises it is more noticeable in fatty products than in lean ones (Hammer, 1981; Matulis *et al.*, 1994) However, Ruusunen *et al.* (2001) have shown that fat content of cooked sausages affects the perceived saltiness in different ways depending on the composition of the formulation. By replacing lean pork with pork fat, thus increasing the fat content and simultaneously reducing the meat protein content, perceived saltiness of sausages increases, but by replacing water with fat on an equal weight basis, the perceived saltiness of the sausage does not change. Therefore, the increase of meat protein content was supposed to reduce perceived saltiness. This result was confirmed with meat patties. More salt was needed in meat patties with high lean meat content to achieve the same perceived saltiness than in products with lower meat content (Ruusunen *et al.*, 2003, 2004). In meat patties, fat content had a smaller effect on perceived saltiness than lean meat content and their effects on perceived saltiness were opposite. It is also well-known that perceived saltiness is high in products with high amounts of loose water.

The theory of water-binding

Hamm (1972) and Offer and Knight (1988) have published comprehensive reviews about the technological effects of salts in meat. The above mentioned reviews concentrate on sodium chloride and phosphates, but other chloride salts and salts of weak acids are discussed, mainly to further elucidate the theories behind the effects of sodium chloride and phosphates. The reviews deal mostly with uncooked meat, and not so much with cooked meat or cooked meat products. Tornberg (2004) has recently presented a review on heat-treated meat products. The present chapter deals with effects of the lowering of the sodium content on the technological properties of meat products. Whiting (1988) also presented a review on solute-protein interactions in meat batter.

The effect of sodium chloride on meat proteins is most probable caused by the fact that chloride ion is more strongly bound to the proteins than sodium ion. This causes an increase in negative charges of proteins. Hamm (1972) concludes that this causes repulsion between the myofibrillar proteins (myofilaments), which results in a swelling of myofibrils or even a partial solubilisation of filaments, the latter is due to the repulsions of individual molecules. The cross-bridges between the filaments prohibit the unlimited swelling of the myofbrils. Polar groups of the side chains of the amino acids (ca. 76-80%) of the proteins bind water molecules on their surfaces by van der Waals' forces. The water molecules, being polar, then orient

themselves so that in a case of a negative ionic group there will be the positive part towards ionic group and the negative part pointing at the solution, and vice versa with positive groups. Additional water molecule layers will be formed on this so-called monomolecular layer with similar orientations. Thus, all the water molecules are more or less influenced by the pulling forces caused by the polar groups of the proteins. On the contrary, non-polar side chains of the amino acids push the polar water molecules causing arched-like structure around the non-polar group. The combined effect is that water molecules are pulled (polar groups) and pushed (non-polar groups) between the filaments creating a tension that forces the water molecules in an ice-like form in the protein network of filaments and transverse elements, like cross-bridges and Z-line. The factors inhibiting the unlimited swelling are the actomyosin cross-bridges between the filaments and Z-lines. The amount of the water bound is determined by the net charge of the proteins causing repulsion that increase the binding, and by the number and strength of cross-bridges that limit the binding.

This sounds reasonable, because the distances between the filaments surfaces are about 20 nm (actin to myosin and actin to actin) or 30 nm (myosin to myosin). This means a thickness of a layer of about 60 to 90 water molecules. The hypothesis explains the effects of salt content and pH, as well as the role of cross-bridges and consequently how water is retained in meat. However, the hypothesis does not take the effect of counter-ions, eg. the sodium ions, into account. Offer and Knight (1988) also claim that the distances between the filaments are too long to establish a repulsive force that would be strong enough to generate the water-binding.

Offer and Knight (1988) suggest an alternative hypothesis also based on the selective binding of chloride ions to the myofibrillar proteins. According to them this does not cause a marked repulsion between the filaments but between the molecules of myosin filaments breaking down the shaft of the filament. This will cause a loosening of myofibrillar lattice. If phosphate is not used, the S1 units of heavy meromyosin are still attached to actin filaments. Offer and Knight postulate that the swelling occurs by an entropic mechanism driven by the free, light meromyosin parts bound to actin filaments.

Offer and Knight (1988) also present another aspect that is close to the hypothesis of Hamm (1972). They start with the same selective binding of chloride ions as Hamm, but because the structural proteins are solid in meat and cannot move, electrical forces pull the counter-ions (sodium ions) very close to the filament surfaces thus creating an uneven distribution of ions in the water phase. This establishes an osmosis-like force within the filament lattice which in turn pulls water molecules into the system. This would cause an unlimited swelling, but the cross-bridges cause an opposite force that Offer and Knight call ‘elastic pressure’. In any case, osmotic pressure created by the uneven distribution of ions and the elastic pressure are equal at any moment. This explains the effects of salt content, cross-bridges, pH and the denaturation effects and resulting shortening of myosin S1–S2 complex as well.

The effects of NaCl on different proteins in meat are very complex, and the complexity increases if different concentration combinations of added NaCl, KCl and phosphates are acting simultaneously (see reviews of Hamm 1972 and Offer and Knight 1988). Conciselly, the solubility of myosin is increased as the NaCl concentration raises from 0.04 to 0.5 M. After the initial aggregation and formation of filaments the structures start to dissociate at salt contents higher than 0.25 M. The swelling of myofibrils starts at 0.5 M without added phosphates and at 0.4 M with added phosphates, where an extensive extraction of myosin also starts to take place.

The swelling depends on pH (Hamm, review 1972; Offer & Knight, review 1988). Without salt there is a maximum at pH 3.0, a minimum (the average isoelectric point of meat proteins) at pH 5.0 and from there a constant increase within the physiological pH range. Due to the selective binding of ions, salts move the isoelectric point. By 2% NaCl the isoelectric point and swelling minimum are at pH 4.0. (Hamm, review 1972). Wilding, Hedges & Lillford (1986) found that hypertonic salt solutions (KCl and KI) induce fibre shrinkage at pHs below the isoelectric point of myofibrillar proteins (pH 5.0), which actually means that the isoelectric point has moved to lower values. With NaCl there is a maximum in swelling as well as in heated gel strength at 6.0 (Hamm, review 1972; Ishioroshi, Samejima & Yasui, 1979) or at pH 6.2 (Puolanne, Ruusunen and Vainionpää, 2001). This seems to be due to the increased sodium ion binding to the negatively charged myofilaments, and the simultaneous weakening of the binding of chloride ions.

Minced meat and restructured products

Minced meat products and restructured meat products are made without or with added phosphates. There is a wide variety of meat products from all meat products made of lower quality of meat, fat, carbohydrates and other polymers, added proteins and added water. There is no particular technological minimum for sodium chloride content; meat patties can be made even without added sodium chloride (Booren, Jones, Mandigo & Olsen, 1981) by pressuring frozen meat to form the patty, or freezing the product after the formulation (Demos, Forrest, Grant, Judge & Chen, 1994). In these products, sodium chloride content, if used, is mainly determined by sensory aspects. Transglutaminase has also been used to increase the gel formation in patties thus increasing the yield (Nielsen, Petersen & Møller, 1995; Tseng, Liu & Chen, 2000).

Schults, Russel and Wierbicki (1972), Schults and Wierbicki (1973) and Kenney and Hunt (1990) found, using centrifuge methods with added phosphate, a steep initial reduction of cooking loss when the salt content increased from 0 to 1%, and the best bind was at 4% NaCl. On the contrary, in sausages a marked difference between phosphate and non-phosphate systems has been found between 1.0–1.5% NaCl (e.g. Puolanne & Ruusunen, 1980; Barbut, Maurer & Lindsay, 1988). Barbut & Mittal (1989) also found that there are differences between the proteins of beef, pork and poultry meat in gelation patterns and responses to salt.

Sodium chloride is, however, important decreasing the cooking loss and improving the texture. Sodium chloride increases the cohesiveness of the batter thus improving the moisture and fat retention. Schwartz & Mandigo (1976) studied several combinations of 0–2.5% NaCl and 0–0.5% sodium tripolyphosphate in restructured pork and found that salt increased TBA values, packaging loss, improved cooked colour, aroma, flavour and eating texture. Among the variables 0.75% NaCl and 0.125% sodium tripolyphosphate were needed when producing restructured pork. Matlock, Terrell, Savell, Rhee Dutson (1984a, b) found that phosphate improved scores of saltiness, juiciness, binding, and cooking yields, but they reported decreased rancidicy due to sodium tripolyphosphate. NaCl alone increased rancidity (Matlock *et al.* (1984b). With phosphate a 15% reduction in sodium content can be achieved (Matlock *et al.* (1984a). According to Ruusunen *et al.* (2004), the increase in NaCl content causes a marked decrease in cooking loss, but the effect on increasing the firmness is less pronounced. Phosphates improve the firmness; the same firmness can be reached in meat balls by ca. 40%, using basic potassium phosphate, less sodium than without phosphates (Ruusunen *et al.*, 2004). Also other ingredients such as soy protein, caseinate, gums etc. can be used to improve the technological properties of the patties, they do not necessarily affect the meat but the entire mixture.

In conclusion, decreasing the sodium content in minced meat products is not a major technological problem. NaCl increases, however, the bind, firmness, cooked yield and taste. The sensory effects such as perceived saltiness can be affected also by other components of the meal, and spices. The NaCl content in the products varies usually from 0% to about 2%. The level for most minced meat products could, however, be below 1.0%. It is also recommendable at homes and in catering to add the salt on plate just before eating in order to obtain the same perceived saltiness by much lower amounts of salt.

Cooked sausages

Sodium chloride increases the water-binding in meat linearly between ionic strengths of 0 to 0.8–1.0 in the water phase (Hamm 1972; Offer and Knight 1988). This corresponds to less than 5% NaCl in lean meat, provided that the water content is about 75%. Part of the water is tightly bound to the monomolecular layer (4 percentage points) and multimolecular layer (4–6 percentage points), which means that this water does not have the physical characteristics of free water and consequently solves less solutes. Therefore, it can be estimated that the ionic strength of salt must be calculated for 65g water in 100g lean meat. This must be taken into account when comparing the water-binding curves; e.g Offer and Trinick (1983).

Added polyphosphates cleave the actomyosin bond thus weakening the myofibrillar structure. The amount of cross-bridges per unit volume will decrease, which according to Hamm (1972) and Offer and Knight (1988) leads to an increased water-binding capacity. With added phosphates the water-binding curve is not

linear in relation to NaCl content. At low contents, up to 1.0% NaCl there is a linear relationship, but then water-binding increases considerably until levelling slowly off at 1.5% (Puolanne and Ruusunen, 1980). This means that a sausage of normal gel-forming capacity can be made with about 0.3–0.5% lower sodium chloride content when phosphates are used, compared with a sausage made without added phosphates. Offer and Trinick (1983) showed in a model study with isolated myofibrils that marked changes in myofibrillar structure take place at the ionic strengths of 0.3–0.4. This means 17.6–23.4 g NaCl/l, and in a sausage with 60% moisture (tightly bound water ca. 5 percentage points) and 20% fat a content the upper limit is 12.9 g NaCl/kg. It should be stressed, however, that although good water-binding can be obtained with this sodium chloride content in raw sausage batter, it does not necessarily form a heat stable gel. Practical experience suggests that about 15 g NaCl/kg is needed.

Another aspect to be considered in cooked sausages is the fat content. If both salt content and fat content are reduced simultaneously (low fat – low sodium product), some problems may arise. If the fat content of the above mentioned sausage is reduced by 30% (from 200 to 140 g fat/kg) and replaced with water, 14.3 g NaCl/ kg is now needed for an ionic strength of 0.4; i.e. the simultaneous reductions of both constituents are not easily achieved. However, compared to the present situation worldwide, an average NaCl content of 1.6–1.8% in cooked sausages would mean a reduction.

Sofos (1983) manufactured frankfurters with 2.5%, 2%, 1.5% and 1% NaCl. A reduction in salt content more than 20% (<2.0% NaCl) resulted in frankfurters of softer and less firm texture. Sofos (1985) was able to compensate the reducing of NaCl by adding sorbate, which is usually added for antimicrobial reasons. Hand, Hollingsworth, Calkins & Mandigo (1987) reduced simultaneously fat content and salt content in frankfurters (without phosphate). Low fat frankfurters with 1.5% NaCl had softer consistency that those containing 2.0 or 2.5% NaCl. They concluded, however, that modifications of the formulation, low fat – low salt frankfurters can be manufactured. Crehan *et al.*, (2000) stated that the high hydrostatic pressure processing can be used to improve the stability of frankfurters with reduced NaCl content (from 2.5 to 1.5%), but cook yield was not affected.

Whiting (1984a) concluded that when salt content is reduced (from 2.5% to 1.5%, without phosphate), water exudates will be affected first, then gel strength, but fat release will not occur until more extreme conditions will be encountered. Whiting (1984b) compensated the reduction of water-binding and gel strength with phosphates, the effect of which was virtually independent of the pH differences the individual phosphates. Puolanne *et al.* (2001) studied the combined effects of sodium chloride, pH and use of phosphates on the water-binding (related to gel strength) of meat using model sausages. Differences in pH were reached by selecting raw meats with different pH, and no adjustments of pH were made. It was clearly seen that the same water binding can be obtained by different combinations, pH being an important variable. In some countries acid phosphates are used for better keeping quality, but this may cause problems in gel strength especially if low-salt sausages are aimed at.

Another strategy is to utilise low-sodium salt mixtures. There are many commercial mixtures, and they usually contain potassium chloride. Hand, Terrell & smith concluded that NaCl (2.8%) can be replaced with 35% KCl (by ionic basis) but not with $MgCl_2$ without detrimental effects on shrinkage. This can be expected due to the high salt levels, but technological difficulties might have been expected if the reference salt content would have been markedly lower. We have studied (Puolanne, Saarela & Ruusunen, 1988) a commercial mixture (Pan-Salt®) that contains 58% NaCl, 27% KCl, 12% $MgCl_2$ or $MgSO_4$. In our study it was shown that the sodium intake can be reduced in sausages without added phosphates by 31–37% thus obtaining the same water-binding. Mg^{2+} ions form an insoluble salt with added phosphates, and therefore this salt mixture is more suitable for sausages without added phosphates. The study showed the effects of different anions and cations as could be expected from the lyotrophic series of ionic binding as given by Hamm (1972) and Offer and Knight (1988). The use of KCl in salt mixtures also gives an additional benefit due to the fact that potassium is a counter-ion to sodium and reduces the harmful effect of sodium on blood pressure.

Finally, the water-binding and heat stability of cooked sausage can be improved by potato starch, soy protein products, gums, caseinate, added meat protein (mainly collagen), pig skin etc. gel-forming ingredients (e.g.

Whiting, 1984b). Depending on the national quality policy this also offers a way of reducing the salt content.
The use of pre-rigor curing can replace the use of phosphates. Chloride ions increase the net charge of the myofibrillar proteins preventing the formation of actomyosin bonds. Jolley, Honikel & Hamm (1981) studied the pre-rigor curing effect and found a curvilinear relationship between NaCl and water-binding in uncooked homogenates with a good binding already at 1.3% NaCl. With cooked homogenates the relationship was linear, and they found that 1.7% NaCl would give the binding similar to the normal 2% in rigor meat homogenates. Puolanne & Terrell (1983 a, b), achieved the pre-rigor effect in experimental cooked sausages with a 1.5% NaCl, but they found that the effect is curvilinear, similar to the water-binding curve in sausages with added phosphate. It should also be mentioned that the formation of an excellent raw sausage batter with 1.0% NaCl can be obtained by using pre-rigor meat, but at least 1.5% NaCl is needed for the heat stability of the gel, similar to rigor meat and added phosphate (Puolanne & Ruusunen, 1980; Puolanne & Terrell, 1983a,b). Similarly Bernthal, Booren & Gray (1981) found that 2% NaCl resulted in a good water-binding in pre-rigor meat, but 1% was not markedly better than 0 or 0.5%. The use of phosphate with pre-rigor meat does not give a marked additional benefit (Puolanne & Turkki, 1983).

It can be concluded that without phosphate the NaCl content can be lowered to 1.5–1.7% and with phosphate to 1.4% without jeopardising the technological quality and yield.

Hams

The connective tissue membranes and cell membrane prevent the free movements of ions in muscular tissue. The diffusion of salts is very slow in meat; it is more the question of days for salt to diffuse than hours (Vestergaard, Risum & Adler-Nissen, 2004; Ockerman *et al.*, 2004). Offer and Knight (1988) mention the "tiger stripe" -type appearance caused by uneven distribution of brine just after the injections. The diffusion is normally accelerated by brine curing and subsequent tumbling for several hours.

Hamm (1981) and Offer and Knight (1988) pointed out that whole meat and a piece of meat behave in a different manner than chopped meat. In chopped meat the connective tissue is to a large extent disrupted and fibres and myofibrils are broken. Puolanne (1999) has calculated that during the normal chopping of cooked sausage batter the cutter knife smashes on average every point of the sausage batter at least once. Hence salt, water and phosphates are able to directly attach the filaments in every part of the batter. In addition, Offer and Knight (1988) concluded that the endomysial connective tissue acts as a mechanical restraint to swelling. They also stated that if myofibrils are exposed to large excess of salt solution, especially in the presence of polyphosphates, myosin molecules formed by depolymerisation of the thick filament will tend to be extracted, and this will not result in swelling. Wilding *et al.* (1986) also claim that endomysial sheath acts as a restraint to myofibrillar swelling.

Grabowska and Hamm (1979) stated that when using 2% NaCl and a meat:water ratio 1:1.5 about 20 % of the myofibrillar proteins are dissolved, and with the addition of 0.3% diphosphate the solubility is 35%. In larger meat pieces such as hams, this brine addition is impossible in practical terms. It can be concluded that in large meat pieces there will be a swelling of the myofibrils in situ. Thus, not only the swelling itself but also the heat stability of the system is a critical factor in determining the yield and juiciness.

In addition, meat contains triphosphatase and diphosphatase, which break down polyphosphates to ortophosphates at rates depending on pH and temperature. Therefore, the effects of phosphates in larger meat pieces are subjected to variation due to the rate of phosphate diffusion and consequently the state of phosphate hydrolysis; in chopped sausages there is not such an variation, and the contact with salt, water and phosphates as added, and myofibrillar proteins is almost instant. With laboratory tests we have, however, found that also in chopped sausage batters about one hour is needed for the salt, phosphate and water to have the maximal effects on the heat stability, which can be speculated to be due to the diffusion in the myofibrillar level (Unpublished observations).

Salt mixtures can be used in hams too, although their sensory characterisitics are more sensitive than those of sausages. Frye, Hand, Calkins & Mandigo (1986) replaced 50% NaCl (2%) with KCl (by ionic basis) in

hams, and concluded that hams with 2% NaCl had the best sensory scores, but a 50% replacement with KCl gave the best bind and acceptable sensory scores. Lin, Mittal & Barbut (1991) were able to obtain the best water-binding and cooked yield by replacing 15–18% NaCl (2%) with KCl in coarsely ground ham product.

As a conclusion, the recommendations given for hams should be about 0.3% higher than for cooked sausages, due to the lower fat content.

Fermented meat products

Salt, nitrite, pH and temperature control the fermentation as well as the safety and the quality of dry fermented meat products (Leistner, Herzog & Wirth, 1971). They are all inter-related, and if one of these factors is reduced it should be compensated with an increase of one or more of the other factors to keep the same safety and technological quality. Salt is such an essential constituent of dry fermented products that there are not so many studies on its reduction.

Petäjä, Kukkonen & Puolanne (1985) concluded that 2.5% NaCl is the lower limit for a good quality salami-type fermented sausage, but with 2.25% the sausages are less firm and the typical aroma is weaker and the yield lower than with higher salt contents. Also Stahnke (1995) found that a low NaCl content favours pH decrease. Cimeno, Astiasarán & Bello (1998, 1999, 2001) replaced NaCl with potassium, magnesium and calcium ascorbates and were able to reduce the NaCl content by about 50%. The only relevant difference was the lower consistency which is to be expected when the chloride ions are replaced with ascorbate ions that do not effectively react with myofilaments.

Peltonen (2003) studied the combined effects of sodium chloride and pH on the water-binding capacity of meat. The minimum water-binding was at pH 4.6–4.7, and the maximum ionic strength was at about 1.0 (Figure 1.). At high ionic strengths, however, there is no water-binding minimum at the range of pH 5.7–4.4. When the drying period starts, the water binding is high (pH 5.6 and ionic strength ca. 0.8-1.0 in the water phase), but starts to decrease with the decrease of pH and increase of ionic strength. This initial phase favours particle cohesion, and later on the decrease of water-binding favours the evaporation of water and thus the drying.

Recently, Olesen, Meyer & Stahnke (2004) found that NaCl (1.5 and 3.0%) had a strong effect on contents of volatile compounds in fermented sausage, and the effect was influenced by the ripening time. The relevance of the findings was not tested by sensory tests.

In dry fermented products a simple reduction cannot be made, because the low water activity has to be reached in order to control the microbial flora. Consequently the technological and microbiological as well as sensory characterics of the supplements compared to NaCl will decide to what extent NaCl can be replaced. Bitterness seems to be the most relevant sensory trait (Gou, Guerrero, Gelabert & Arnau (1995), but effects on microbial flora should not be forgotten.

It can be concluded that the regulation of the fermentation is the decisive factor in fermented sausages. This will limit the lowering to the level >2.0% NaCl.

Concluding remarks

In most countries and in most cases, sodium contents of meat products can be lowered markedly. High fat content, non-meat ingredients, pressure treatment etc. may allow even lower NaCl values for different products than mentioned in the text above. Perceived saltiness and overall acceptability of the low-salt products may, however, weaken. Particularly important is that increased meat protein content reduces perceived saltiness. The sodium content in consumers normal diet must also be taken into account; have they used to high or low salt content in their diet. This means that the values given above must always be tested product by product, by consumer segment and by nationality. Shelf life will also be affected when

lowering the salt content of meat products. By using salt mixtures the intake of sodium (NaCl) can be markedly reduced.

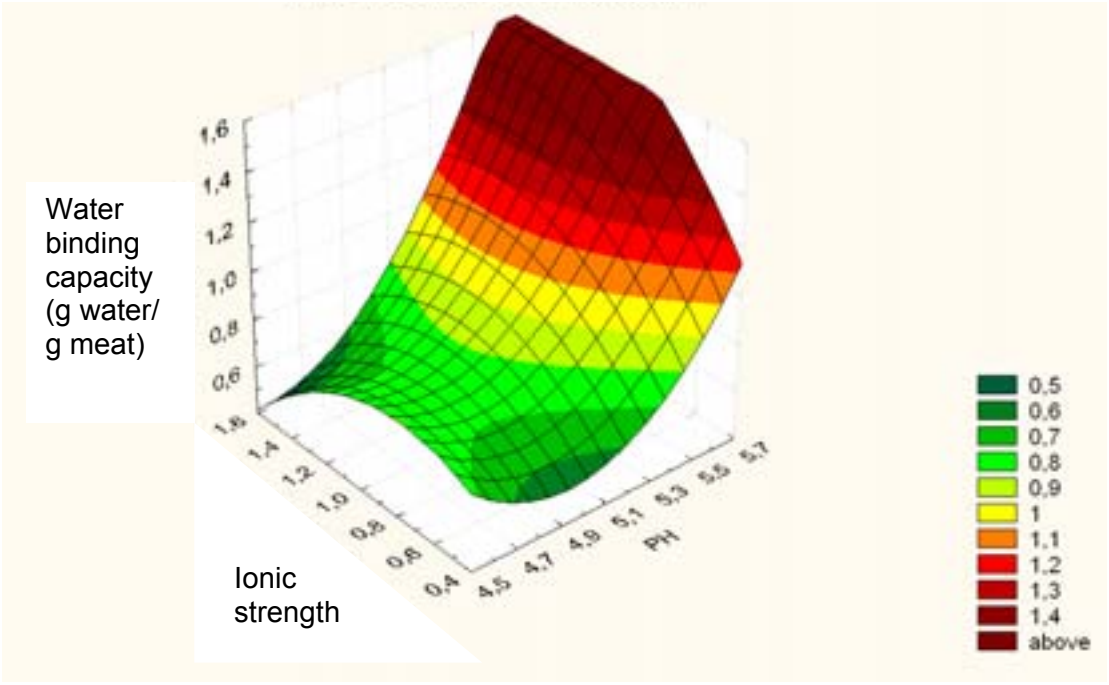

Figure 1. The effects of pH and ionic strength on the water-binding capacity of meat. (Peltonen, 2003)

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BREED AND FEEDING EFFECT ON FATTY ACID COMPOSITION OF INTRAMUSCULAR FAT IN YEARLING BULLS

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Background

Pasture diets have sustained lower live weight gains relative to those achieved with high-concentrate diets, and the cattle finished at pasture have produced carcasses with a low fat content (Steen & Kilpatrick, 1998). But pasture-finished cattle may produce beef with a more desirable fatty acid (FA) composition in terms of its beneficial effect on human health (Kinsella, 1988), especially in relation to the content of *n-3* polyunsaturated fatty acids (PUFA) (French et al., 2000; Steen *et al*., 2003; Varela *et al*., 2004). On the other hand, breed can also affect muscle fat quality, where FA variations are mostly related to intramuscular fat levels and consequently to neutral and polar lipids ratios (Scollan *et al*., 2001).

Objectives

The purpose of the present work was to study the effect of breed and feeding system on intramuscular fat and total fatty acid contents of *Longissimus thoracis* muscle in beef cattle.

Materials and methods

Animal management

Eight yearling bulls from "Asturiana de los Valles" (AV) (beef breed adapted to extensive production systems) and eight yearling bulls from "Asturiana de la Montaña" (AM) (small to medium-sized hardy animals, adapted to mountain systems) were studied. Four AV and 4 AM animals were reared under extensive conditions on ryegrass and clover pastures and received a finishing diet during the last 60 days composed of concentrate meal (84% barley meal, 10% soya meal, 3% fat, 3% minerals, vitamins and oligoelements) and barley straw *ad libitum*. The other eight animals (4 AV and 4 AM) were managed under intensive system (concentrate meal, same composition) and barley straw *ad libitum* in the housing facilities of the Institute (S.E.R.I.D.A.). Animals were slaughtered with an average weight of 554 kg for intensively reared AV and 504 kg for extensively reared AV animals, and 463 kg for intensively reared AM and 461 kg for extensively reared AM animals. Slaughtering was performed in a commercial abattoir according to a routine procedure, and after dressing the carcasses were chilled at 3ºC for 24h.

Measurements

Twenty four hours *post-slaughter* the left half carcass was quartered and the part of the rib joint comprised between the 6th and 9th ribs extracted and transported to the laboratory. The 6th rib was excised and *Longissimus thoracis* (LT) muscle was separated, aged at 4ºC for 7 days and then minced with an electrical chopper, vacuum packed and kept at –20ºC until determination of intramuscular fat content by near infrared spectroscopy (Oliván *et al*., 2002). The LT of the 8th rib was extracted, vacuum packed and frozen at –80ºC for subsequent FA composition analysis by gas chromatography (GC).

Total fatty acid analysis

The FAs were extracted in 5M KOH in methanol/water (50:50) at 60ºC for 1 hour and esterified at 40ºC during 10 min with 2M trimethylsilyl-diazomethane in *n*-hexane according to Elmore *et al*. (1999) with some modifications. Separation of fatty acid methyl esters (FAMEs) was performed on a Varian CX3400 GC with a flame ionisation detector (FID) and a split/splitless injection port (50:1). GC analysis was performed using a B-PX 70 for FAME column (120m x 0.25mm i.d., 0.2µm film thickness) with programmed oven

temperature. Injector and detector ports were set at 270°C and 300°C, respectively. The carrier gas was hydrogen and the flow rate 1.6 ml/min measured at the initial temperature. Esterified FAs were identified according to similar peak retention times using standards and quantified according to internal standard method ($C_{23:0}$ methyl ester) added prior to saponification.

Statistical analysis

The statistical analysis was conducted using the SPSS11.5 program (2002). Principal component analysis was applied to explain, with a limited number of factors, the variation produced by breed type and feeding system on intramuscular fat level and on total fatty acid composition.

Results and discussion

The biplot (Figure 1) represents the first two principal components, which explained 72.8% of the variation observed on intramuscular fat level and fatty acid composition (individuals, groups and ratios). The first principal component (PC1) explained 55.6% of the variation observed, whilst the second principal component (PC2) explained an additional 17.2%.

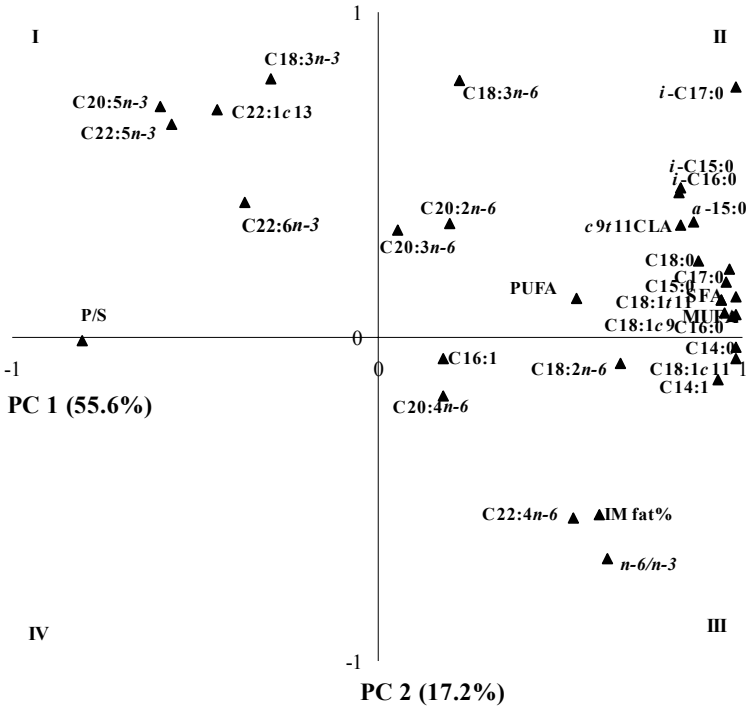

Figure 1. Biplot representation of principal components (PC1 & PC2) of different variables studied on the *Longissimus thoracis* muscle**.**

IM fat %: intramuscular fat percentage; *c*9,*t*11 CLA: *c*9,*t*11 C18:2; ∑SFA = C14:0 + C15:0 + C16:0 + C17:0 + C18:0; ∑MUFA = C14:1*c*9 + C16:1*c*9 + C17:1*c*10 + C18:1*t*11 + C18:1*c*9 + C18:1*c*11 + C22:1*c*13; ∑PUFA = C18:2*n-6* + C18:3*n-6* + C20:2*n-6* + C20:3*n-6* + C20:4*n-6* + C22:4*n-6* + C18:3*n-3* + C20:5*n-3* + C22:5*n-3* + C22:6*n-3* + *c*9*t*11 CLA*; n-6/n-3* = (C18:2*n-6* + C18:3*n-6* + C20:2*n-6* + C20:3*n-6* + C20:4*n-6* + C22:4*n-6*) / (C18:3*n-3* + C20:5*n-3* + C22:5*n-3* + C22:6*n-3*); P/S = ∑PUFA / ∑SFA.

The first principal component (PC1) was positively related to saturated, branched and monounsaturated FAs, and *c*9,*t*11 CLA contents. In consequence, it was also positively related to groups of aforementioned FAs (SFA & MUFA). In a lower degree, intramuscular fat level (IM fat %), PUFAs group and *n-6/n-3* ratio were also positively related to PC1. On the other hand, P/S ratio was negatively related to this principal component.

The second principal component (PC2), in general, was positively related to *n-3* FAs (in a low degree to $C_{22:6}$*n-3*) and *i*-$C_{17:0}$. However, it was negatively related to intramuscular fat level (IM fat %), *n-6/n-3* ratio and $C_{22:4}$*n-6*.

Figure 2 represents the projection of principal components (PC1 & PC2) for meat samples studied where meat samples were identified with feeding system and breed type labels. From these preliminary results it can be observed that feeding system was the most remarkable effect. In general, as there were some exceptions, extensively reared AV and AM breeds were separated from intensively reared AV and AM breeds. Both breeds (AV & AM) showed a similar behaviour from fat quantity and quality point of view when animals fed pasture. However, it is important to note that variability was higher in intensively fed animals in comparison to extensively fed ones. So taking into account the difficulty of the data interpretation, PC1 seemed to be negatively related to extensively reared animals and positively to intensively reared animals. And consequently, pasture fed animals could be related to *n-3* FAs and P/S ratio. This association between extensive system and *n-3* FAs was also seen by Steen et al. (2003) in pastured animals from crosses of the continental beef breeds and Varela et al. (2004) in extensively reared Rubia Gallega breed. On the other hand, animals fed with concentrate were related to PC1, and therefore, to SFAs, branched FAs, MUFAs, PUFAs (particularly *n-6* FAs), *c*9,*t*11 CLA, *n-6/n-3* and IM fat. Intensively fed animals of the rustic breed (AM), which basically laid in quadrant III, could be related to IM fat (%) and *n-6/n-3* ratio. In general feeding system effect seemed to be more clear in AM breed than in AV breed, as in the last one, when animals were fed with concentrate, high dispersion of the data was observed. On the other hand, the differentiation between both breeds was difficult on each feeding regime, and animals from both breeds appeared mixed in both feeding systems, concentrate and pasture.

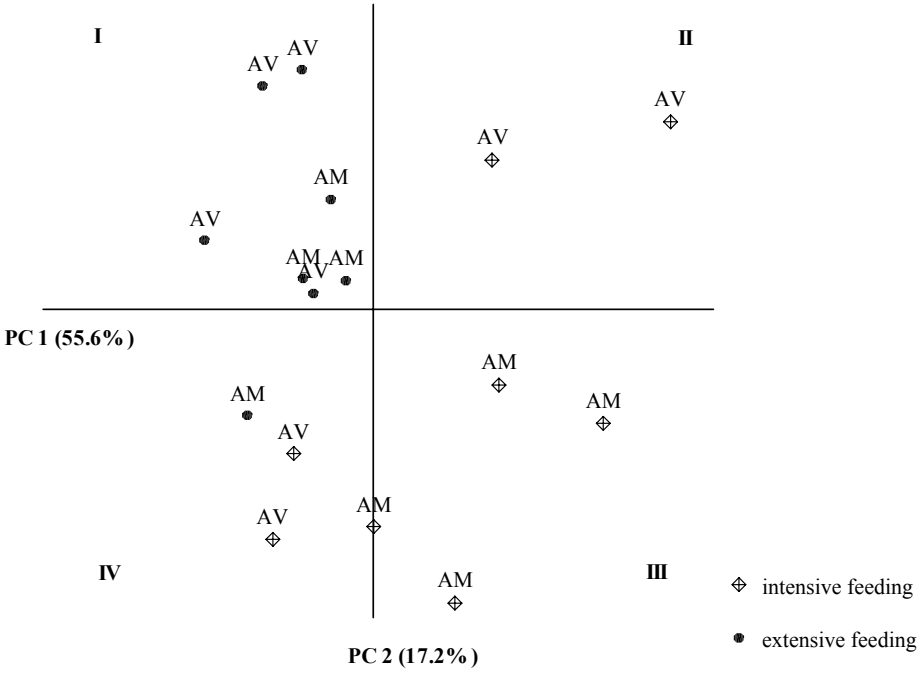

Figure 2. Projection of principal components (PC1 & PC2) of meat samples studied. AV: Asturiana de los Valles; AM: Asturiana de la Montaña.

Conclusions

These preliminary results from few animals are indicating that feeding system effect was more pronounced than breed type effect on intramuscular fat quality. Extensively produced meat showed a low fat content, high *n-3* PUFAs content and high P/S ratio, where pasture fed AV animals produced meat was the best adapted to human nutritional requirements. Breed effect was not significant in intensively, nor in extensively, reared animals.

Acknowledgements

N.A. acknowledges a predoctoral fellowship awarded by the Spanish Ministry of Science and Technology (INIA) and thanks Dr. Celaya from S.E.R.I.D.A. for his advice and support on statistical analysis.

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CONJUGATED OCTADECADIENOIC ACID ISOMERS (CLA) IN PORTUGUESE VEAL FROM AROUQUESA AND BARROSÃ AUTOCHTHONOUS BREED CALVES SLAUGHTERED IN EARLY AUTUMN

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Background

The conjugated linoleic acids (CLA), a group of positional and geometric octadecadienoic isomers with conjugated double bonds, are produced either in the rumen by the activity of microbial enzymes on polyunsaturated fatty acids (linoleic acid, 18:2 and linolenic acid, 18:3) or by endogenous desaturation (via delta-9 desaturase) of rumen derived octadecenoate isomers. Recent research has focused on the nutritional role of the CLA isomers because of their health benefits. These effects include potential anticarcinogenic, antiatherosclerotic and immune-modulating properties (Prates and Mateus, 2002; Demirel *et al*., 2004). Rumenic acid (*cis*-9,*trans*-11 CLA) has been associated with anticarcinogenic effects, while *trans*-10,*cis*-12 CLA isomer possesses an important role in the lipid metabolism (Fritsche and Fritsche, 1998). Meats from national demarcated regions with Protected Designation of Origin (PDO) are traditional meats (extensive production systems) produced in a delimited region whose quality is essentially due to the geographic environment. Furthermore, these PDO meats have supposedly unique characteristics, mainly in lipid fraction, linked to the local production systems and animal breeds.

Objectives

The aim of this study was to characterise the total CLA contents and CLA isomeric profiles of Portuguese traditional Arouquesa-PDO and Barrosã-PDO veal, both obtained from autochthonous calves fed extensively during summer (with the least abundant green pastures) and slaughtered in early autumn (October).

Materials and methods

Arouquesa-PDO and Barrosã-PDO veal were obtained from Arouquesa (7-8 months of age, 230 ± 28 kg live weight) and Barrosã (8-9 months of age, 192 ± 35 kg live weight) breed calves, produced in the north of Portugal (Fig. 1). The calves were reared on a traditional production pasture-based system according to the rules established in the product specifications. Muscles samples were collected from the ribeye (T1-T3 *longissimus thoracis* muscle, LT) and loin (L4-L6 *longissimus lumborum* muscle, LL) portions of *longissimus dorsi* muscle and from the distal region of *semitendinosus* muscle (ST) 2-3 days after slaughter (+1 °C). All muscle samples were ground using a food processor (3 × 5 s), vacuum packed and stored at -80 °C until analysed.

Total lipids were extracted from meat (dry matter) by ultrasonication, using methylene-chloride (4:1 v/v) (3×) and *n*-hexane (1×), as was previously described in Fritsche *et al.* (2000). Lipid contents of the test samples were calculated, in duplicate, by weighing the residues obtained after solvent evaporation under a stream of nitrogen. Methyl ester solutions of fatty acids were obtained by alkaline transesterification with sodium methoxide.

The methyl esters of CLA isomers were individually separated and quantified by triple column silver-ion (ChromSpher 5 Lipids, 4.6 mm ID × 250 mm, 5 µm particle size, Chrompack, Bridgewater, NJ, USA), using an HPLC system (HP 1100 Series, Hewlett-Packard, Palo Alto, CA, USA) equipped with autosampler and diode array detector adjusted at 233 nm, with a solvent (0.1 % acetonitrile in hexane) flow rate of 1 ml/min and injection volumes of 20-30 µl. The CLA isomers identification and quantification (external standard technique) were performed as described by Alfaia *et al.* (2003).

The data were analysed using the GLM procedure of SAS (1989). Total lipids and total CLA contents and the proportion of each CLA isomer were studied by analysis of variance, including the effects of breed and muscle type and their interaction. When the *F*-test was significant, the least-squares means were compared at a significance level of 5% ($P<0.05$).

Results and discussion

The intramuscular fat contents of the ribeye (T1-T3, LT) and loin (L4-L6, LL) portions of *longissimus dorsi* muscle and the distal region of *semitendinosus* (ST) muscle of Arouquesa-PDO and Barrosã-PDO veal are presented in Table 1. These data do not allow us to identify breed differences "*per se*" but the overall effect of breed and local production system (traditional production system). The content of total lipids (mg/g muscle) was similar ($P>0.05$) in the three muscles of both breeds. Our values were lower or similar to those reported by Roseiro *et al.* (2002) for Barrosã veal (2.97%, in *longissimus dorsi* muscle) but much lower than those reviewed by Chizzolini *et al*. (1999) for beef (6.3%, in *longissimus dorsi*, and 3.9%, in *semitendinosus* muscle). According to the criteria set by Food Advisory Committee (1990) (less than 5% of fat) these meats-PDO may be considered lean.

The isomeric distribution of individual CLA isomers (Fig. 2), the total content of CLA (mg/g muscle), as well as the content of some specific CLA (mg/g fat), are depicted on Table 1.

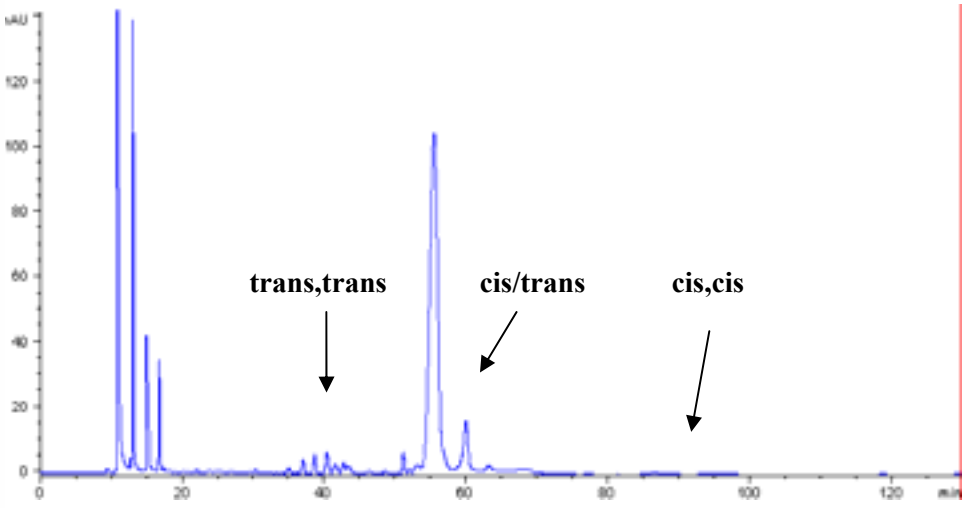

Figure 2. Typical Ag^{+}-HPLC chromatogram of CLA isomeric profile in veal from Arouquesa and Barrosã breed calves slaughtered in early autumn.

Figure 1. Geographical distribution of Arouquesa (A) and Barrosã (B) breed calves. Adapted from Direcção-Geral de Veterinária website.

The total CLA content was higher ($P<0.05$) in Arouquesa-PDO veal than in Barrosã-PDO veal, yet no significant differences were observed among the muscles within a breed. Furthermore, no significant differences were observed on specific CLA content in meat from LT, LL and ST muscles compared traditional veal obtained from calves of a different breed. The sum of *cis,trans* CLA isomers contributed around 93% (Barrosã breed) and 90.5% (Arouquesa breed) to total CLA. Total *trans,trans* CLA isomers contributed only with 9.0% and 6.4% in Arouquesa-PDO and Barrosã-PDO veal, respectively. The percentage of total *cis,trans* isomers is mainly due to the amounts of *cis*-9,*trans*-11 CLA isomer (84.7% to 85.5% for Barrosã-PDO and 79.3% to 80.6% for Arouquesa-PDO veal). The predominant CLA isomer, *cis*-

9,*trans*-11, showed significant differences between Arouquesa-PDO and Barrosã-PDO veal, and the variations in the amount of *cis*-9,*trans*-11 probably reflects either differences in the pasture composition or breed differences in delta-9 desaturase expression (Taniguchi *et al*., 2004). The interaction between breed and muscle type was significant for the proportions of *cis/trans*-12,14 and *trans*-11,*cis*-13 isomers. Finally, no significant differences in total *cis,cis* CLA isomers within each muscle and between the breeds were observed, and the mean amounts detected, mainly composed of the *cis*-9,*cis*-11 isomer, were lower than 0.6%.

Table 1. Analysis of variance and least-square means of total lipids and total CLA contents (mg/g muscle), specific CLA contents (mg/g muscle) and its individual isomers (% CLA) of intramuscular fat of the ribeye (T1-T3, LT) and loin (L4-L6, LL) portions of *longissimus dorsi* and the distal region of *semitendinosus* (ST) muscles of two traditional Portuguese breed calves slaughtered in early autumn.

	Arouquesa-PDO veal (n=15)			**Barrosã-PDO veal (n=12)**			Level of significance		
	LT (T1-T3)	LL (L4-L6)	ST (distal region)	LT (T1-T3)	LL (L4-L6)	ST (distal region)	B	M	B × M
Total lipids (mg/g muscle)	**23.6 ± 6.58**	**23.9 ± 10.00**	**24.6 ± 10.16**	**25.0 ± 6.29**	**20.0 ± 4.71**	**20.4 ± 6.42**	**ns**	**ns**	**ns**
Total CLA contents (mg/g muscle)	**0.206± 0.072**	**0.205± 0.102**	**0.221 ± 0.131**	**0.194 ± 0.120**	**0.164 ± 0.062**	**0.145 ± 0.046**	*	**ns**	**ns**
Specific CLA contents (mg/g fat)	**8.73 ± 1.77**	**8.66 ± 2.23**	**8.84 ± 2.57**	**7.56 ± 3.19**	**8.29 ± 2.49**	**7.28 ± 1.63**	**ns**	**ns**	**ns**
CLA isomers (% CLA)									
t12,t14	1.49 ± 0.88	1.69 ± 1.04	1.52 ± 0.47	0.97 ± 0.58	1.61 ± 0.68	1.21 ± 0.46	ns	ns	ns
t11,t13	2.24 ± 1.39	2.04 ± 1.24	1.52 ± 0.47	1.81 ± 0.64	1.09 ± 0.75	1.21 ± 0.60	**	*[#]	ns
t10,t12	0.49 ± 0.40	0.73 ± 0.72	0.44 ± 0.37	0.40 ± 0.36	0.52 ± 0.52	0.49 ± 0.49	ns	ns	ns
t9,t11	3.29 ± 2.05	3.58 ± 2.40	3.15 ± 1.88	1.88 ± 0.70	1.88 ± 0.73	2.65 ± 2.38	**	ns	ns
t8,t10	0.45 ± 0.37	0.26 ± 0.19	0.44 ± 0.66	0.36 ± 0.31	0.34 ± 0.28	0.18 ± 0.13	ns	ns	ns
t7,t9	0.52 ± 0.20	0.47 ± 0.31	0.48 ± 0.26	0.52 ± 0.27	0.66 ± 0.40	0.77 ± 0.48	*	ns	ns
t6,t8	0.37 ± 0.20	0.29 ± 0.25	0.36 ± 0.31	0.24 ± 0.17	0.29 ± 0.12	0.21 ± 0.13	ns	ns	ns
total t,t	***8.85 ± 3.41***	***9.06 ± 2.58***	***9.07 ± 2.38***	***6.18 ± 1.52***	***6.39 ± 1.42***	***6.74 ± 2.46***	***	**ns**	**ns**
c/t12,14	1.92 ± 1.78 [a]	0.75 ± 0.55 [b,c]	1.98 ± 1.56 [a]	1.35 ± 0.91 [a,c]	2.30 ± 2.08 [a]	1.73 ± 0.85 [a,c]	ns	ns	*
t11,c13	2.73 ± 2.28 [a,c]	3.89 ± 1.35 [a]	2.47 ± 2.28 [c,d]	2.68 ± 2.56 [a,c]	1.08 ± 1.22 [b,d]	0.75 ± 0.87 [b]	***	ns	*
c11,t13	0.41 ± 0.44	0.40 ± 0.54	0.46 ± 0.67	0.28 ± 0.28	0.33 ± 0.36	0.45 ± 0.35	ns	ns	ns
t10,c12	0.45 ± 0.46	0.55 ± 0.49	0.58 ± 0.62	0.38 ± 0.45	0.51 ± 0.73	0.36 ± 0.49	ns	ns	ns
c9,t11	80.62 ± 5.37	79.26 ± 4.47	80.19 ± 4.55	84.71 ± 4.51	85.48 ± 2.93	85.31 ± 3.39	***	ns	ns
t7,c9	4.62 ± 2.36	5.61 ± 2.56	4.73 ± 2.44	3.79 ± 1.44	3.46 ± 1.67	4.20 ± 2.31	*	ns	ns
total c/t	***90.74 ± 3.49***	***90.46 ± 2.60***	***90.41 ± 2.51***	***93.18 ± 1.94***	***93.17 ± 1.34***	***92.81 ± 2.37***	***	**ns**	**ns**
total c,c	***0.41 ± 0.24***	***0.48 ± 0.23***	***0.52 ± 0.37***	***0.64 ± 0.54***	***0.44 ± 0.26***	***0.45 ± 0.35***	**ns**	**ns**	**ns**

B, overall effect of breed and local production system; M, muscle type; ns not significant; means in the same row with different superscripts are different ($P<0.05$); * $P<0.05$; ** $P<0.01$; *** $P<0.001$; [#] (LT=LL, LL=ST and LT>ST).

Conclusions

These preliminary results indicate that when evaluating the CLA isomeric profile, and more specifically the content of the CLA in configuration *cis*-9,*trans*-11 in meat, one must take into account the overall effect of breed and local production system. From the nutritional point of view, the fat from the Barrosã veal may be more healthful because of its higher proportion of *cis*-9,*trans*-11 CLA isomer. Although no significant differences were found for specific CLA contents, the total CLA contents and the sum of *trans,trans* and *cis,trans* isomers were significantly influenced by the traditional production system. The results also showed that in general no significant differences occur within CLA isomeric profile in LT, LL and ST muscles for Arouquesa and Barrosã veal.

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Acknowledgements

Sampling assistance (Dr. Carlos Venâncio, ANCRA - Associação Nacional dos Criadores da Raça Arouquesa; Eng. João Paulo Teixeira, CAPOLIB - Cooperativa Agrícola de Boticas, CRL) and financial support (project CIISA/2002/52.Carne-Bioactivos) are acknowledged.

COMPARISON OF TOTAL CONTENT OF LIPID AND CHOLESTEROL IN PORTUGUESE BOVINE MEATS-PDO FROM FOUR DIFFERENT AUTOCHTHONOUS BREEDS (BARROSÃ, AROUQUESA, ALENTEJANA AND MERTOLENGA)

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Background

Portuguese bovine meat production is based either on intensive rearing of crossbred and meat specialized exogenous breeds or on traditional extensive rearing of autochthonous breeds. The traditional meat production system of the Portuguese bovine autochthonous breeds represents an important socio-economical factor of wealth to the rural populations as it provides meat, milk and labor. In a recent past, the introduction of exotic bovine breeds specialized in meat or milk production, such as Charolais, Limousine and Friesian, have created a threat to the preservation of Portuguese autochthonous bovine livestock and to the traditional meat production system, characterized by the utilization of local agricultural resources with little or no costs in pellet feeding. In the present days, autochthonous bovine population is increasing thanks to active producer associations, and the meat of these breeds is being commercialized under the Protected Designation of Origin (PDO) certification, which obliges producers to follow the traditional rearing methods. Arouquesa and Barrosã, from the North Atlantic territory of Portugal, and Alentejana and Mertolenga, from the South Mediterranean territory of Portugal, are four commercially important autochthonous Portuguese breeds (Figure 1). It is supposed that each meat-PDO has unique characteristics, particularly in its lipid fraction, thanks to traditional, unique production system and to the breed own characteristics. However, for the consumers, the meat-PDO represents an expensive choice, which can only be justified by enhanced sensorial and nutritional characteristics.

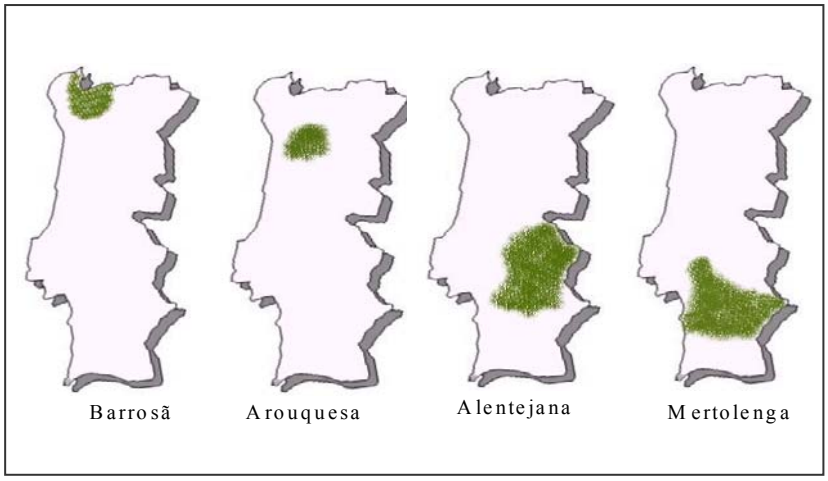

Figure 1. Geographical distribution of the Portuguese autochthonous bovine breeds analyzed in this study.

Objectives

The aim of this work was to compare the total content of lipids and cholesterol in Arouquesa-PDO veal, Barrosã-PDO veal, Alentejana-PDO beef and Mertolenga-PDO beef, all obtained from autochthonous bovines fed extensively during winter and early-mid spring (with the most abundant green pastures) and slaughtered in late spring (June).

Materials and methods

Meat samples from Arouquesa (113 ± 18 kg of carcass weight, 8.5 ± 0.9 months) and Barrosã (106 ± 21 kg, 7.9 ± 1.8 months) breed calves, and from Alentejana (357 ± 32 kg, 20 ± 2 months) and Mertolenga (236 ± 31 kg, 23.6 ± 2.8 months) breed bulls, were taken from the ribeye (*longissimus thoracis*; T1-T3), from the loin (*longissimus lumborum*; L1-L3) and from the distal portion of the *semitendinosus* muscle. The samples were collected in late spring, 2-3 days after slaughter, and stored at -80ºC until analysed.

Total lipids were extracted from meat (dry matter) by ultrasonication, using methylene-chloride (4:1 v/v) (3×) and *n*-hexane (1×), as was described by Fritsche *et al.* (2000). Total lipid content of the samples was calculated, in duplicate, by weighing the residues of solvent evaporation under a stream of nitrogen.

Total cholesterol was also extracted from meat (dry matter) with *n*-hexane after direct saponification with 0.5 M KOH solution for 15 min at +80ºC (Fletouris *et al.*, 1998). Cholesterol quantification was performed by normal-phase HPLC (column Zorbax Rx Sil, 4.6 mm ID × 250 mm, 5 µm particle size, Chrompack, USA), using an auto-sampler and diode array detection (DAD) at 206 nm, a solvent flow rate of 1 ml/min and injections volumes of 30 µl (Figure 2). The total cholesterol content in meat was expressed as a mean of two replicates with a variation coefficient lower than 3.5%.

The statistical analysis of the total lipids and total cholesterol contents were performed using the GLM procedure of SAS (1989) at a significance level of 5% ($p<0.05$). When the *F*-test of analysis of variance was significant, the least-squares means were compared at the same significance level.

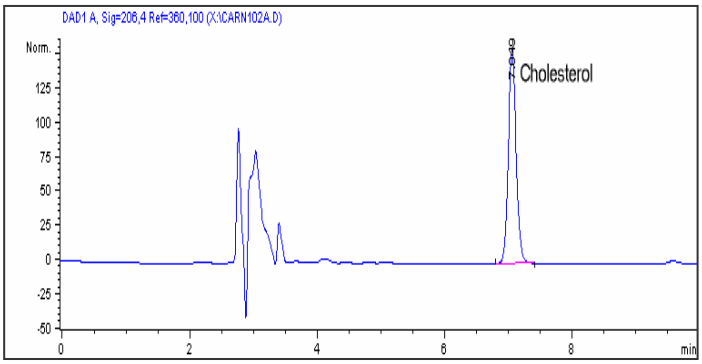

Figure 2. Typical normal-phase HPLC chromatogram of total cholesterol in a meat sample, detected at 206 nm.

Results and discussion

These data do not allow us to identify breed differences "*per se*" but the overall effect of breed and local production system (traditional production system). Total cholesterol content was higher in *semitendinosus* muscle than in the other muscles analyzed in all four breeds (Table 1). The total cholesterol content of Alentejano and Mertolengo bulls are similar to those reported for Podolian young bulls (Cifuni *et al.*, 2004) and feedlot beef cattle (Rule *et al.*, 2002). Arouquesa and Barrosã veal had slightly lower total cholesterol content than the average reported by Chizzollini *et al.* (1999). Total lipid content of meat did not show a common pattern in the different muscles of the four breeds (see Table 1). However, according to the criteria set by the Food Advisory Committee (1990) (less than 5% of fat), all meats analyzed were lean. This trait alone can be considered a quality characteristic of these meats-PDO, because it is independent of the season and availability of nutrition. The analysis of specific cholesterol content showed that *longissimus thoracis* had the highest content, while *longissimus lumborum* and *semitendinosus* had similar specific cholesterol contents in all breeds, except for Alentejana bulls. The specific cholesterol contents distribution among the different muscles analyzed did not show a regular pattern. The total lipid content and total cholesterol content showed a highly significant positive correlation ($R^2=0.135$, $P<0.0001$, $n=180$), although, according to earlier work, marbling depots seemed to have minimal effect on the total cholesterol content of meat (Kinney Sweeten *et al.*, 1990). This controversy may be explained by the fact that cholesterol deposits are mainly in the membranes not in the cytoplasm of the adipose tissue. The statistical analysis performed to evaluate the possible interaction between muscle and breed, revealed a significant interaction between the two factors for all analyzed parameters, which means an absence of a common pattern for the different muscles of the breed calves and bulls analyzed.

Table 1. Means of total lipids (TL, mg/g meat), total cholesterol (TC, mg/g meat) and specific contents of total cholesterol (SC, mg/g lipids) measured in *semitendinosus* (ST), *longissimus lumborum (LL)* and *longissimus thoracis (LT)* muscles of four Portuguese bovine meats-PDO.

	Arouquesa			Alentejana			Mertolenga			Barrosã		
	ST	LL	LT	ST	LL	LT	ST	LL	LT	ST	LL	LT
[1] TC	0.61^{a}	0.54^{e}	0.58^{b}	0.49^{d}	0.45^{e}	0.43^{e}	0.50^{d}	0.44^{e}	0.44^{e}	0.61^{a}	0.52^{cd}	0.57^{bc}
[2] TL	30.8^{a}	23.7^{b}	17.3^{cde}	14.1^{abc}	20.7^{def}	10.9^{h}	17.9^{edc}	16.3^{defg}	12.1^{gh}	22.5^{bc}	16.3^{defg}	14.9^{efgh}
[3] SC	23.8^{a}	25.5^{a}	35.7^{bcd}	41.2^{d}	25.2^{a}	41.5^{d}	29.5^{ab}	28.8^{ab}	38.8^{cd}	30.1^{ab}	32.9^{bc}	40.9^{d}

[1] – Significant interaction ($p< 0.01$) between muscle type (M) and breed (B); standard error of means (s.e.m.) = 0.0107
[2] – Significant M*B interaction ($p< 0.0001$), s.e.m. = 1.75
[3] - Significant M*B interaction ($p< 0.04$), s.e.m. = 2.647

Conclusions

We conclude that the Portuguese autochthonous breeds studied, although not specialized for meat production, represent an important source of lean meat with similar cholesterol content. These breeds are produced with the local resources in a production system where the specialized breeds cannot compete.

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Acknowledgements

Sampling assistance (Dr. Luís Bagulho Silva, Carnalentejana S.A.; Eng. João Potes, Eng. Roberto Santos e Eng. Paulo Ventura, Montado Alentejano e Tradição - Produtos Pecuários, S.A.; Dr. Carlos Venâncio e Eng. Manuel Cirnes, ANCRA - Associação Nacional dos criadores da Raça Arouquesa; Eng. João Paulo Teixeira, CAPOLIB -Cooperativa Agrícola de Boticas, CRL) and financial support (project CIISA/2002/52.Carne-Bioactivos) are acknowledged.

ANTIHYPERTENSIVE ACTIVITIES GENERATED FROM PORCINE SKELETAL MUSCLE PROTEINS BY LACTIC ACID BACTERIA

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Background

Many kinds of bioactive peptides are known to be generated from food proteins (Korhonen & Pihlanto, 2003). Especially from milk proteins, such peptides (*e.g.*, antihypertensive, antimicrobial and mineral binding peptides) have been discovered considerably. In some countries (*e.g.*, Japan), food industry has shown a great interest in bioactive peptides from food proteins for developing novel functional foods.

Angiotensin I-converting enzyme (ACE) inhibitory peptides are representative bioactive peptides generated from food proteins. ACE plays an important physiological role in regulating blood pressure (Figure 1). Several inhibitors of ACE have been found to be effective as antihypertensive pharmaceuticals. ACE inhibitory peptides derived from food proteins have been reported to create antihypertensive effects in spontaneously hypertensive rats by oral administration (Yamamoto *et al*., 1999). The antihypertensive effect of ACE inhibitory peptides derived from the casein of sour milk, was demonstrated in hypertensive human patients (Hata *et al*., 1996). This product has been developed into a new physiologically functional food in Japan. Research has also been conducted to characterize the ACE inhibitory activity derived from other foodstuffs, such as maize, eggs, gelatin, fish and fish products. However, little is still known about the derivation of such peptides from muscle proteins of domestic animals.

Recently, we have found that enzymatic hydrolysates of porcine skeletal muscle proteins exhibited potent ACE inhibitory activity (Arihara *et al*., 2001). Among muscle protein hydrolysates produced with eight different proteases, the digest of thermolysin showed the most potent inhibitory activity. ACE inhibitory peptides, named myopentapeptide A (Met-Asn-Pro-Pro-Lys) and B (Ile-Thr-Thr-Asn-Pro), were isolated from the thermolysin hydrolysate. Moreover, hydrolysates of porcine myosin as well as some peptides identical to a sequence of myosin showed antihypertensive activity in spontaneously hypertensive rats (Nakashima *et al*., 2002).

The results of these studies suggest that ACE inhibitory and antihypertensive peptides are easily generated from muscle proteins by enzymatic digestion. Thus, in meat products, such as fermented meat products with long-term ripening, these kinds of peptides may be generated. In fact, we have already detected ACE inhibitory activity in several commercial fermented meat products (unpublished data). Naturally occurring ACE inhibitory activity has also been detected in well-ripened cheese.

Objectives

In the present study, we investigated the generation of ACE inhibitory and antihypertensive activities in porcine skeletal muscle homogenates fermented with lactic acid bacteria. Efforts were also made to purify and identify the corresponding peptides from the homogenates. Such activities and substances could be utilized for producing new healthy meat products, which might open up a new market in the meat industry.

Materials and methods

Materials and Reagents

Fresh pork trim (ham) was obtained from a local packer. Hippuryl-L-histidyl-L-leucine (Hip-His-Leu) and ACE (from rabbit lung) were obtained from Sigma Chemical Co. (St. Louis, MO). Other chemicals were obtained from Wako Chemicals Co. (Tokyo, Japan).

Preparation of Fermented Homogenates

Fermented muscle homogenate was prepared as shown in Figure 2. Porcine skeletal muscle was homogenated in a Waring-type blender with four volumes of distilled water. Glucose (2%) and one of 5 strains (*Lactobacillus gasseri* JCM1131, *L. rhamnosus* FERM P-15120, *L. acidophilus* IAM12475, *L.*

helveticus JCM1554, *L. delbrueckii* subsp. *bulgaricus* NCFB2483) of lactic acid bacteria were added to the homogenate. These 5 strains of lactic acid bacteria were selected according to their proteolytic activities in our previous study (unpublished data). After 72 h of fermentation at 37°C, homogenates were heated at 98°C for 10 min to inactivate the bacterial and muscle protease activities. After removal of insoluble materials by centrifugation, the supernatants were utilized for further experiments.

Assay of ACE Inhibitory Activity

The ACE inhibitory activity was measured by a spectrophotometric assay according to Cushman and Cheung (1971) with modification by Nakamura *et al*. (1995). This assay is based on the liberation of hippuric acid from Hip-His-Leu catalyzed by ACE. The method was slightly modified in the present study. A sample solution of peptides (15 µl) was mixed with 125 µl of 100 mM sodium borate buffer (pH8.3) containing 7.6 mM Hip-His-Leu and 608 mM NaCl and then preincubated for 5 min at 37°C. The reaction was initiated with the addition of 50 µl of ACE dissolved in distilled water (50 m units/ml), and the mixture was incubated for 30 min at 37°C. The reaction was stopped by adding 125 µl of 1N HCl. The hippuric acid liberated by ACE was photometrically determined at 228 nm after ethyl acetate extraction. The concentration of ACE inhibitors needed to inhibit 50% of ACE was defined as the IC_{50} value.

Antihypertensive Effect in Spontaneously Hypertensive Rats

Male spontaneously hypertensive rats (SHR), 10 week old, were purchased from Charles River Japan Inc. (Yokohama, Japan). The SHR were housed in cages on a cycle of 12 h of light and 12 h darkness. The temperature and humidity in the cages were maintained at 24°C and 50 to 60%, respectively. The SHR were fed a standard laboratory diet (CE-2; Clea Japan, Inc., Tokyo, Japan), and tap water was freely available. A solution containing peptides was adjusted to 50 mg of solid material per ml, and was administered on the rats at a dose of 1 ml ie., 150 mg/kg of body weight. The *in vivo* antihypertensive activity was measured by monitoring the systolic blood pressure (SBP) of the SHR, from 15 to 28 week old (280 to 390 g in body weight). Rats given the sample solution via a gastric metal zonde were put in a thermostatted box at 40°C for 15 min, and the SBP was measured with a tail cuff equipped with a programmed electrosphygmomanometer (BP-98; Sftron Co., Tokyo, Japan, Figure 4). Phosphate-buffered saline (PBS) was used as a control in SHR.

Purification of ACE inhibitory Peptide

The supernatant solution of fermented muscle homogenate was fractionated by high-performance liquid chromatography (HPLC) with reversed-phase mode (column: CAPCELL PAK C_{18} UG120 4.6 x 150 mm; Shiseido, Tokyo, Japan) as described previously (Arihara *et al.*, 2001). Elution was performed with a linear gradient system from solvent A (0.1% trifluoroacetic acid in CH_3CN) at a flow rate of 1m/min, and absorbance was detected at 215 nm (first HPLC run). The active fraction was lyophilized, dissolved with distilled water, and rechromatographed under the same conditions as described above (second HPLC run). The peptide samples were further purified by HPLC with the same system except for the elution solution (third HPLC run). Elution was performed with a linear gradient (solvent A: 0.015% ammonia in distilled water, solvent B: 0.015% ammonia in CH_3CN).

Analysis of Peptide

The molecular formula of the peptide was confirmed from its fast atom bombardment mass spectrum (FAB-MS) obtained using an HX-110 spectrometer (JEOL Ltd, Tokyo, Japan). The sequence of the peptide was analyzed by automated Edman degradation using a 470A Protein Sequencer (Applied Biosystems, Inc., Forster City, CA).

Synthesis of Peptide

The synthesized peptide used in this study was prepared by the solid phase method with a 430A Peptide Synthesizer (Applied Biosystems, Inc.). Hydrogen fluoride was used for removing the side chain-protecting groups and for cleaving the peptide from their solid support. The synthesized peptides were purified by HPLC on a reversed-phase column (CAPCELL PAK C_{18} UG120 4.6 x 150 mm; Shiseido, Tokyo) with a linear gradient of CH_3CN (0 to 20%) in 0.1% trifluoroacetic acid.

Results and discussion

All muscle homogenates fermented with any of the *Lactobacillus* strains (*L. gasseri* JCM1131, *L. rhamnosus* FERM P-15120, *L. acidophilus* IAM12475, *L. helveticus* JCM1554, *L. delbrueckii* subsp. *bulgaricus* NCFB2483) showed ACE inhibitory activities higher than non-fermented muscle homogenate (Figure 4).

Among these 5 strains, the homogenate fermented with *L. rhamnosus* showed the highest inhibitory activity. Further experiments were then carried out on the muscle homogenate fermented with *L. rhamnosus*. The single oral administration of this homogenate to SHR significantly decreased their systolic blood pressure (Figure 5). The peptide responsible for the ACE inhibitory activity was purified from the homogenate by the combination of HPLC with reversed-phase mode. Octapeptide with the ACE inhibitory activity was purified and its amino acid sequence was determined (Val-Phe-Pro-Met-Asn-Pro-Pro-Lys). A search for sequence homology in databases revealed that the same sequence existed in the primary structure of the porcine skeletal muscle myosin heavy chain. The ACE inhibitory activity (IC_{50}) against the synthesized octapeptide was determined as 66.0 uM.

In the dairy industry, many physiologically functional foods have been developed. Asic studies on the tertiary function of milk components and on physiologically functional dairy prdoducts have been extensively conducted (Arai, 1996). However, there have been few such studies on meat products. Although many low-fat and low-salt meat products have been developed, there have been no efforts to introduce physiologically functional properties into meat products. We have reported recently that the concept of probiotics (cultures of live microorganisms that benefit the host by improving properties of indigenous microflora) has great potential in the meat industry (Arihara *et al*., 1998; Sameshima *et al*., 1998). *L. rhamnosus* FERM P-15120, the strain selected in this study, is a probiotic lactic acid bacteria isolated from human intestinal tract (Sameshima *et al*., 1998). Therfore, fermented meat products prepared with this strain are expected to have both probiotic and antihypertensive properties.

By using bioactive components, properties having potential health benefits can be introduced into meat products, thus improving the nutritional value of the products. Utilization of ACE inhibitory activity and substances from meat proteins may lead to the development of new healthy meat products.

Conclusions

This study demonstrated that peptides with ACE inhibitory and antihypertensive activity may be generated from meat by bacterial fermentation. Furthermore, fermentation of meat by lactic acid bacteria resulted in the generation of the ACE inhibitory peptide. The results of this study suggest that ACE inhibitory and antihypertensive activities generated by fermentation could be utilized to develop physiologically functional foods. Although bioactive peptides, such as ACE inhibitors, have not yet been utilized in the meat industry, meat products with such acitivity could open up a new market in the near future. It is expected that increasing interest will be shown in basic research and potential applications of bioactive peptides for meat products.

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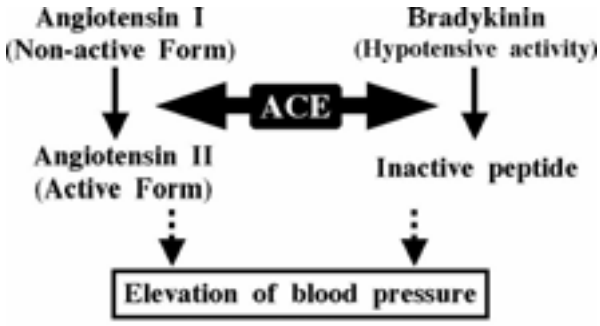

Figure 1. Role of angiotensin I-converting enzyme

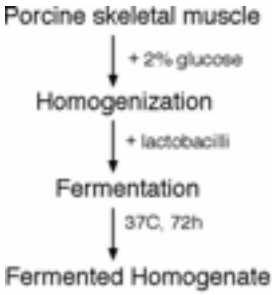

Figure 2. Preparation of fermented muscle homogenates.

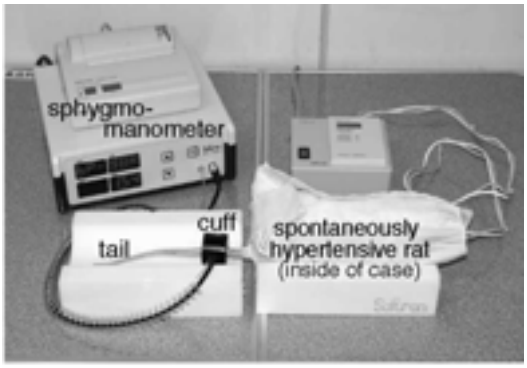

Figure 3. Electrosphygmomanometer used for the measurement of SBP.

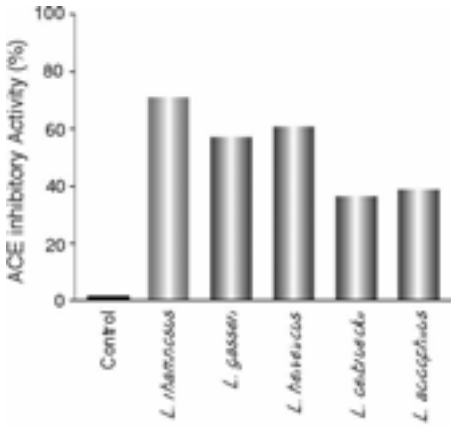

Figure 4. ACE inhibitory activities of fermented muscle homogenates.

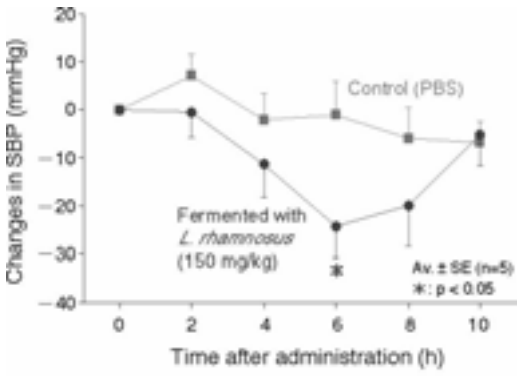

Figure 5. Antihypertensive activities of fermented muscle homogenates given to SHR in single oral administration.

FATTY ACID CONTENT AND COMPOSITION OF MUSCLE LIPIDS OF GOAT KIDS FED SUNFLOWER OIL SUPPLEMENTED DIET

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Background

Consumers, becoming more health conscious, are increasingly paying attention to quality aspects of meat, particularly to lean meat. Goat meat is a good source of lean meat due to its very little intramuscular fat content. However, the leanness of goat meat may be a disadvantage in promoting goat meat products, which may have inferior juiciness, palatability, "mouth feel", and tenderness. Therefore, a shift in the partitioning toward deposition of more inter- and intramuscular fat would increase carcass quality as well as consumer acceptance. High concentrate diets enhanced mostly internal fat (Bas *et al*., 1982), but did not influence muscle composition (Muller *et al*., 1985) in goat kids. Using diets rich in 18:2 showed a decrease of the total lipid content and an increase in the amount of phospholipids (PL) in muscles of calves and lambs (Jenkins and Kramer, 1990; Ponnampalam *et al*. 2001). In contrast, in our previous study with kids (Marinova *et al*., 2001), sunflower oil supplemented diet increased the intermuscular fat and total fat content in all meat cuts.

However, not only fat content, but also its fatty acid profile affects the quality of meat. The interest in fatty acids with respect to consumer health lies in the proportions of polyunsaturated (P) and saturated (S) fatty acids, their ratio (P/S), the ratio of n-6/n-3 fatty acids, as well as the content of cholesterol. In addition to the beneficial effects of polyunsaturated fatty acids, the conjugated linoleic acid (CLA) isomers have received much attention for their potential health promoting effects. Vegetable oils as natural sources of the essential linolenic and linoleic acids (being precursors of CLA) are now attracting increased research attention. Feeding calves and lambs diets supplemented with unprotected sources of 18:2, resulted in an increase of muscle PL fraction, but decreased the n-6/n-3 ratio (Jenkins and Kramer, 1990; Ponnampalam *et al*. 2001). Recently it was demonstrated that an unprotected sunflower oil supplemented diet significantly increased CLA content in the triacylglycerol (TG) fraction of various lamb tissues (Peterson at al., 2002). However, no data related to the effect of vegetable oils on the lipid content and composition in goat muscles are available.

Objectives

The object of this study was to examine the effect of dietary enrichment of n-6 fatty acids (sunflower oil) on the content and fatty acid composition of muscle triacylglycerols and structural phospholipids, as well as cholesterol level, in growing goat kids.

Material and methods

Two groups of five male kids (age 3 months) were fed iso-nitrogenous diets for 21 days, as described in a previous paper (Marinova *et al.,* 2001). The diets contained either no added fat (control), or sunflower oil (2.5% of as-fed basis weight of concentrate) (experimental). Samples from *M. longissimus dorsi* (LD)*, M. semimebranosus (*SM) *and M. supraspinalis* (SP) were taken after slaughter at 24h *post mortem*, and lipids were extracted according to the method of Bligh and Dyer (1959). Aliquots of the lipid extracts were submitted to cholesterol and phospholipid assays, using the methods of Sperry and Webb (1950) and Bartlet *et al*.(1959), respectively. Methyl esters of PL and TG isolated by preparative TLC, were obtained using a 0.01% solution of sulfuric acid in dry methanol at 47^{o}C for 14h, as described by Christie (1973). The fatty acid composition of the lipid fraction as well as the content of TG, were analyzed by gas chromatography using triarahidin as an internal standard. The effect of treatment (control ν sunflower oil supplemented group) was subjected to Student's test for determination of significance.

Results and discussion

The intramuscular fat (i.f) content, and the contents of the single lipid fractions distinguished between the three muscles (Table 1). In m.SP, where the total fat content was highest, the concentrations of PL and cholesterol were also higher, but the TG level lower, compared with the other two muscles.

Table 1. Contents of intramuscular fat, phospholipids, triacylglycerols and cholesterol in M. *longissimus dorsi, M. semimembranosus and M. supraspinalis,* of goat kids in response to feeding sunflower oil

Muscles	*M. longissimus dorsi*		*M. semimembranosus*		*M. supraspinalis*	
			Groups[a]			
Items	Control	Experimental	Control	Experimental	Control	Experimental
Internal fat [b]	16.20±10.1	22.50±11.6	15.60±3.60	19.30±4.9	20.10±4.7	25.50±7.7
PL [b]	4.44± 0.51	4.90± 0.89	5.81±0.79	6.30±0.85*	6.08±1.81	6.30±1.08
Cholesterol [b]	0.54± 0.18	0.85± 0.06	0.80±0.15	1.16±0.07*	0.92±0.14	1.21±0.11*
TG [c]	2.15± 0.29	3.33± 0.41	1.44±0.19	3.19±0.62	1.29±0.13	3.79±0.78

[a] Control: no added sunflower oil; experimental : sunflower oil supplemented diet (2.5% of weight of concentrate as fed-basis) ; [b] PL – phospholipids; mg/g fresh tissue; [c] TG – triacylglycerols" µ mol/g fresh tissue; * $P < 0.05$.

Sunflower oil supplementation tended to increase (although to a different extent in different muscles) all lipid fractions. Jenkins and Kramer (1990); Ponnampalam *et al*. (2001) also reported higher PL level after corn or sunflower treatment, but reduced muscle TG levels. Contrary to the negligible changes in PL fractions, the TG concentrations were increased by 55%, 120% and 193%, (Table 1), respectively for m.LD ,m.SM and m.SP.

Table 2. Fatty acid composition (M %) of triacylglycerols from *M. longissimus dorsi, M. semimembranosus and M. supraspinalis* of goat kids in response to feeding sunflower oil

Muscles	*M. longissimus dorsi*		*M. semimembranosus*		*M. supraspinalis*	
			Groups[a]			
Fatty acids	Control	Experimental	Control	Experimental	Control	Experimental
14:0	4.65±1.38	3.12±0.41	2.01±0.74	1.32±0.06	4.90±1.43	4.01±0.33
16:0	25.79±0.68	26.27±0.77	28.48±0.58	26.81±0.34*	26.18±1.37	26.71±0.60
16:1	3.64±0.27	2.56±0.21*	3.32±0.66	2.58±0.12	2.98±0.38	2.56±0.29
17:1	1.18±0.12	0.90±0.12	-	-	-	-
18:0	13.23±0.96	15.62±0.95	15.79±1.26	18.19±1.33	14.72±2.04	16.20±1.21
18:1	48.40±3.25	49.06±0.34	48.24±2.05	46.96±1.38	48.38±2.55	47.78±0.70
18:2	3.11±0.82	2.47±0.13	2.16±0.41	4.14±0.44*	2.84±0.29	2.74±0.30
UFAb	56.33	54.99	53.72	53.68	54.20	53.08

[a] Control: no added sunflower oil; experimental : sunflower oil supplemented diet (2.5% of weight of concentrate as fed-basis) ; [b] UFA - Unsaturated fatty acids; *$P<0.05$.

The increase of fat in kids' muscle after sunflower oil treatment leads to an increase in the relative diameter of larger cells and to a corresponding decrease in that of the smaller cells, most noticeably in SP muscle (Marinova, 2003). Paterson *et al*. (2002) reported that sunflower oil supplementation, as a source of unprotected 18:2, resulted in a significant increase of CLA content in different lamb tissues, and suggested that the biological activity of CLA may be more related to deposition of fat rather than to specific effects on PL alterations. The increased intramuscular fat could have a beneficial effect on the marbling of lean goat meat, although the tendency of getting more cholesterol would have to be considered.

Similarly to other livestock species reared for meat production, the major fatty acids in TG muscle of kids were 16:0, 18:0 and 18:1 (Table 2). The sum of unsaturated fatty acids (UFA) did not differ between the three muscles, although small variations in the proportions of 14:0, 18:0 and 18:2 were observed. In experimental animals, the only significant changes ($p<0.05$) were the reduction in the proportions of 16:1 in LD and 16:0 in SM muscles. The reduction of 16:0 in SM was apparently compensated for by an increase of

18:0 and 18:2 contents. Paterson *et al*. (2002) reported similar changes with sunflower oil in the rib muscle of lambs. Sunflower oil also tended to decrease the proportion of 18:1 in SP muscle, while increasing 18:0 in all three muscles. However, in SM muscle, no increase of 18:2 was observed in the other two muscle TG fractions. It may be speculated that blood flow delivered different amount of 18:2 to different muscles, or, the decreased proportion of 18:2 in LD and SP muscles was due to a conversion of 18:2 to CLA, which substitutes 18:2 in TG molecules, as was suggested by Peterson at al. (2002). Unfortunately we were not able to determine the CLA content.

Table 3. Fatty acid composition (M%) of phospholipids from *M. longissimus dorsi, M. semimembranosus and M. supraspinalis* of goat kids in response to feeding sunflower oil

Muscles	*M. longissimus dorsi*		*M. semimembranosus*		*M. supraspinalis*	
			Groups[a]			
Fatty acids	Control	Experimental	Control	Experimental	Control	Experimental
14:0	0.85±0.04	0.96±0.42	0.11±0.01	0.53±0.03**	0.90±0.28	1.25±0.27
15:0	0.42±0.12	0.52±0.09	-	-	0.24±0.02	0.39±0.15
16:0	22.03±0.64	23.15±1.39	20.57±0.50	19.74±0.23	18.55±1.06	19.04±1.00
16:1 7	0.72±0.13	1.35±0.18*	1.00±0.20	1.54±0.22	0.70±0.07	1.17±0.14*
16:1 9	1.00±0.24	0.78±0.13	1.18±0.19	0.78±0.08	0.84±0.14	1.00±0.13
17:0	1.48±0.29	1.08±0.06	0.88±0.12	0.91±0.10	1.14±0.23	1.08±0.19
17:1	0.87±0.09	0.83±0.15	1.01±0.16	0.82±0.10	0.84±0.18	0.57±0.11
18:0	17.05±0.95	17.53±1.12	17.31±0.63	18.13±0.40	16.50±0.84	15.95±0.51
18:1	30.62±2.18	29.04±3.01	31.71±2.57	28.86±1.97	30.88±1.91	31.95±1.92
18:2	13.83±1.38	13.52±1.75	14.93±2.13	17.41±0.95	17.96±1.05	18.32±1.59
18:3	1.84±0.19	1.80±0.30	2.26±0.40	1.92±0.17	2.27±0.49	1.74±0.16
20:3	0.60±0.03	0.67±0.13	0.45±0.02	0.72±0.07*	0.52±0.07	0.44±0.04
20:4	7.75±0.62	7.28±1.49	8.21±1.40	7.81±0.35	7.79±0.54	6.60±0.49
20:5	0.94±0.16	1.49±0.48	0.38±0.20	0.83±0.30	0.87±0.42	0.50±0.21
PUFA[b]	24.95	24.76	26.23	28.69	29.41	27.60
P/S[c]	0.60	0.57	0.67	0.73	0.79	0.73
n-6/n-3	7.98	6.53	8.94	9.43	8.37	11.32

[a] Control: no added sunflower oil; experimental : sunflower oil supplemented diet (2.5% of concentrate as fed-basis) ; [b] polyunsaturated fatty acids; [c] P/S – polyunsaturated/saturated ratio; *P<0.05.

FA profiles of PL in the three muscles of goat kids reflected the metabolic type of muscles (Table 3). The highest proportion of 16:0, and the lowest proportion of 18:2 were found in LD, whereas no differences in the content of 18:1 were observed between muscles. The linoleic acid concentration in the three muscles was higher than that reported for muscle PL of goats of different breeds and ages (Banskalieva *et al*., 2000). The SP muscle being oxidative type had a higher level of PL (Table 1), as well as a higher proportion (29.6%) of polyunsaturated fatty acids (PUFA) than the LD and SM muscles of oxidative-glycolytic type (24.95% and 26.23%, respectively). The n-6/n-3 ratios in the LD, SM and SP were 7.98, 8.94 and 8.74, respectively, ie. higher than the minimum of 4.0 officially recommended. High n-6/n-3 ratio with great variation (4.38 to 19.62) caused by differences in age, breed, muscles, diet, etc. have also been reported earlier in goats (Banskalieva, *et al*., 2000). The P/S ratios of 0.60, 0.67 and 0.79 of LD, SM and SP, respectively, were somewhat comparable to the recommended beneficial value of 0.7, indicating that as a meat producing species, goats have a rather optimal P/S ratio.

The sunflower oil treatment had no significant effect on the major fatty acids (16:0,18:0 and 18:1) in muscle PL fractions of kids. The relative proportion of 18:2 tended to increase in SM and SP resulting in a slight elevation in the total content of polyunsaturated FA in SM, but not in the other two muscles. However, an opposite tendency ie., decrease was observed in the proportions of 18:3n-3, 20:2n-6 and 20:4n-6. The increased P/S ratio reflects the net effect in the sum of polyunsaturated FA of a muscle. Despite the slightly diminished P/S ratios in LD and SP, they remained close to the recommended beneficial value of 0.7. The tendency of 20:4 to decrease may suggest that the activity of Δ6-desaturase (participating in conversion of 18:2 to 20:4) is affected, or some amount of 18:2 was converted to CLA (Peterson at al. (2002). Although small, the variations in the amount of n-6 fatty acids were compensated by a slightly increased incorporation

of some n-3 fatty acids in the PL fraction. As a result, the n-6/n-3 ratios tended to decrease in LD and SM, but not in SP muscle.

Conclusions

The results of this study show that the P/S ratio in PL fractions of the muscle of goat kids was comparable to the recommended beneficial value set for this ratio. However, the higher proportions of n-6 fatty acids resulted in the higher n-6/n-3 ratio compared to other species. Sunflower oil supplementation increased mostly TG fractions, which may have a positive effect on the marbling of goat meat. Only in the SM muscle of the treated kids, was a tendency of increased incorporation of 18:2 into the TG and PL fractions observed. Using an unprotected source of 18:2 did not change the P/S ratios in muscle PL fractions, but increased the value of n-6/n-3 ratio in SP, whereas in the other two muscles (LD and SM) an opposite tendency was observed.

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ABOUT SOME REGULAR FEATURES OF CHANGE OF BEEF FAT FRACTIONAL COMPOSITION DURING FERMENTATIVE GLYCEROLYSIS

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Background

Beef fat having high level of saturated fatty acids has limited use in current food diets, because its frequent consumption can provoke occurrence of cancer. Therefore, the search for possible ways of incorporation of transformed beef fat into combined meat products with balanced fatty acid composition is an urgent problem. One way of resolving this problem is the creation of technology of enzymatic modification of beef fat in the mixture of lipids having high functional-technological properties that are needed in meat industry. Enzymatic glycerolysis changes the lipid composition of the fats, which leads to accumulation of fractions of mono- and diglycerides having wide use as food additives. It was interesting to study the kinetics of beef fat fractional composition change during enzymatic glycerolysis.

Objectives

The purpose of the work was to study kinetic regularities of accumulation and consumption of individual lipid fractions during glycerolysis of beef fat in the presence of enzymatic preparation Liposym TL IM.

Materials and methods

Sterilized melted beef fat, food grade glycerol, and enzymatic preparation Liposym TL IM (Novozymes company, Denmark) were used for investigations. Lyposym TL IM is a granulated enzymatic preparation of purified 1,3 specific lipase immobilized on silica gel from Thermomyces lanuginosus, being produced during deep cultivation of GM strain Aspergillus oryzae. The preparation is mechanically stable, is intended for use in technological processes with batch loading, and has an activity of 175 IUN/g. As a unit of interesterification Novo (Interesterification Unit Novo, IUN) was taken the amount of enzyme, transforming 0.01% mass of tristearin per min during interesterification of soy oil by completely hydrogenized soy oil in a ratio 73: 27 (mass.%) at 70ºC in the absence of solvents.

Glycerolysis was carried out in a thermostatted glass reactor provided with a three-blade stirrer with constant rotation speed. Molar ratio fat-glycerol varied from 1:1 to 1:10, whereas the enzyme-substrate ratio E:S was changed in the range from 1:100 to 1:5. During investigation of kinetic characteristics of the process of glycerolysis was carried out at 45, 50, 55, 60, as well as 70ºC, periodically taking out samples for analysis of fractional composition by the method of thin-layer chromatography. Plates with the developed spots were scanned with the help of the system GelVue 2, by the area of the spots' percentages of fractions of tri-, di monoglycerols and fatty acids in the mixture of lipids were calculated. Calibration curves were constructed according to commercial standards Sigma.

Results and discussion

From literature it is known that the ratio of triglycerides to glycerol is the determining factor in obtaining different fractional composition of lipids. The amount of glycerol supplied to the reactor significantly influenced the yield of target fractions of mono- and diglycerides, The highest yield of fractions of mono- and diglycerides in this case was observed with mole ratio fat-glycerol 1:5, and did not increase with further increase of glycerol fraction in reaction mixture, which allows to recommend this ratio as the optimum. The process of enzymatic glycerolysis should be carried out with enzyme-substrate ratio that allows the achievement of maximum yield of target fractions with minimum doses of enzyme. The calculated enzyme-substrate ratio constituted 1:40 (mass.).

To clarify the influence of temperature on the velocity of glycerolysis, the dependencies of concentration change of separate lipid fractions in time were studied [1,2].

Fig. 1a shows experimental kinetic curves of the destruction process of triglycerides. Graphical differentiation of the above dependencies allowed to construct differential kinetic curves in co-ordinates (dP/dt; t), where the value dP/dt reflects the rate of destruction of triglycerides at the given time period (Fig.1b). We could with high correlation coefficients to approximate the curves with broken lines containing two rectilinear areas: horizontal and inclined, which may be considered as areas characterizing two stages of the process of glycerolysis. Observing the constant rate of change of triglycerides concentration at the first stage we can suppose that the number of elementary acts of triglycerides interaction with the active centers of enzymes per unit of time is also the constant value. Therefore, one can conditionally call the first stage as the stage of the effective work of the enzyme. In this case the break on differential curves will correspond to the time of effective work of enzyme, and the value of (dP/dt) on the first stage can be interpreted as the effective velocity of triglycerides destruction. The values of effective lengths of glycerolysis process were obtained by extrapolation of differential kinetic curves on abscissa axis.

The view of dependencies of triglycerides concentration change from time allowed us to suppose that the process of their destruction can be described by the equation of reaction of pseudo-first order. To check this supposition kinetic curves of Fig. 1a were linearized in semi-logarithmic co-ordinates. Determination of the value of inclination angle tangent of obtained straight lines allowed us to calculate values of effective constants of triglycerides destruction rates (Table 1).

From Table 1 it follows that the largest time period of effective operation of enzyme was at 45ºC and constituted 50 min, however, this temperature did not ensure high reaction rate. Temperature increase up to 60ºC led to the increase of effective initial rate 10-fold, and the time of the effective work of enzyme decreased to 5 min. Further temperature growth caused decrease of the rate of change of triglycerides concentration. Comparing experimental and differential curves of triglycerides conversion at 60ºC (Fig.1a and b) one can suppose that after 10-15 min the process of triglycerides hydrolysis was balanced by the process of their synthesis that was also evidenced by stabilization of concentration of triglycerides at 60ºC at the level 30%, i.e. 1.3 –1.5 times higher than at 45-55ºC.

The above approach was applied for description of kinetic curves of change of free fatty acids fraction concentration. The time of effective work of enzyme as determined from analysis of dependencies of the change of free fatty acids accumulation velocity was 2 times higher, than in the case of triglycerides destruction (Table 1).

Kinetic dependencies of monoglycerides fraction accumulation are interesting for discussion. Since in the system simultaneously occurred the processes of glycerolysis and autolysis, then one can consider formation of monoglycerides, on the one hand, as a result of synthesis from glycerol and free fatty acids, and on the other hand, as a result of incomplete hydrolysis of tri- and diglycerides. Simultaneously with the accumulation of monoglycerides the process of their hydrolysis to glycerol and fatty acids could also occur, therefore, the description of monoglycerides formation mechanism and the calculation of kinetic parameters of processes of their synthesis and destruction is a complex problem.

Fig.2(a) shows dependencies of change of mass share of monoglycerides fraction from time at different temperatures. Unlike triglycerides and fatty acid fractions there are no clear and simple dependency of monoglycerides yield from temperature. Thus, at temperatures 45-55ºC during the first hour the concentration of monoglycerides increased, and during the second hour: at 45ºC – decreased, at 50ºC – stabilized and at 55ºC continued to rise. At 60ºC the increase in monoglycerides concentration took place only during first 20 minutes, after which the growth stopped and total yield did not exceed 4-5%. At 70ºC during first 15 minutes there was no monoglycerides fraction at all, but then their concentration began to increase slowly, reaching only as little as 3%.

The process of monoglycerides formation looks more vividly on differential kinetic curves, presented in Fig. 2b. It is interesting to note that effective time for enzyme work, during which there was observed constant velocity of accumulation of monoglycerides fractions actually did not depend upon the temperatures in the interval 45 – 60ºC and constituted 15-20 minutes. The most effective initial velocity of accumulation of monoglycerides was marked at 50ºC , the least - at 60ºC. The dependencies dP/dt = f(t) constructed at 50-60ºC (Fig.2b) were satisfactorily approximated by broken line from three lengths. The appearance of

medium length on differential curve was associated with the fact that after the stage with constant velocity of monoglycerides formation, with the above temperatures there was an abrupt decrease of the velocity by 2 or more times during 5-10 min. Further the velocity of monoglycerides formation decreased much slower, which was seen from the third area of differential curve.

The process of monoglycerides accumulation at 45ºC, 50ºC and 55ºC was satisfactorily described by the reaction equation of pseudo-first order. The values of effective constants of monoglycerides accumulation velocities are presented in Table.1.

The dependence of accumulation of diglycerides fraction from temperature was of more complex nature.. The highest yield of diglycerides was reached at 55ºC, in 2 hours. Comparatively high values of diglycerides concentrations, about 22%, and correspondingly low yield of monoglycerides at 70ºC, can, probably be explained by two predominating processes: triglycerides hydrolysis on the first stage, and also the synthesis of diglycerides from monoglycerides and fatty acids. The absence of clear correlation between the yield of diglycerides fraction and glycerolysis temperature, is probably associated with multiple elementary acts leading to their formation and destruction.

Conclusions

As a result of investigations were determined the conditions of glycerolysis of beef fat in the presence of preparations Lipozym TL IM which ensure production of modified fat with maximum content of fractions of mono- and diglycerides. Optimum conditions of glycerolysis: correlation fat-glycerol 1:5 (mol.), correlation enzyme-substrate 1:40 (mass.), at temperature of 53-58ºC. Under the above conditions modified beef fat was obtained with the fractional lipids composition as follows: monoglycerides – 27-32%, diglycerides – 26-31%, triglycerides – 25-30%, free fatty acids – 7-12%. Such composition of the product allows to speak about good prospects of use of modified fat as a component of a stabilizing functional additive for production of emulsified meat products.

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Table 1. Main kinetic characteristics of glycerolysis of beef fat

T, ^{0}C	Effective initial velocity of reaction (horizontal length), % mass/min		Time of effective work of enzyme, min.			Effective time length of the process, min			Effective constant of velocity, min.$^{-1}$		
	dP_{TG}/dt	dP_{MG}/dt	TG*	FA**	MG***	TG	FA	MG	TG	FA	MG
45	0.75	0.32	50	90	20	78	110	60	0.016	0.013	0.024
50	1.8	0.7	10	55	15	32	67	26	0.022	0.032	0.077
55	2.2	0.4	6	45	15	14	58	37	0.025	0.032	0.03
60	8	0.2	5	18	20	16	27	25	0.02	0.112	-
70	0.6	-	0	15	-	60	30	-	0.01	0.032	-

*TG – triglycerides; ** FA – fatty acids; ***MG – monoglycerides

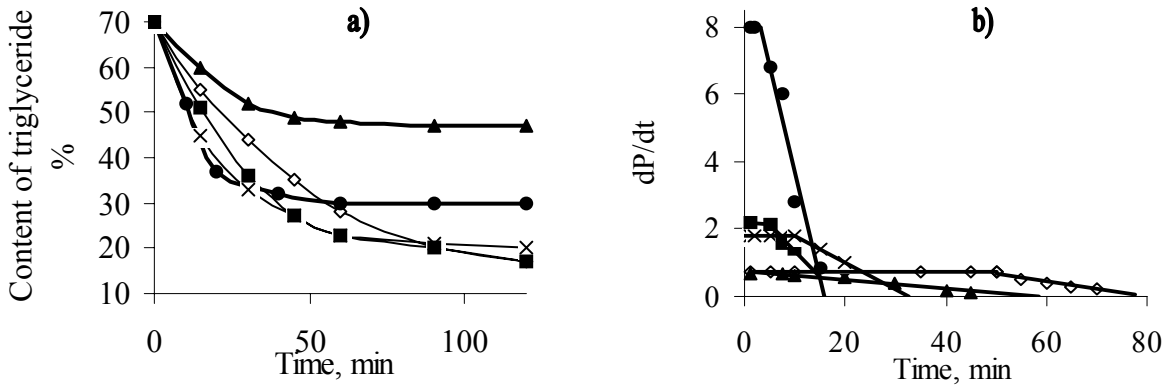

Figure1. Integral (a) and differential (b) kinetic dependencies of change of triglycerides mass share of fraction from time during glycerolysis of melted beef fat. (◊) 45º0C, (×) 50ºC, (■) 55ºC, (●) 60ºC, (▲) 70ºC.

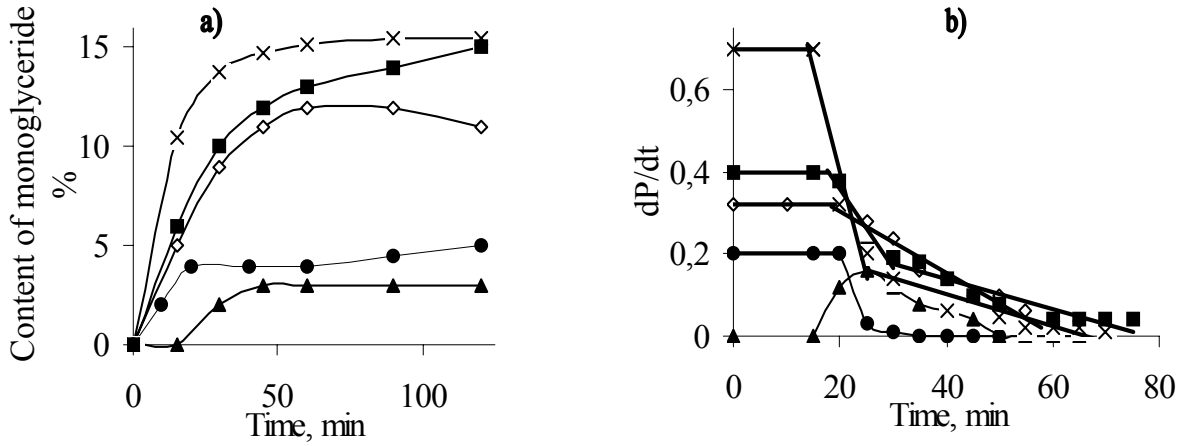

Figure 2. Integral (a) and differential (b) kinetic dependencies of change of mass fraction of monoglycerides mass share of fraction from time during glycerolysis of melted beef fat.
(◊) 45ºC, (×) 50ºC, (■) 55ºC, (●) 60ºC, (▲) 70ºC.

ASSESMENT OF EFFICIENCY OF CHICK-PEA USE FOR SELENIUM ENRICHMENT OF STEER BEEF AND POULTRY MEAT

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Background

Interest of researchers to selenium – microelement essential for normal activity of an organism – has increased lately. Activity spectrum of selenium inside the organism is rather wide. It fulfils catalytic, structural and regulatory functions; interacts with vitamins, enzymes and biological membranes; is involved in reduction-oxidation processes; fat, protein and carbohydrate metabolism.

To enrich feeds with selenium, various selenium-containing additives, primarily organic selenium compounds, are being used widely in animal husbandry. Moreover, plants with a high content of selenium are known, such as coconuts (8100.0 μg/kg), pistachio nuts (4500.0 μg/kg), soybeans (600.0 μg/kg), wheat bran (1100.0 μg/kg), edible boletuses (1000.0 μg/kg), daily selenium requirement for man being 70 μg. Chick-pea (*Cicer arietinum L.*) has a selenium content of ~700 μg/kg (Table 1).

The main form of digestible selenium in vegetable food is selenium-methionine. It is assimilated 5-10 times better than other forms of selenium. For this reason, plants are considered the priority sources of selenium.

Objectives

The objective of these investigations was to compare the capacity of cattle and poultry tissues as well as organs to accumulate selenium of both vegetable and chemical origin.

Materials and methods

Broiler chicken and young bulls kept on feeds (42 and 90 days, respectively), containing DAFS-25TM (diacetophenonilselenide) preparation and chick peas as selenium-enriching additive, served as the object of investigations.

Samples were analyzed using the following methods:
- selenium content – by fluorimetric method;
- iron content – according to GOST (State Standard) 26928-86;
- total amino acid composition – by Mure and Shtein on LC 3000 automatic amino acid analyzer ("Eppendorf-Biotronic", Germany);
- fatty acid composition – by Folch method on HP 6890 gas chromatograph ("Hwelett Packard");
- color (a-redness) – with "Spectroton" spectrocolorimeter;
- protein, fat, moisture content, and pH – by generally accepted methods.

Results and discussion

Investigation of physical-chemical indices of poultry and beef meat did not show significant difference between test and control groups in terms of moisture, protein and ash content. However, the fat content was significantly lower in broiler chicken meat compared to beef.

As far as selenium content in muscular tissue is concerned, the groups differed from each other (Table 2). Increase in selenium content of poultry meat of the test group was found, the maximum amount being determined for meat and skin samples – by 46.7% higher, compared to the control sample, and by 14.5% higher, compared to the skinless sample. It is obvious, that selenium from chick-pea was assimilated by broiler chicken organism, and accumulated in tissues. Connective tissue, in this case, had higher accumulative capacity, in comparison with the muscular tissue.

Identical data were obtained for red meat. Fattening of Aberden Angus steers on 10 % chick peas diet also resulted in a certain increase of selenium content in muscular tissue. In three months of fattening the amount of selenium in meat of the test group became 33.8% higher than that of the control group.

For comparative study on efficiency of use of diverse-origin biologically active substances, degree of synthetic (DAFS-25, 1st test group) and natural (chick peas, 2nd test group) selenium accumulation was investigated. In the process of fattening it was established, that up to the 15-months' age steers of the 2nd test group outweighed (in live weight) animals of the control group by 19.6 kg (P>0.99), and those of the 1st test group, by 12.8 kg (P>0.95). The average daily gain in live weight of steers by groups was 926.0 ± 10.75, 967.0 ± 11.62, and 1 057.5 ± 10.24 g for the control, 1st and 2nd test groups, respectively. Yield of main carcass tissues is given in Table 3. Steers of the 2nd test group, exceeding analogues of the control and the 1st test group by 13.1 kg, or by 7.3% (P>0.99), and by 8.9 kg, or 4.8% (P>0.95), were characterized by the highest content of muscular tissue in carcasses. However, at the same time, the content of interior fat increased by 4.1 kg, or 39.8%, and by 1.8 kg, or 14.3%, respectively.

The chemical composition data reliably confirmed a higher fat content in meat samples of the 2nd test group, compared to analogues of the control and the 1st test group, by 9.9% (P>0.999) and 8.4% (P>0.999), respectively.

Results of investigations of selenium and iron content in steer meat are of interest (see Table 2). It was established that the maximum increase in selenium content (by 34%), compared to the control, was observed when fattening with chick-pea, whereas the fattening with DAFS-25 resulted in a correspondent increase of 15%. Reduction of iron level both in poultry and steer meat of test groups was surprising considering that the iron level was equal in all feeds. Results of investigations of selenium in steer liver, heart and *longissimus dorsi* demonstrated that the level of selenium content in the heart muscle, when fattening both with chick-pea and DAFS-25, was practically the same or slightly higher, in contrast to selenium level in muscular tissue, where it was increased with the increase of its ingress into the organism. Selenium level in liver of the test animals did not exceed that of the control ones. This may be an indication of non-accumulation or obtaining its optimal level even during usual fattening.

Results of investigation of color characteristics of the steer back *longissimus dorsi* after freezing, storage in the frozen state for 24 hours and the consequent defrosting showed, that during the first day the maximum value of the redness index was noted in control, and the minimum, in samples with DAFS fattening, what correlates with the data on iron content. In 3 days the above index decreased to the same level in all samples. In 5 days decrease was in progress. At the same time, the redness index of meat samples of steers obtaining feed with chick-pea was the highest, while in control samples it decreased by 62%, in samples with DAFS-25 fattening – by 54%, and in samples with chick-pea fattening – by 41%, that is, these samples were decolored more slowly, compared to the others. This may be explained by the high selenium content, which perhaps exhibited antioxidant properties.

Conclusions

Comparative evaluation of effect of diets with chick-pea and DAFS-25 selenium-containing preparation on gain in live weight, meat quality and selenium accumulation by organs and tissues of farm animals and poultry demonstrated the following:
- selenium assimilation from chick-pea was more effective, compared to DAFS-25 additive;
- the maximum selenium accumulation took place in muscular tissue;
- the presence of selenium positively affects the stability of meat colour.

In the future, further investigations on selenium influence over iron assimilation by animal organism, as well as study on technological characteristics of meat products manufactured from such meat, are planned to be carried out. In this way, a possibility of meat selenium enrichment in the animal lifetime was shown. Usage of natural selenium-containing plants is preferable in this case.

Acknowledgements

The authors thank collaborators of the North-Caucasian branch of VNIIMP for assistance in carrying out of investigations.

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Table 1. The chemical composition of Chick-pea

Content	Mean	SD
Protein, %	23,7	0,80
Fat, %	5,0	0,15
Cellular tissue, %	4,0	0,03
Starch, %	46,4	0,39
Sugar, %	5,6	0,99
Ash, %	3,0	0,20
Moisture, %	6,9	0,12
Selenium, µg/kg	660	23,3

Table 2. Selenium content in meat of poultry and steers fed selenium-containing additives

Sample	Iron, mg/kg			Selenium, µg/kg		
	Control	Chick-pea	DAFS-25	Control	Chick-pea	DAFS-25
Chicken meat with skin	8.37 ± 1.2	6.95 ± 0.95	--	129.5 ± 3.5	190.0 ± 5.6	--
Chicken meat skinless	7.39 ± 1.2	6.33 ± 1.3	--	122.0 ± 4.2	166.0 ± 12.7	--
Beef	10.41 ± 4.03	10.15 ± 3.5	9.44 ± 1.4	183.0 ± 21.0	244.8 ± 34.7	211.0 ± 20.8

Table 3. Tissue yield from steer carcasses depending on the type of additive containing selenium

Index	Group					
	control		chick-pea		DAFS-25	
	Mean	SD	Mean	SD	Mean	SD
Slaughter mass, kg	395.8	4.1	403.1	3.95	414.6	4.27
Carcass yield, %	55.7	0.25	55.9	0.19	56.6	0.27
Fat yield, %	2.6	0.02	3.1	0.02	3.5	0.01
Bone yield, %	16.1	0.06	15.9	0.13	15.3	0.08

COMMON DIETS: NUTRITION, AND OBESITY

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Scientific data suggest positive relationships between a vegetarian diet and reduced risk for several chronic degenerative diseases and conditions, including obesity, coronary artery disease, hypertension, diabetes mellitus, and some types of cancer. Vegetarian diets, like all diets, need to be planned appropriately to be nutritionally adequate.

Position statement
It is the position of The American Dietetic Association (ADA) that appropriately planned vegetarian diets are healthful, are nutritionally adequate, and provide health benefits in the prevention and treatment of certain diseases.

Vegetarianism in Perspective
The eating patterns of vegetarians vary considerably. The lacto-ovo-vegetarian eating pattern is based on grains, vegetables, fruits, legumes, seeds, nuts, dairy products, and eggs, and excludes meat, fish, and fowl. The vegan, or total vegetarian, eating pattern is similar to the lacto-ovo-vegetarian pattern except for the additional exclusion of eggs, dairy, and other animal products. Even within these patterns, considerable variation may exist in the extent to which animal products are avoided. Therefore, individual assessment is required to accurately evaluate the nutritional quality of a vegetarian's dietary intake.
In addition to the health advantages, other considerations that may lead a person to adopt a vegetarian diet pattern include concern for the environment, ecology, and world hunger issues. Vegetarians also cite economic reasons, ethical considerations, and religious beliefs as their reasons for following this type of diet pattern. Consumer demand for vegetarian options has resulted in increasing numbers of foodservices that offer vegetarian options. Presently, most university foodservices offer vegetarian options.

Health Implications of Vegetarianism
Vegetarian diets low in fat or saturated fat have been used successfully as part of comprehensive health programs to reverse severe coronary artery disease (3,4). Vegetarian diets offer disease protection benefits because of their lower saturated fat, cholesterol, and animal protein content and often higher concentration of folate (which reduces serum homocysteine levels) (5), antioxidants such as vitamins C and E, carotenoids, and phytochemicals (6). Not only is mortality from coronary artery disease lower in vegetarians than in non-vegetarians (7), but vegetarian diets have also been successful in arresting coronary artery disease (8,9). Total serum cholesterol and low-density lipoprotein cholesterol levels are usually lower in vegetarians, but high-density lipoprotein cholesterol and triglyceride levels vary depending on the type of vegetarian diet followed (10).

Vegetarians tend to have a lower incidence of hypertension than non-vegetarians (11). This effect appears to be independent of both body weight and sodium intake. Type 2 diabetes mellitus is much less likely to be a cause of death in vegetarians than non-vegetarians, perhaps because of their higher intake of complex carbohydrates and lower body mass index (12).

Incidence of lung and colorectal cancer is lower in vegetarians than in non-vegetarians (2,13). Reduced colorectal cancer risk is associated with increased consumption of fibre, vegetables, and fruit (14,15). The environment of the colon differs notably in vegetarians compared with non-vegetarians in ways that could favourably affect colon cancer risk (16,17). Lower breast cancer rates have not been observed in Western vegetarians, but cross-cultural data indicate that breast cancer rates are lower in populations that consume plant-based diets (18). The lower estrogen levels in vegetarian women may be protective (19).

A well-planned vegetarian diet may be useful in the prevention and treatment of renal disease. Studies using human being and animal models suggest that some plant proteins may increase survival rates and decrease proteinuria, glomerular filtration rate, renal blood flow, and histologic renal damage compared with a non-vegetarian diet (20,21).

Nutrition Considerations for Vegetarians

Plant sources of protein alone can provide adequate amounts of essential amino acids if a variety of plant foods are consumed and energy needs are met. Research suggests that complementary proteins do not need to be consumed at the same time and that consumption of various sources of amino acids over the course of the day should ensure adequate nitrogen retention and use in healthy persons (22). Although vegetarian diets are lower in total protein and a vegetarian's protein needs may be somewhat elevated because of the lower quality of some plant proteins, protein intake in both lacto-ovo-vegetarians and vegans appears to be adequate (16).

Plant foods contain only nonheme iron, which is more sensitive than heme iron to both inhibitors and enhancers of iron absorption. Although vegetarian diets are higher in total iron content than non-vegetarian diets, iron stores are lower in vegetarians because the iron from plant foods is more poorly absorbed (23). The clinical importance of this, if any, is unclear because iron deficiency anemia rates are similar in vegetarians and non-vegetarians (23). The higher vitamin C content of vegetarian diets may improve iron absorption.

Although plant foods can contain vitamin B-12 on their surface from soil residues, this is not a reliable source of B-12 for vegetarians. Much of the vitamin B-12 present in spirulina, sea vegetables, tempeh, and miso has been shown to be inactive B-12 analog rather than the active vitamin. Although dairy products and eggs contain vitamin B-12, research suggests that lacto-ovo-vegetarians have low blood levels of vitamin B-12. Supplementation or use of fortified foods is advised for vegetarians who avoid or limit animal foods (24).

Because vitamin B-12 requirements are small, and it is both stored and recycled in the body, symptoms of deficiency may be delayed for years. Absorption of vitamin B-12 becomes less efficient as the body ages, so supplements may be advised for all older vegetarians.

Lacto-ovo-vegetarians have calcium intakes that are comparable to or higher than those of non-vegetarians (25,26). Calcium intakes of vegans, however, are generally lower than those of both lacto-ovo-vegetarians and omnivores (26). It should be noted that vegans may have lower calcium needs than non-vegetarians because diets that are low in total protein and more alkaline have been shown to have a calcium-sparing effect (27). Furthermore, when a person's diet is low in both protein and sodium and regular weight-bearing physical activity is engaged in, his or her calcium requirements may be lower than those of a sedentary person who eats a standard Western diet. These factors, and genetic influences, may help explain variations in bone health that are independent of calcium intake.

Because calcium requirements of vegans have not been established and inadequate calcium intakes are linked to risk for osteoporosis in all women, vegans should meet the calcium requirements established for their age group by the Institute of Medicine (28). Calcium is well absorbed from many plant foods, and vegan diets can provide adequate calcium if the diet regularly includes foods rich in calcium (29). In addition, many new vegetarian foods are calcium-fortified. Dietary supplements are advised for vegans only if they do not meet calcium requirements from food.

Diets that do not include fish or eggs lack the long-chain n-3 fatty acid docosahexanoic acid (DHA). Vegetarians may have lower blood lipid levels of this fatty acid, although not all studies are in agreement with this finding (34,35). The essential fatty acid linolenic acid can be converted to DHA, although conversion rates appear to be inefficient and high intakes of linolenic acid interfere with conversion (36). The implications of low levels of DHA is not clear. However, it is recommended that vegetarians include good sources of linolenic acid in their diet.

Figure 1 below presents food sources of nutrients that are often of concern for vegetarians.

Iron	Milligrams per serving
Breads, cereals, and grains	
Whole wheat bread, 1 slice	0.9
White bread, 1 slice	0.7
Bran flakes, 1 c	11.0
Cream of wheat, 1/2 c cooked	5.5
Oatmeal, instant, 1 packet	6.3
Wheat germ, 2 Tbsp	1.2
Vegetables (1/2 c cooked)	
Beet greens	1.4
Sea vegetables	18.1-42.0
Swiss chard	1.9
Tomato juice, 1 c	1.3
Turnip greens	1.5
Legumes (1/2 c cooked)	
Baked beans, vegetarian	0.74
Black beans	1.8
Garbanzo beans	3.4
Kidney beans	1.5
Lentils	3.2
Lima beans	2.2
Navy beans	2.5
Soyfoods (1/2 c cooked)	
Soybeans	4.4
Tempeh	1.8
Tofu	6.6
Soymilk, 1 c	1.8
Nuts/seeds (2 Tbsp)	
Cashews	1.0

Calcium	Milligrams per serving
Legumes (1 c cooked)	
Chickpeas	78
Great northern beans	121
Navy beans	128
Pinto beans	82
Black beans	103
Vegetarian baked beans	128
Soyfoods	
Soybeans, 1 c cooked	175
Tofu, 1/2 c	120-350
Tempeh, 1/2 c	77
Textured vegetable protein, 1/2 c	85
Soymilk, 1 c	84
Soymilk, fortified, 1 c	250-300
Soynuts, 1/2 c	252
Nuts and seeds (2 Tbsp)	
Almonds	50
Almond butter	86
Vegetables (1/2 c cooked)	
Bok choy	79
Broccoli	89
Collard greens	178
Kale	90
Mustard greens	75
Turnip greens	125
Fruits	
Dried figs, 5	258
Calcium-fortified orange juice, 1 c	300

F*IG 1. Food sources of nutrients. Sources: Package information and data from: Pennington J.* Bowe's and Church's Food Values of Portions Commonly Used*. 16th ed. Lippincott-Raven; 1994.* Provisional Table on the Content of Omega-3 Fatty Acids and Other Fat Components in Selected Foods, 1988. *Ibadan Oyo State: Nafdac Dept of [a]Red Star Yeast and Products, a division of Universal Foods Corp, Ikeja, Lagos.*

Conclusions

Diets that are high in carbohydrate and low to moderate in fat tend to be lower in energy. The lowest energy intakes were observed for those on a vegetarian diet. The diet quality as measured by HEI was highest for the high carbohydrate groups and lowest for the low carbohydrate groups. The BMIs were significantly lower for men and women on the high carbohydrate diet; the highest BMIs were noted for those on a low carbohydrate diet.

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FIBER TYPE PROFILE AND CHEMICAL COMPOSITION OF THREE MARONESA VEAL MUSCLES

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Background

In recent years, consumers have become more concerned about dietary fat and cholesterol associated with meat consumption, since these compounds are referred to as playing important roles in predisposition of human coronary disease (Salvatori *et al*. 2004), obesity and cancer (Chizzolini *et al*. 1999; Erickson, 1998). The Maronesa, an autochthonous cattle breed reared in the north part of Portugal is used for meat production uniquely based on calves produced under Protected Denomination of Origin (PDO). This type of veal is perceived as healthy due to the friendly extensive system applied on animal production, and is highly appreciated for its extraordinary eating quality.

A number of different factors, such as muscle location, muscle fibre type composition, sex and nutritional status, have been reported to influence the content of cholesterol and fat in meat. According to Calkins (2003) the amount of fat and the fibre characteristics of the muscle may contribute to the eating quality experienced by consumers.

Objective

The aim of this study was to evaluate the lipid composition, and the content of total collagen and haem pigment in various muscles with respect to muscle fibre types in Maronesa-PDO meat.

Material & Methods

About 1 hour after slaughter, samples from *Longissimus dorsi* (Ld), *Supra spinatus* (Ss) and *Biceps femoris* (Bf) of eight Maronesa calves of both sexes, with ages ranging from 6 to 9 months were taken for histochemical analysis. Transverse serial sections (10μm) were cut in a cryostat at –24ºC and stained for myofibrillar ATPase after pre-incubation at pH 4.45 as described by Brooke & Kaiser, 1970. The succinic dehydrogenase protocol described by Sheehan & Hrapchak (1987) was also used to define fibre metabolic properties. The percentage of each fibre type was calculated from a minimum of 400 units, by counting the total number of each type and dividing it by the total number of fibres. Differentiation into type I, type IIa and type IIb was based on staining intensity.

Samples for chemical analysis were collected 24 h after slaughter. Total lipids were extracted by the procedure of Folch *et al*. (1957) and then separated into neutral and polar fractions as described by Juaneda & Rocquelin (1985). Cholesterol was determined by HPLC according to Roseiro *et al*. (2002). Total collagen was measured following the ISO 3496:1994 procedure.

The samples were analysed for pigment content according to Hornsey (1956). Results were expressed as mg haematin/100g wet tissue, using a standard curve of a commercially available haematin instead of the factor used by Hornsey (1956).

Data were analysed using one-way analysis of variance (ANOVA) and significant differences were determined using Tukey´s HSD post hoc test requiring a probability value of less than 5 % ($p<0.05$) (Statistica 6.0-StatSoft Inc., 2001).

Results & Discussion

Muscle fibre type composition and metabolic profile

Fibre type composition and metabolic profile of the analysed muscles are indicated on Table 1. The Ss muscle showed a mean percentage of type I fibres significantly lower (P<0.01) than the other two muscles, yet it was the most oxidative unit of the three. This could be due to the fact that oxidative pattern is not only determined by fibre type I content but also by the incidence of such type of metabolism among types IIa (Zerouala & Stickland, 1991) and IIb fibres. On the other hand, the percentage of glycolytic fibres, classified by SDH procedure, was much lower than that of type IIb fibres, classified by ATPase. Ruusunen & Puolanne (1997) made a similar observation. These authors, by using NADH method to evaluate metabolic fibre type profile, reported that some IIb fibres analysed by the myosin ATPase method were in fact oxidative by metabolism.

According to the histochemical analyses, all studied muscles can be considered, predominantly, having oxidative metabolism. This may be related to the age of the animals. Fiedler *et al.* (1998) observed a decrease in the proportion of the oxidative fibres and concomitantly an increase in the glycolytic metabolism of the muscle along growth. Similarly, Johnston *et al*. (1981) showed that Ld and Bf muscles from older animals presented predominantly glycolytic metabolism. Nevertheless, in our case, Ld muscle showed lower mean percentage of IIb and a higher mean percentage of IIa fibers than those observed by Guinot *et al*. (1992), for Friesian-Holstein calves (carcass weight of 105-120). These results suggest that weight at slaughter may not have an important effect on muscle fibre type profile and that this issue may be affected to a larger extent by other factors such as breed, diet and handling. A study from Roseiro *et al* (2004) on Barrosã calves of similar age and weight at slaughter showed muscles with lower oxidative metabolism, higher percentage of type IIb and lower percentage of type IIa fibres than those of Maronesa calves.

Table 1. Cholesterol and intramuscular fat contents of different muscles of Maronesa calves.

	Muscle			F-value	P
	Ld	Bf	Ss		
Intramuscular characteristics					
Total lipids (g/100g)	1.38^{b}	1.48^{b}	1.80^{a}	8.11	*
Neutral lipids (g/100g)	0.71^{b}	0.73^{b}	1.02^{a}	3.10	*
Polar lipids (g/100g)	0.66^{b}	0.79^{ab}	0.84^{a}	3.65	*
Cholesterol (mg/100g)	48.50^{b}	51.50^{ab}	62.20^{a}	2.79	*
Haem pigment (mg/100g)	19.61	19.89	21.23	0.31	Ns
Total collagen (mg/g)	6.45^{b}	11.89^{ab}	13.38^{a}	3.95	*
ATPase (pH 4.6)					
Fibre type I (%)	21.42^{a}	24.99^{a}	16.69^{b}	3.60	*
Fibre type IIa (%)	33.84	27.27	35.86	2.99	Ns
Fibre type IIb (%)	44.74	47.73	47.45	0.55	Ns
SDH					
Oxidative (%)	55.41^{b}	55.88^{b}	68.40^{a}	12.04	**
Glycolytic (%)	44.59^{a}	44.12^{a}	31.70^{b}	12.04	**

In same row, means with different letters are significantly different. * p<0.05; ** p< 0.01; ns not significant.

Intramuscular chemical characteristics

As presented in Table 1, the amount of intramuscular fat, cholesterol and collagen was affected by anatomical location (P<0.05). The Ss muscle, the most oxidative among the analysed, showed significantly higher total (1.80 mg/100g) and neutral (1.02 mg/100g) lipid contents than Ld (1.38 and 0.71 mg/100g, respectively) and Bf (1.48 and 0.73 mg/100g, respectively). In relation to the polar lipids and cholesterol, Ss muscle also presented higher mean values (P<0.05) than LD muscle but did not differ significantly from those obtained from the Bf.

The oxidative fibres have greater aerobic metabolism, which is supported by lipids. For this reason they contain higher levels of intra-fibre lipids than their glycolytic counterparts. Although other authors have observed no significant differences in cholesterol content between muscles with distinct anatomical location

(Cifuni *et al*., 2004; Bohac & Rhee, 1988), Browning *et al*. (1990) did find differences among them, as we did. As previously reported, the content of total lipids, phospholipids and triacylglycerols depend on the metabolic type of muscles. The oxidative muscles contain more lipids and more triglycerides than the glycolytic ones (Alasnier *et al*., 1996).

Total haem pigment is an indicator of the redness of muscle and is closely related to its oxidative activity (Meynier & Gandemer, 1991). Although there were no significant differences in the oxidative activity between muscles, the Ss muscle, the most oxidative one, showed mean haem pigment content higher than Ld and Bf muscles.

As presented in Table 1, the collagen content of muscles was ranked as Ss>Bf>Ld. The same tendency for Bf and Ld muscles was observed by Seideman (1986). Beatty *et al.* (1966) found that the proportion of white fibres in the muscles were positively correlated to their collagen content. In this study, despite the small number of animals involved, the Ss and Bf muscles presented a higher proportion of white fibres (IIb) than Ld suggesting a possible relationship between these two issues. According to Kirchofer *et al*. (2002), muscles with increased type IIb fibres have more connective tissue and are less tender than muscles with more type I fibers. Many researchers have reported significant relationships between collagen content of the meat and its tenderness. However, the usefulness of collagen as a predictor of meat tenderness is still controversial. Muscles with more α-white fibres had more connective tissue, less intramuscular fat, and were less tender than muscles with more β-red fibres (Calkins *et al*. 1981).

Acknowledgements

This research was supported by the Fundation for the Science and Technology (FCT). The authors are grateful to the Associação de Criadores do Maronês (ACM) and to the Hospital de Santa Maria.

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HORSEMEAT AS A PROMISING RAW MATERIAL FOR PRODUCTION OF CANNED MEAT FOR CHILDREN

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Background

Horsemeat is not a traditional raw material for production of canned meat products for children. Pediatricians recommend beef for infants because it corresponds best to their nutrient status.
But it is known that horsemeat has pronounced dietetic properties. Protein of horsemeat by its amino acid balance is not inferior to beef. Horse fat is easily melted (melting point of lamb fat – 44-55^{0}C, beef fat – 40-50^{0}C, pork fat – 33-46^{0}C, horse fat – 30-43^{0}C), contains more than 50% of unsaturated fatty acids, among them - up to 20% linoleic and linolenic which are indispensable.
Horsemeat possesses hypoallergenic properties, high therapeutic efficiency in cases of celiac diseases, lactase deficiency and ability to decrease cholesterol level in blood that was confirmed by many clinical tests, carried out by technologists of the Institute together with pediatricians and specialists in nutrition.
Besides, horsemeat is more wholesome from hygiene point of view because horses are not sensitive to parasite diseases as well as to spongiform encephalopathy.

Objectives

The evaluation of nutrients adequacy, biological value of horsemeat as compared to beef and pork and substantiation of usefulness of horsemeat for child nutrition.

Materials and methods

Calculation of amino acid and fatty acid balance was carried out according to computer technique of Lipatov N.N. /1/ as compared to the reference - mature woman's milk.
Medical and biological evaluation of canned pork and horsemeat samples for children was carried out at the Chair of ecology of a man and environment hygiene of the I.M.Sechenov Moscow medical academy with the use of growth-mass and biochemical indices (Sukhanov B.P., 1987, Korolev A.A., 1997) on model groups of animals: growing white male rats of Vistar line with the initial body weight 70±5 g during 28 days. Biological value was judged from the value of coefficient of protein efficiency /2/. The content of available lysin was determined by the method of binding of color "orange", based on combination of two reactions: the first one between negatively charged group of azo dye and positively charged main nitrogen containing groups of lysin, histidine, arginin and the end groups of food proteins suspended in acid medium; the second reaction of propionilation of free amino groups of lysin with propionic anhydride, providing their blocking. The content of available lysin was determined by difference of the amount of dye for the first and second reaction.
Determination of taurin was carried out on liquid amino acid analyzer Biotronic LC-5000.

Results and discussion

A characteristic of amino acid balance of meat raw materials proteins is presented in Table 1.

Table 1.

№	Trimmed meat	Indispensable amino acids, g/100 g of protein									Cmin, %	Rp, Un.	σ, g/100g of product
		Protein, %	iso	leu	lys	meth+cys	phen+tyr	thre	trp	val			
1	Horse meat	19.5	4.6	9.8	7.5	4.0	8.6	4.6	1.5	5.2	78.16	0.84	6.96
2	Beef	18.7	4.2	7.95	8.54	3.87	7.81	4.32	1.13	5.86	75.33	0.79	9.18
3	Non-fat Pork	16.4	4.7	7.79	8.75	3.79	7.68	4.73	1.37	6.1	79.49	0.81	8.5
4	Semi-Fat Pork	14.4	4.95	7.51	8.66	3.76	7.69	4.57	1.34	5.81	76.63	0.79	9.19
Reference													
Mature woman milk		1.4	4.5	9.0	7.0	3.9	8.6	4.0	4.6	5.0	100.0	1.0	0

As follows from presented data the protein of horsemeat as evaluated with the help of such indices of amino acid composition as minimum score (Cmin), coefficient of utility (R_p), coefficient of comparable redundancy (σ), shows rather high amino acid balance and is not inferior to beef and pork.

To describe the amino acid composition more completely investigations for determination of taurin - non-protein amino acid that is essential for infants - were carried out. Taurin is formed from sulfur-containing amino acids – methionine and cystine. It is a part of bile acids, relates to non-volatile compounds of meat and comes to the organism with foods of animal origin. Vegetable foods (vegetables, fruit, cereals etc.) and milk do not contain taurin. For children of this age conjugation of bile acids with taurin is typical, because taurin conjugates do not deposit under the conditions of acid medium in proximal part of small intestines, thus assisting in better fat absorption. Thus, with sufficient supply of taurin with foods biological value of protein increases at the expense of economic use of sulfur-containing amino acids, and assimilatiom of fats improves as well. The investigations carried out demonstrate a higher level of taurin in horsemeat - 134±5.3 mg/100g of product as compared to beef - 102±3.0 mg/100g of product.

Characteristic of fatty acid balance of fat in different kinds of meat is presented in Table 2.

Table 2.

№	Trimmed meat	Fat, %	**Fatty acids, g/100g of fat**						**Coefficient Of fatty acid** Balance, R_L, fract.units	
			Σ SFA	**Σ** MUFA	**Σ** PUFA	**lino-**leic	**lino-**lenic	**arachi-**donic	I=1..3	i =1..6
1	Horsemeat	8.3	33.87	45.66	14.94	11.2	0.35	0.24	0.86	0.93
2	Beef	16.0	44.5	46.4	3.5	2.5	0.87	0.13	0.63	0.43
3	Non-fat pork	15.8	36.54	47.26	11.19	10.07	0.684	0.432	0.89	0.80
4	Semi-fat pork	40.0	35.5	46.2	10.98	9.85	0.71	0.42	0.868	0.804
Reference										
Mature woman milk		3.8	41.78	43.03	12.42	10.85	0.62	0.95	1.0	1.0

Fatty acid composition of horsemeat as evaluated from the coefficient of fatty acid balance, by the content of sums of saturated, monounsaturated and polyunsaturated fatty acids (I = 1 … 3) and especially linoleic, linolenic and arachidonic (i = 1…6) is not inferior than the fat of beef and pork and can be acknowledged as highly adapted to fat of woman's milk.

Table 3 presents the composition of elements of beef, pork, horsemeat which are used for production of canned foods for children.
As is seen from Table 3, by its microelement composition horsemeat is close to beef, but contains more iron and copper which take part in the process of blood forming, which is a positive factor in curing and prophylactics of anemia.
Results of medical and biological evaluation of canned pork and horsemeat are presented in Table 4.
Analysis of data presented in Table 4 shows that canned horsemeat is not inferior by its biological value to canned pork and beef, and the available lysin tends to increase.
According to clinical tests carried out under the supervision of Dr. Sc. (Med.) Ladodo M.S and Dr. Sci. (Med.) Borovik T.E. high therapeutic efficiency of horsemeat is shown in curing of patients suffering food allergy, anemia, different forms of malabsorbtion of intestines.

Conclusions

Comparative evaluation has shown that horsemeat by its food and biological value is not inferior to beef and pork and can be used not only for healthy children of early age, but also in curative nutrition of children suffering food allergy, anemia, different forms malabsorbtion of intestines.

Table 3. Composition of elements

Mineral elements	Mature woman milk	Horsemeat	Beef	Pork
Macroelements(mg/100g)				
Potassium (K)	45.5	370.0	355.0	316.0
Sodium (Na)	18.0	50.0	73.0	64.8
Calcium (Ca)	25.5	13.0	10.2	8.0
Magnesium (Mg)	3.0	23.0	22.0	27.0
Sulfur (S)	-	-	230.0	220.0
Phosphorus (P)	13.0	185.0	188.0	170.0
Chlorine (Cl)	39.0	-	59.0	48.6
Microelements (µg/100g)				
Iron (Fe)	40.0	3100.0	2900.0	1940.0
Iodine (J)	10.0	-	7.2	6.6
Cobalt (Co)	-	3.0	7.0	8.0
Manganese (Mn)	0.35	30.0	35.0	28.0
Copper (Cu)	30.0	206.0	182.0	96.0
Molybdenum (Mo)	-	-	11.6	13.0
Fluorine (F)	10.0	-	63.0	69.3
Chromium (Cr)	-	-	8.2	13.5
Zinc	140.0	-	3240,0	2070.0
Vitamins				
A, mg/100g	0.055	-	Traces	Traces
E, mg/100g	0.43	0.8	0.57	0.54
B_6, mg/100g	0.018	-	0.37	0.4
B_{12},µg/100g	0.05	-	2.6	-
Biotin, µg/100g	0.48	-	3.04	-
Niacin, mg/100g	0.2	3.0	4.7	2.8
Pantothenic acid,mg/100g	0.45		0.5	-
Riboflavin, mg/100g	0.06	0.1	0.15	0.16
Tiamin, mg/100g	0.02	0.07	0.06	0.6
Folacin, µg/100g	1.4	-	8.4	4.4
Choline, mg/100g	14.0	-	70.0	-

Table 4

Indices	**Puree from horsemeat**	**Puree from pork**	**Puree from beef**
Coefficient of protein efficiency (KEB)	2.92 (0.27)	2.98 (0.31)	3.00 (0.3)
Net utilization of protein	74.80 (1.72)	75.23 (1.44)	69.83 (1.3)
Digestibility (D), %	95.60 (1.40)	96.70 (1.24)	91.80 (0.9)
Cholesterol in blood serum, mg %	80.31 (4.08)	86.95 (3.81)	85.60 (3.7)
Available lysin, mg/100g of product	51.78 (0.51)	51.76 (0.53)	51.78 (0.50)

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EFFECTS OF MODIFICATION OF FATTY ACID COMPOSITION OF ANIMAL PRODUCTS ON ADOLESCENT FATTY ACID INTAKE IN BELGIUM

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Background

Over the last decades, much research has been devoted to the potential of modifying the fatty acid composition of animal food products, to better meet human nutritional guidelines. This research has mainly focused on increasing the content of polyunsaturated fatty acids (PUFA), aiming at increasing the P/S ratio (polyunsaturated/saturated fatty acids), on lowering the ratio of n-6/n-3 PUFA and on increasing the content of fatty acids with reported beneficial effects, e.g. long-chain PUFA and conjugated linoleic acids (Raes et al., 2004). There is little doubt that n-3 fatty acids are important in human nutrition (Connor, 2000).

There is growing interest in the animal feed industry to implement strategies fulfilling these objectives. However, the impact of such strategies on the actual fatty acid intake patterns of human population groups, and consequently the potential human health benefits, remain unclear. An assessment of the impact of strategies to modify the fatty acid composition of foods of animal origin on the fatty acid intake pattern of human populations is therefore desirable (Gibney, 1999). The outcome of such assessment could assist in evaluating the potential health benefits, which in turn need to be outweighed against the extra costs for implementing these strategies in the animal industry and against alternative strategies. A preliminary study was carried out in this regard, using human food consumption data and alternative fatty acid composition data of animal food products.

Objectives

To examine the impact of alterations in the n-6/n-3 PUFA ratio of animal foods on the pattern of fatty acid intake in the Belgian population. An alternative fatty acid composition profile for the major animal foods and two scenarios of implementation compared to a standard situation were considered.

Materials and methods

Food consumption data

Consumption data from a survey done in 1997 in a random sample of Flemish adolescents (aged 13-18 years; 129 boys and 212 girls) from the region of Ghent in Belgium were used. The dietary assessment method used was a 7-day diary, which was completed according to a standardised protocol. The database-architecture for this survey was generated on the basis of a commonly used Dutch dietary assessment tool, providing a detailed level of entries for food items (n=745 recorded in this study) (Unilever, 1992). A number of 527 foods contained fat following the food composition tables and were further used in this study.

Food composition data and fatty acid composition of animal products

Nutrient composition data routinely used in Belgium are those from the Belgian and the Dutch food composition tables (NEVO, 1993; NUBEL, 1992, 1995). However, differentiated data on the n-6 and n-3 PUFA are not available at the level of detail wanted in this study. The following approach was therefore adopted. The fat content of all food items was taken from the local food composition tables and was assumed to remain constant for the various scenarios analysed in this study. The origin of the lipid fraction was determined according to vegetable, land animal or marine origin or combinations thereof. The lipid fraction of land animal origin was further specified according to beef, pork, poultry, milk, eggs, sheep and horse. Subsequently, fatty acid content of the food items was obtained by linking the fat content and the contribution of the various lipid fractions to fatty acid proportions. For vegetable and marine fat sources, fatty acid proportions were taken from the McCance & Widdowson's and USDA food composition tables.

For land animal fat sources, two fatty acid composition profiles were considered for beef, pork, poultry, milk and eggs in the different scenarios envisaged in this study, i.e. a 'standard' and a 'n-3 enriched' fatty acid profile (Table 1). Because of the low intake of sheep and horse meat in Belgium, no alternative fatty acid composition was considered for these meats. Fatty acid composition was defined in terms of the sum of saturated fatty acids (SFA), the sum of monounsaturated fatty acids (MUFA), C18:2n-6 (linoleic acid, LA), C18:3n-3 (α-linolenic acid, LNA), C20:4n-6 (arachidonic acid, AA) and the sum of the long-chain n-3 PUFA C20:5n-3 (EPA), C22:5n-3 (DPA) and C22:6n-3 (DHA). The data were derived from a range of literature sources and from own information (for references see review of Raes et al., 2004). Values for the n-3 enriched profile were considered target values that could be obtained in commercial practice if feeds are including LNA rich sources, e.g. linseed and grass, at rather high levels that, however, do not compromise animal performances. The fatty acid composition of the meats was based on estimated contributions of intramuscular fat and subcutaneous fat (mainly for pork) and the differences in fatty acid composition between these fat depots. The use of fish oil or algae to increase the content of long-chain n-3 PUFA was not considered, hence higher levels of these fatty acids are resulting from elongation and desaturation of LNA.

Table 1. Standard (stand) and n-3 PUFA enriched (n-3) fatty acid profiles of the land animal fats assumed in this study (% w/w of total fatty acids)

	Beef		Pork		Poultry		Milk		Eggs		Sheep	Horse
	Stand	n-3	Stand	n-3	Stand	n-3	Stand	n-3	Stand	n-3	Stand	Stand
SFA	39.0	38.0	39.0	37.0	30.0	28.0	68.0	58.5	31.0	30.0	36.0	36.0
MUFA	39.0	38.0	39.0	37.0	38.0	36.0	25.0	32.0	41.5	39.0	35.0	34.0
C18:2n-6 (LA)	8.5	8.5	14.0	14.0	17.0	14.0	1.5	3.0	20.0	17.5	6.0	13.0
C20:4n-6 (AA)	1.5	1.5	0.4	0.4	3.0	3.0	0.0	0.0	2.0	1.0	2.2	1.7
C18:3n-3 (LNA)	1.0	2.0	1.5	5.0	2.5	7.0	0.5	1.3	1.0	7.0	1.5	7.5
LCn-3 (EPA, DPA, DHA)	0.5	1.0	0.3	0.5	2.0	4.5	0.0	0.0	1.5	2.5	2.8	1.6

Scenarios and calculations

Descriptive statistics and distributions for the intake of SFA, MUFA, LA, AA, LNA and LCn-3 PUFA were calculated for three scenarios, 1/ standard fatty acid composition of all land animal fats (Stand), 2/ n-3 enriched pork only (Pork+), 3/ n-3 enrichment of all land animal fats (All+). Scenario 2 and 3 correspond to a situation where all fattening pigs and all beef cattle, fattening pigs, poultry, dairy cows and layers, respectively, are fed diets high in LNA to achieve the fatty acid composition assumed in Table 1.

Results and discussion

Mean values for the intake of summarized and individual fatty acids in the different scenarios are given in Table 2. Mean values correspond reasonably well with data from an earlier study on the fatty acid composition of the Belgian diet, albeit in different population groups (Staessen et al., 1998). As expected, the largest changes in the mean fatty acid intake were observed for LNA and the LCn-3 PUFA. For LNA, the intake in g/p/d increased by 18% and 36% in the Pork+ and All+ scenario, respectively, compared to the standard scenario. The corresponding values for the relative increase of the LCn-3 PUFA intake are 6.5% and 35%. These changes were accompanied by a 6% decrease in the SFA intake in the All+ scenario, and relatively small increases in the intake of MUFA in the All+ scenario and in the intake of LA in the Pork+ and All+ scenario.

Table 2. Mean values for the fatty acid intake in the Standard, Pork+ and All+ scenario (gram/person/day, g/p/d and Energy%, E%)

Scenario	Standard		Pork+		All+	
	g/p/d	E%	g/p/d	E%	g/p/d	E%
SFA	35.8	14.4	35.6	14.3	33.6	13.5
MUFA	29.2	11.7	29.0	11.6	30.2	12.2
C18:2n-6 (LA)	14.1	5.65	14.5	5.83	14.7	5.90
C20:4n-6 (AA)	0.24	0.10	0.24	0.10	0.22	0.09
C18:3n-3 (LNA)	2.16	0.87	2.57	1.04	2.99	1.21
LCn-3 (EPA, DPA, DHA)	0.29	0.12	0.32	0.13	0.41	0.17

When the mean intake values are compared to nutritional recommendations (De Hoge Gezondheidsraad, 2003), none of the scenarios meet the criterium of SFA Energy% (E%) < 10. All scenarios meet the criteria MUFA E% > 10 and LA E% > 2. The mean value for the LNA E% is lower than the recommended value > 1 in the standard scenario, but exceeds this value in the Pork+ and All+ scenario. The recommended value for EPA+DHA E% > 0.3 was clearly not met in any of the scenarios. In addition, DPA was included in our data, making a significant contribution to the sum of the LCn-3 PUFA in land animal products. The average n-6/n-3 ratio dropped from 6.6 to 5.4 and 4.6 in the Pork+ and All+ scenario compared to the standard situation.

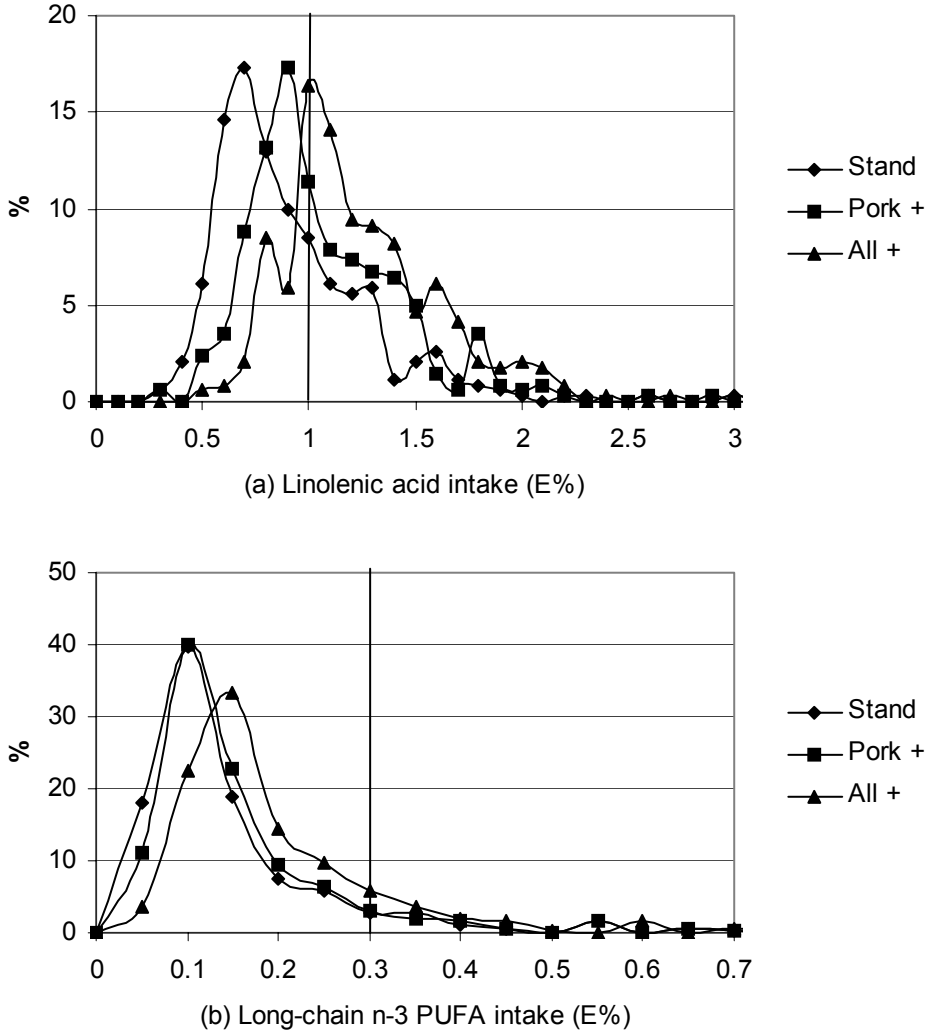

Figure 1. Distribution of the adolescent average daily intake of (a) LNA and (b) LCn-3 PUFA for the three scenarios (Energy%, E%). Vertical lines represent recommended minimum intake values.

The population distributions of the intake of LNA and LCn-3 PUFA are shown in figure 1. Interestingly, the largest increase in intake of these fatty acids occurs at the lowest intake levels. At high intake levels, there is almost no change, illustrating probably that a high intake of LNA and LCn-3 PUFA is not derived from land animal products, but from vegetable food sources and fish, respectively. High intake levels of the LCn-3 PUFA are only observed in a small part of the population, corresponding to the low average fish consumption in Belgium (De Henauw and De Backer, 1999). Staessen et al. (1998) calculated that meat was the most important source of n-3 PUFA in the Belgian diet. Hence, for a low-fish consumption population like in Belgium, strategies to increase the content of n-3 PUFA in land animal foods may be worthwhile. This may be even more valid in view of the increasing share of fish derived from aquaculture and the expected shift in fish feeding practices towards more n-6 fat sources.

This preliminary study has to be considered as a simulation study and an extrapolation to actual food intake patterns in the entire Belgian population is therefore speculative. Furthermore, the outcome of this kind of

assessment exercises is strongly determined by the assumptions made that may be debated at several points. Particularly the changes in fatty acid composition of the land animal fats in the alternative scenarios that were assumed may largely affect the outcome, e.g. a quite high reduction in the SFA content of 'n-3' milk was assumed, which may be responsable for the large drop in SFA intake in the All+ scenario. Similarly, the moderate increase in intake of LCn-3 PUFA in the Pork+ scenario compared to the relatively large increase in the All+ scenario may be due to a larger increase for the content of LCn-3 PUFA that was assumed for eggs, poultry and beef compared to pork. In addition, it is not very likely that an altered animal nutritional strategy will be adopted by the whole sector. Some modelling would be interesting in this respect, taking into account distributions in fatty acid composition instead of fixed mean values. Nevertheless, this preliminary study has shown the potential of this approach to evaluate the effects of alternative feeding strategies in the animal industry on human intake patterns. The potential impact on human health of these shifts in fatty acid intake is of course difficult to establish. In several human clinical studies using land animal foods with modified fatty acid composition following changes in the animals' diets, significant changes in the fatty acid composition of plasma and erythrocytes have been demonstrated (Sim and Nakai, 1994; Stewart et al., 2001; Weill et al., 2002). Hence, modification of the fatty acid composition of land animal foods may be a useful approach.

Conclusions

In this simulation study using Belgian food consumption data for adolescents, a relatively large but achievable enrichment in n-3 PUFA of either all pork or all major land animal products was calculated to increase the average daily intake of α-linolenic acid by 18% and 36%, respectively, and of the sum of long-chain n-3 PUFA by 6.5% and 35%, respectively. Although the outcome is largely affected by the assumptions that are made, this kind of assessment may help in evaluating the potential impact of new animal feeding strategies on human dietary intake and consequently on human health.

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FATTY ACID COMPOSITION OF LAMB MEAT FROM SPAIN, BRITAIN, GERMANY AND URUGUAY

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Background

The fatty acid composition of intramuscular fat can be influenced by factors such as diet (Rhee, 2000), breed (Robelin, 1986), age (Link et al., 1970) and the level of fatness of animals (Nürnberg et al., 1998). On the other hand, the fatty acid composition influences the nutritive value and the palatability of the meat. In relation to the nutritive value, consumption of saturated fatty acids (SFA) has been associated with an increase of plasma cholesterol and plasma low density lipoprotein levels, which are linked at the same time to a major risk of coronary heart disease. Conjugated linoleic acid (CLA), which arises from microbial hydrogenation of dietary linoleic acid in the rumen, exhibits anticarcinogenic properties when is included in the diet at low levels (French et al., 2000). Additionally, meat flavour is influenced by the saturation rate of fatty acids (Purchas et al., 1979). The polyunsaturated fatty acids (PUFA) are more susceptible to oxidation than monounsaturated fatty acids (MUFA), their oxidation is primarily responsible for the oxidative meat flavour deterioration (Gatellier et al,, 2001).

Objectives

The purpose of this study was to analyse fatty acid composition in commercial lamb types representing different production systems from Spain, German, United Kingdom and Uruguay to assess the extent of "natural" dissimilarity in their fatty acid composition.

Materials and methods

Five groups of 20 lambs slaughtered at usual commercial weight were used from four countries: Spain, United Kingdom, Germany and two types of Uruguay, heavy and light lambs, which are representative of their typical production system conditions. Spanish lambs were from Rasa Aragonesa breed, produced in an intensive-housed system, weaned and kept on concentrates and cereal straw *ad libitum* until slaughtering. The carcass weight was 10.2 ± 0.2 kg. British lambs were Dorset breed, mainly reared on a grass-based system, using strategic concentrate supplementation, being carcass weight 22.8 ±1.7 kg. German lambs were commercial lambs (crossbreed between Merino Landschaf x Suffolk or Schwarzköpfe); with a carcass weight of 23.2 ± 3.6 kg. They were reared on grass complemented with concentrate. Uruguayan lambs were from Corriedale breed, they were raised under extensive improved grazing conditions, producing two types of lambs; the carcass weight of light and heavy lambs were 11.1 ± 1.4 and 19.6 ± 2.2 kg., respectively.

Intramuscular fat was extracted from *longissimus lumborum* muscle (Hanson and Olley, 1963). Methyl esters of the samples were formed according to Morrison and Smith (1964), using nonadecanoic acid (19:0) prior to saponification as internal standard. Chromatographic analysis of methyl esters was performed using a Perkin-Elmer gas chromatograph (Perkin-Elmer, USA). Fatty acids were identified from standards and quantified using the internal standard.

One-way ANOVA was performed using GLM procedure from the Statistical Analysis System package (SAS, 1996). Differences between the means were determined using the Student-Newman-Keuls test. PRINCOMP procedure was used to principal component analysis, the variables were standardized.

Results and discussion

The least square means of the fatty acid composition (expressed as proportion by weight of total fatty acids) of the lambs and fatty acid ratios from typical production systems of the countries studied are shown

in table 1 and 2, respectively. Spanish and Uruguayan light lambs showed the lowest intramuscular fat proportion (2.41% and 3.05%, respectively) related to the lowest weights and ages at slaughtered in comparison with the rest of the lamb types, where the Uruguayan heavy lambs had the highest intramuscular fat proportion (5.92%) with intermediate position to United Kingdom (4.32%) and German lambs (4.25%).

Spanish lambs showed the highest proportion of C18:2 and lowest of C18:0, while the Uruguayan and German lambs had the highest percentage of C16:0. The proportions of C18:3 and C20:5 were higher with grass fed lambs (mainly Uruguayan) compared with lambs reared intensively using concentrates (Spanish and German lambs). These differences in fatty acid composition could be mainly related to differences in the feeding production system (grass or concentrate). Thus, Díaz et al. (2002) found lambs fed with concentrate showed lower percentage of C18:0 and higher C18:2 than lambs fed with pasture. Grass contains high levels of C18:3, precursor of the *n*-3 fatty acids series, while concentrate generated high level of 18:2 precursor of *n*-6 fatty acids series (Rhee, 2000).

The CLA isomer, *cis-9, trans-11 18:2*, was detected in all the lambs types evaluated. However, Spanish lambs had only 0.40 % of the total of fatty acids, while the other lambs, showed more than twofold (0.94 % and 0.79 % for light and heavy Uruguayan lambs, 0.97 % for German and 1.05 % for British lambs). French et al. (2000) showed the linear increment of intramuscular CLA concentration when the proportion of concentrate in the diet decreased. The high concentrations of rapidly fermentable sugar and soluble fibre of forage creates a rumen conditions which promotes a greater production or decreased utilization of CLA by the rumen (Kelly et al., 1998).

The highest P/S ratio was for Spanish lambs, due to their lower proportion of SFA and higher of PUFA. It could be due to differences in feed, age and fatness level. Thus forage stimulates ruminal activity promoting the biohydrogenation of the fatty acids, which in turn, increases the concentration of SFA (Choi et al., 1997). Link et al. (1970) showed in muscle that the proportion of PUFA decreased with increasing animal age and concomitant increases in intramuscular neutral lipid deposition. The increment of SFA with age and decrement of PUFA could be the reason why older animals (German, British and Uruguayan heavy lambs) showed low PUFA proportions and P/S ratio. With regard to fatness Nürnberg et al. (1998) found a negative relationship between fat content and PUFA, and Marmer et al. (1984) found that triacylglycerols, which increased with fatness, are less unsaturated than phospholipids in muscle membranes. The ratio *n*-6/*n*-3 was very high in Spanish lambs (8.42) related to the other lambs (lower than 2.5). According to Kemp et al. (1981), the use of concentrate resulted in raised concentrations of *n*-6 PUFA and grass diets increased *n*-3 PUFA.

Principal component (PC) analysis was performed on intramuscular fat to study the relationship between fatty acid percentages and to examine the relationships between the types of lambs compared. Figure 1 displays the projection of the fatty acid data in the plane defined by the two first principal components (PC). The first PC explained 34.97 % of the variability of the fatty acid composition. This component was mainly characterized by C18:2, C20:4 in the right hand side and antithrombotic relation (ATT) in the left one; where these variables were placed far from the origin of the first PC. The second PC explained the 21.50 % of the variability, it was defined by long chain fatty acids (C22:5, C22:6 and C20:5) and in the opposite direction by MUFA, which were placed far from the origin of the second PC. However, Bas and Morand-Fehr (2001), using PCs analysis to study fatty acid composition, found that the first two PC explained about 45% of the total variance in subcutaneous and intramuscular fat, and about 60% in perirenal adipose tissue. The projection of the fatty acid data in the plane defined by the two first principal components of the five groups studied is shown in figure 2. Spanish lambs were clearly separated from rest of lamb types and were placed on the right hand, close to short chain fatty acids and *n*-6. The Uruguayan light lambs were located down in the figure, close to long chain fatty acids, whereas Uruguayan heavy and British lambs are slightly moved on the left side, where ATT, C18:0, SFA and CLA lay and German lambs were located between Spanish lambs and the other three groups.

Conclusions

The Spanish lambs fed with concentrate had lowest SFA proportion and higher PUFA proportion, therefore they had a better ratio P/S compared with the rest of the lambs types. The Uruguayan light lambs were similar to Spanish lambs in relation to PUFA proportion, and Uruguayan heavy, German and British lambs showed similar proportions of CLA, SFA and PUFA as well as P/S. Uruguayan heavy lambs had lower *n*-6/*n*-3 ratio in comparison with the German lambs, being the United Kingdom lambs placed between them.

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Tables and figures

Table 1. Fatty acid composition of *longissimus lumborum* muscle in percentage by weight of total fatty acids of lambs from typical production system from diverse countries.

	Spain	Germany	UK	Uruguay Light	Uruguay Heavy	CME	Sign.
Fat %	2.41 ^{c}	4.25 ^{b}	4.32 ^{b}	3.05 ^{c}	5.92 ^{a}	1.720	***
C10:0	0.24 ^{a}	0.23 ^{a}	0.16 ^{b}	0.22 ^{a}	0.22 ^{a}	0.004	***
C12:0	0.42 ^{a}	0.30 ^{b}	0.16 ^{c}	0.27 ^{b}	0.12 ^{c}	0.014	***
C14:0	3.77 ^{a}	3.62 ^{a}	2.36 ^{b}	3.60 ^{a}	2.55 ^{b}	0.759	***
C14:1	0.15 ^{a}	0.12 ^{b}	0.06 ^{c}	0.11 ^{b}	0.07 ^{c}	0.002	***
C15:0	0.47 ^{a}	0.49 ^{a}	0.43 ^{a}	0.41 ^{a}	0.32 ^{b}	0.009	***
C16:0	22.58 ^{b}	23.65 ^{ab}	23.43 ^{b}	24.73 ^{a}	24.66 ^{a}	2.302	***
C16:1	1.81 ^{a}	1.39 ^{b}	1.32 ^{b}	1.42 ^{b}	1.44 ^{b}	0.080	***
C17:0	1.31 ^{a}	1.04 ^{b}	1.10 ^{b}	1.07 ^{b}	1.02 ^{b}	0.035	***
C17:1	0.98 ^{a}	0.64 ^{c}	0.75 ^{b}	0.56 ^{c}	0.59 ^{c}	0.019	***
C18:0	12.56 ^{d}	18.79 ^{ab}	19.78 ^{a}	16.62 ^{c}	17.49 ^{bc}	4.327	***
C18:1	39.63 ^{a}	39.05 ^{a}	40.51 ^{a}	35.81 ^{b}	40.56 ^{a}	6.640	***
C18:2	9.48 ^{a}	5.45 ^{b}	3.92 ^{c}	6.01 ^{b}	4.18 ^{c}	1.990	***
C18:3	0.56 ^{c}	1.48 ^{b}	1.62 ^{b}	3.37 ^{a}	3.19 ^{a}	0.352	***
CLA	0.40 ^{c}	0.97 ^{ab}	1.05 ^{a}	0.79 ^{b}	0.94 ^{ab}	0.096	***
C20:0	0.09 ^{ab}	0.10 ^{a}	0.09 ^{ab}	0.11 ^{a}	0.07 ^{b}	0.001	***
C20:3	0.28 ^{a}	0.14 ^{cd}	0.17 ^{c}	0.22 ^{b}	0.10 ^{d}	0.005	***
C20:4	3.99 ^{a}	1.22 ^{c}	1.13 ^{c}	1.94 ^{b}	0.86 ^{c}	0.593	***
C20:5	0.34 ^{c}	0.51 ^{c}	0.94 ^{b}	1.29 ^{a}	0.86 ^{b}	0.122	***
C22:5	0.68 ^{b}	0.58 ^{b}	0.81 ^{b}	1.14 ^{a}	0.60 ^{b}	0.076	***
C22:6	0.24 ^{ab}	0.21 ^{b}	0.22 ^{b}	0.31 ^{a}	0.17 ^{b}	0.010	***

^{a,b,c}Least square means in the same row with different superscript letters are different (P<0.05);

Table 2. Fatty acid ratios of *longissimus lumborum* muscle from typical production system from diverse countries.

	Spain	Germany	UK	Uruguay Light	Uruguay Heavy	CME	Sign.
SFA	41.44 [b]	48.23 [a]	47.51 [a]	47.04 [a]	46.44 [a]	5.540	***
MUFA	42.58 [a]	41.21 [a]	42.64 [a]	37.90 [b]	42.66 [a]	7.170	***
PUFA	15.58 [a]	9.60 [b]	8.80 [b]	14.27 [a]	9.96 [b]	8.015	***
P/S	0.38 [a]	0.20 [c]	0.19 [c]	0.31 [b]	0.21 [c]	0.006	***
***n*-6/*n*-3**	8.42 [a]	2.47 [b]	1.54 [bc]	1.36 [bc]	1.07 [c]	2.284	***
ATT	0.17 [e]	0.55 [d]	1.05 [b]	0.80 [c]	1.19 [a]	0.037	***

[a,b,c]Least square means in the same row with different superscript letters are different (P<0.05); SFA: Saturated fatty acids; MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; P/S: PUFA/SFA; *n*-6/*n*-3: (C18:2+C20:3+C20:4)/(C18:3+C20:5+C22:5+C22:6); ATT: (C20:3+C20:5)/C20:4.

Figure 1. Projection of the fatty acid data in the plane defined by the two first principal components

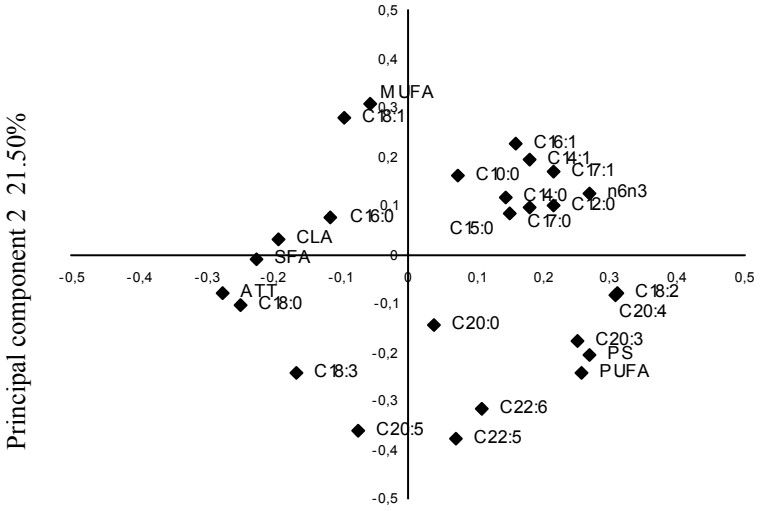
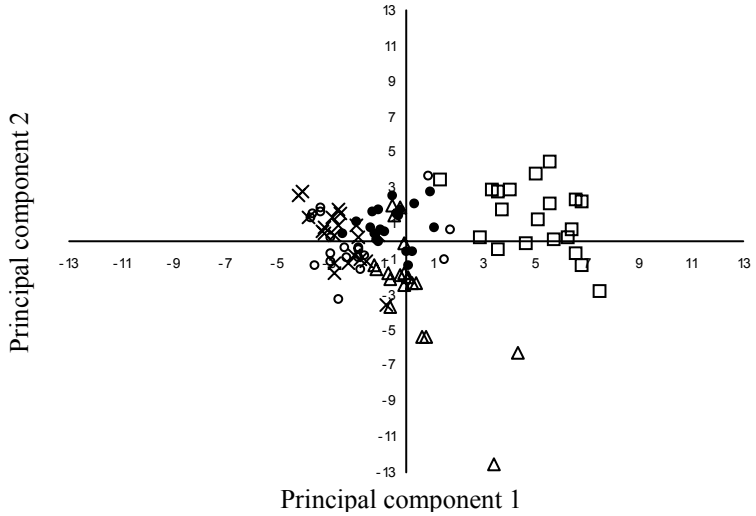


Figure 2. Projection of the fatty acid data of the five groups studied in the plane defined by two principal components. ▵Uruguayan light lambs. ×Uruguayan heavy lambs. ▫Spanish lambs.
○ British lambs. ● German lambs.



EFFECT OF ROSEMARY EXTRACT ON THE FORMATION OF HETEROCYCLIC AROMATIC AMINES IN FRIED BEEF PATTIES

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Background

Epidemiological studies have shown that the daily diet can be responsible for various types of cancer. Heterocyclic Aromatic Amines (HAA) are especially found in the crust of fried meat and fish. These substances are formed during the Maillard reaction from creatinine, carbohydrates and amino acids. Several HAA have been shown to be carcinogenic in long-term animal studies on rodents and non-human primates (Adamson et al., 1990). The International Agency for Research on Cancer has classified several HAA as possible human carcinogens and recommends the reduction of these compounds in exposure of humans (IARC, 1993). Besides the important physical parameters such as temperature and heating time, natural antioxidants should inhibit the formation of HAA, because free radicals are involved in the complex Maillard reaction and the formation of HAA.

Objectives

The objective of this study was to examine the possibility of reducing the formation of HAA in beef patties by using a commercial rosemary extract. Additionally, the beef patties were tested for sensory parameters such as taste, smell, juiciness and colour.

Materials and methods

Preparation of beef patties

Plain beef, roughly desinewed and defatted, was coarsely minced through a 3 mm plate. 1.2 % salt and the liquid extract of Rosmarinus officinalis (Tab.1) (Flavor Guard LO W/S, Chr. Hansen, Germany) in concentrations of 0.5, 0.75, 1.0 and 2.0 g/kg were added to the minced beef, respectively. The control batch was prepared without the extract. After mixing 80 g ± 1 g of the material, the beef patties (16 mm thick x 85 mm diameter; estimated fat content of 12 %) were formed with a special mould.

Heating devices

The patties were put between two pieces of tin foil, which were coated with sunflower oil. The two grill plates of a double contact grill (Nevada-grill, Neumärker, Hemer, Germany) produce a temperature of 230 °C. The patties were immediately fried on both sides simultaneously for 5 min to a core temperature of 72 °C and to a surface temperature < 190 °C at the end of the frying process.

Colour measurement of beef patties

One hour after heating, the L*(brightness)-, a*(redness)- and b*(yellowness)- values were determined by using a Chromameter CR 200 (Minolta, Ahrensburg, Germany).

Determination of HAA

The method of HPLC analysis with some modifications was based on the method described by Gross and Grüter (1992). The quantification was carried out with an external calibration (Norharman and Harman) or standard addition (MeIQx, 4,8-DiMeIQx, PhIP).

Sensory test

Sensory testers evaluated colour, juiciness, taste and smell of the fried samples with a scale from 0 (very unpleasant) to 6 (very pleasant). After frying, the samples were frozen for a week and reheated in a microwave for the sensory test.

Determination of weight loss during frying

The beef patties were weighed before and 1 hour after heating.

Results and discussion

There are different kinds of rosemary extracts in trade. Some of these extracts are in powder form and contain maltodextrin as carrier. These extracts were not suitable for our investigations because maltodextrins as carbohydrates can influence the Maillard reaction and the HAA formation consequently (Skog and Jägerstad, 1990). The different extracts also include strongly differing concentrations of carnosol, carnosolic acid and other antioxidant compounds such as phenolic diterpenes. These substances have a very high antioxidant potential and inhibit the warmed-over flavour in the patties, which were frozen and reheated in the microwave. The rosemary extract used was a water dispersible liquid, which contains lecithin and polyglycerides as emulgators. But the extract contains a lower content of carnosol and carnosolic acid (Tab. 1), while the products in powder form contain up to 17 %.

Figure 1 shows the effect of the used rosemary extract on the formation of HAA. PhIP was the only compound significantly reduced by adding the rosemary extract. The lowest concentration had approximately the same effect on the PhIP formation as the highest. The lowest used concentration was the recommendation of the extract producer for patties. Higher concentrations of the rosemary extract in the patties showed no further reduction effect on PhIP. The three highest concentrations of extract addition increased the amount of the MeIQx formation slightly. The PhIP content was reduced by 64 % in the average of all used extract amounts. Norharman and Harman were found in all beef patties but the contents increased with higher addition of rosemary extract.

The colour (Fig. 2) and the weight loss of the patties showed no significant differences between the batches with or without extract. Figure 3 shows the result of the sensory evaluation. All patties were evaluated equally pleasant as the controls without rosemary extract. The taste of the patties with extract was described pleasantly spicy. Even the higher amounts of extract addition did not result in over-spiced products.

Some authors have reported reductions in the content of HAA in fried ground beef patties as a result of adding oleoresin rosemary (Balogh et al., 2000). In this study, rosemary oil, when used in two concentrations (1 % and 10 % based on fat content), reduced PhIP formation by 44 % in fried meat. Contrary to these findings, some authors have reported that the formation of PhIP increases slightly with the addition of rosemary flavour. Additionally, no correlation could be found between the oxidative properties of the flavours and the formation of PhIP (Zöchling et al., 2002).

Conclusions

In the present study, only the content of PhIP decreased significantly with the addition of commercial rosemary extract. All other HAA, especially Norharman and Harman, increased slightly with the addition of rosemary extract. The PhIP content in the fried beef patties was reduced by 64 % in the average of all used extract amounts. The flavour and colour of the patties with rosemary extract were found pleasant.

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Abbreviations

HAA : Heterocyclic Aromatic Amines, MeIQx : 2-amino-3,8-dimethylimidazo [4,5-*f*]quinoxaline,
4,8-DiMeIQx: 2-amino-3,4,8-trimethylimidazo [4,5-*f*]quinoxaline,
PhIP: 2-amino-1-methyl-6-phenylimidazo [4,5-*b*] pyridine

Table 1. Specification of the rosemary extract

Ingredient	Concentration
Fat	> 97 %
Protein	< 0.2 %
Carbohydrate	< 1.0 %
Water	< 1.0 %
Carnosol	0.83 %
Carnosolic acid	0.78 %

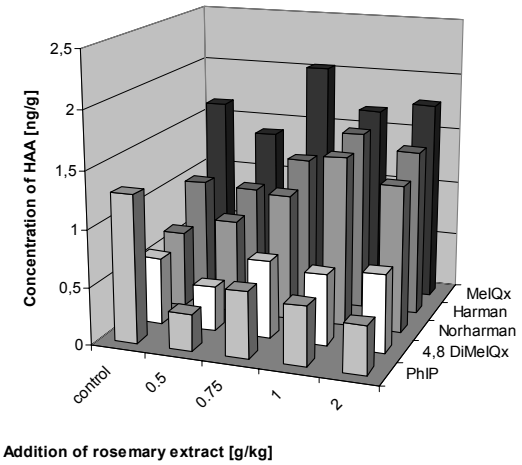

Figure 1. Concentration of HAA in fried beef patties for different additions of rosemary extract

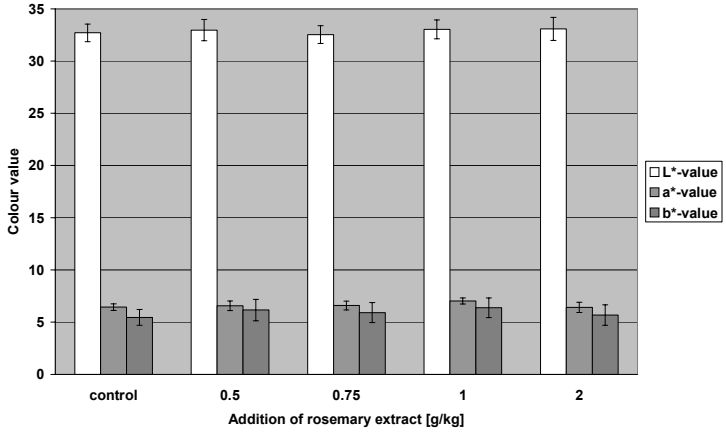

Figure 2. Colour measurement of fried beef patties for different addition of rosemary extract

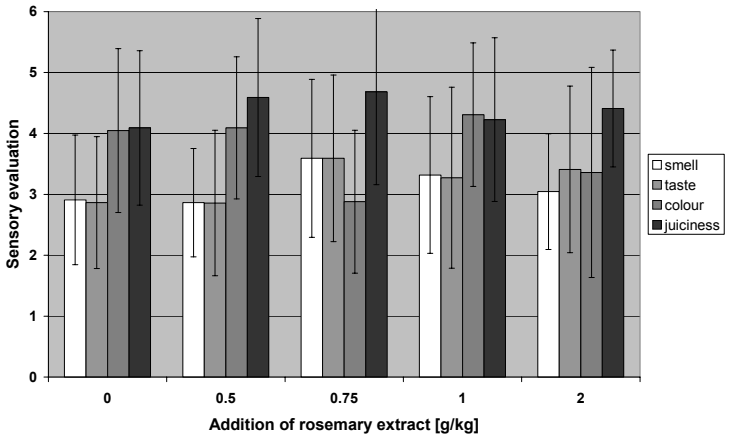

Figure 3. Sensory evaluation of fried beef patties for different addition of rosemary extract (scale from 0 (very unpleasant) to 6 (very pleasant))

THE EFFECT OF GENDER ON THE MEAT QUALITY CHARACTERISTICS OF KUDU *(TRAGELAPHUS STREPSICEROS)*

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Background

The kudu, one of Africa's most majestic animals shows strong sexual dimorphism, the male bears enormous spiral horns, which attains full length (≈120 cm) at six years of age, reaches a larger size (≈250 kg live weight) than the female (≈180 kg live weight) and is more predominantly adorned with manes of hair on the back and neck. Kudu are predominantly browsers, but will occasionally graze. Kudu occur throughout the savannah regions in central Africa south of the equatorial forests, through East Africa to Ethiopia, Sudan and Chad down to the Eastern Cape (South Africa), and are common throughout bushveld areas where there are stands of bush, they do not occur in open grassland and forest. Within South Africa, this species is hunted regularly for local consumption, particularly in the form of biltong (slices of meat, lightly salted and air-dried, similar to jerky). Of the R843m generated by the game industry in South Africa during 2000, 53.4% was generated by the biltong hunting industry and a further ≈3% by the game meat industry (Bothma, 2002). Kudu meat is also a regular item in most South African restaurants that serve game meat and is also frequently exported (Hoffman & Bogalke, 1999).

Objectives

Although this species is consumed regularly, very little data seems to have been published as pertaining to the muscle chemical composition and other quality attributes of its meat. In the present investigation, the proximate and fatty acid chemical composition of the *M. longissimus dorsi et lumborum* of eighteen animals are presented.

Materials and methods

Eighteen kudu were harvested early in the evening during winter in the Tussen die Riviere Nature Reserve in the Free State Province, South Africa. Of these, eight were males (varying in live mass from 111 to 179 kg) and ten females (live mass from 68 to 152 kg).

Standard harvesting techniques were utilised (Hoffman, 2003) with the animals all being killed instantaneously with a head shot using a .270 calibre rifle fitted with a telescopic sight. Live mass was recorded on the hot carcasses after being bled, approximately 60 min *post mortem*. The animals were then eviscerated, skinned and cleaned, followed by the removal of the head, gut and other edible and non-edible parts of the body. These included the kidneys, liver, lungs, internal fat as well as the leg from the hoof to the knee. The carcasses were then moved into a cooling facility overnight. The dressed carcasses were removed from the cooler (set at 4°C) early the next morning (≈12 h *post mortem*) and weighed for calculation of the dressing percentage and for removal of the muscle samples for chemical analysis. For chemical analysis the *M. longissimus dorsi et lumborum* (MLD) was removed from between the 12th and 13th rib to between the 4th and 5th lumbar vertebra.

The lean meat samples (MLD) were placed in polyethylene bags, vacuum-sealed and placed in a freezer at –20°C until further chemical analyses could be carried out. Proximate analysis was conducted on the MLD samples. After removing the subcutaneous fat and superficial connective tissue, the frozen muscle samples were cut into smaller portions, minced three times through a 2 mm sieve to ensure homogeneity, and analysed chemically. Total percentage moisture, protein and ash were determined according to standard AOAC methods (AOAC, 1997). The moisture content was analysed by drying a 2.5 g sample at 100°C for a period of 24 h. The protein (N x 6.25) content was determined by the block digestion method (AOAC, 1997), while ashing was done at 500°C for a period of 5 h. The total fat content was determined by extracting the fat with a 2:1 mixture of chloroform:methanol (Lee, Trevino & Chaiyawat, 1996).

The fatty acid content was determined using the same method described by Tichelaar *et al*. (1998). After thawing the meat, the lipids in a 2 g sample were extracted with chloroform/methanol (CM 2:1; v/v). All the extraction solvents contained 0.01% butylated hydroxytoluene (BHT) as an antioxidant. A polytron mixer (Kinematica, type PT 10-35, Switzerland) was used to homogenize the sample within the extraction solvent. Heptadecanoic acid (C17:0) was used as an internal standard to quantify the individual fatty acids. A sub-sample of the extracted lipids was transmethylated for 2 h at 70°C using methanol/sulphuric acid (19:1; v/v) as transmethylating agent. After cooling, the resulting fatty acid methyl esters (FAME) were extracted with water and hexane. The top hexane phase was transferred to a spotting tube and dried under nitrogen. The FAME were purified by TLC (silica gel 60 plates) and analysed by GLC (Varian Model 3300 equipped with flame ionisation detection) using a 60 m BPX70 capillary columns of 0.25 mm internal diameter (SGE, Australia). Gas flow rates were, hydrogen, 25 ml/min; and hydrogen carrier gas 2-4 ml/min.

Temperature programming was linear at 3°C/min, with an initial temperature of 150°C, a final temperature of 220°C, an injector temperature of 240°C and a detector temperature of 250°C. The FAME in the total lipids were identified by comparison of the retention times to those of standard FAME mixture (Nu-Chek-Prep Inc., Elysian, Minnesota).

A standard analysis of variation was performed on the various parameters measured and differences were tested for by means of student's t-test using SAS version 8.2 (SAS, 2002) statistical software.

Results and discussion

Due to the fact that non-trophy status bulls (young animals) were cropped, there was no significant difference for the live weight (121.30±10.531 vs 137.79±7.680 kg), carcass weight (72.40±6.684 vs 79.25±4.709 kg) or dressout percentage (59.55±0.942 vs 57.45±0.651 %) between the females and males respectively. This value is similar to the 58% reported for other game species such as the impala (Hoffman, 2000). The proximate chemical composition of the MLD is shown in Table 1. None of the parameters differed significantly between the sexes and the low lipid values indicate that kudu meat could be considered as a healthy lean meat.

Table 1. The average (±se) chemical composition of the *M. longissimus dorsi et lumborum* of female and male kudu.

Parameter	Female (n=10)	Male (n=8)	Pr > |t|
Moisture (%)	74.15 ± 0.285	74.49 ± 0.162	0.3420
Protein (%)	24.29 ± 0.278	23.58 ± 0.181	0.0591
Lipid (%)	1.56 ± 0.093	1.58 ± 0.056	0.8802
Ash (%)	1.29 ± 0.021	1.23 ± 0.032	0.0897

In Table 2 the fatty acid content of the MLD is shown. The following fatty acids were not detected: C22:0, C22:4n-6, C24:0 and C24:1n-9. Only two of the longer chained polyunsaturated fatty acids (C20:3n-6 and C20:%n-3) differed between the females and males, the later having a higher concentration each time. Of the kudu muscle's fatty acids, 37% were saturated, 22% monounsaturated and 41% polyunsaturated. The level of arachidonic acid was particularly high (≈8%) and was 0.47 mg per 100 g meat. The kudu had a very high mean desirable fatty acid (stearic acid plus all unsaturated fatty acids) content of 83%. The mean polyunsaturated to saturated ratio (1.12) were all well above the recommended 0.45 advocated by the British Department of Health (Enser *et al*., 1998). The n-6:n-3 PUFA ratio (2.34) was also well below the British department of Health's recommended figure of 4.

Table 2. The mean (±se) fatty acid content (%) of the *M. longissimus dorsi et lumborum* of female and male kudu.

Fatty acid	Female (n=10)	Male (n=8)	Pr > |t|
C16:0	17.53 ± 0.688	16.10 ± 0.343	0.1058
C16:1n-7	0.70 ± 0.129	0.52 ± 0.054	0.2637
C18:0	20.00 ± 1.157	19.72 ± 1.086	0.8642
C18:1	21.94 ± 1.455	19.91 ± 0.967	0.2873
C18:2n-6	19.03 ± 1.313	20.53 ± 0.786	0.3701
C18:3n-3	4.67 ± 0.383	4.85 ± 0.395	0.7463
C18:3n-6	0.08 ± 0.028	0.05 ± 0.026	0.4774
C20:0	0.20 ± 0.035	0.11 ± 0.418	0.1147
C20:1n-9	0.10 ± 0.030	0.06 ± 0.033	0.3313
C20:2n-6	0.12 ± 0.032	0.15 ± 0.083	0.7227
C20:3n-6	0.92 ± 0.049	1.14 ± 0.050	0.0076
C20:4n-6	7.74 ± 0.455	8.44 ± 0.376	0.2702
C20:5n-3	2.50 ± 0.256	3.17 ± 0.147	0.0492
C22:5	2.42 ± 0.150	2.75 ± 0.139	0.1349
C22:6n-3	2.06 ± 0.2391	2.50 ± 0.227	0.2022

Conclusions

The chemical composition of kudu *M. longissimus dorsi et lumborum* indicates that this meat could be classified as nutrient dense. Of particular interest is the high protein (≈24 g per 100g meat) and low fat content (≈1.5 g per 100 g meat) of the meat. The fatty acid profile of the meat is also very advantageous for human consumption by being highly unsaturated (≈ 63%) with a positive n-6:n-3 ratio. Eighty-three per cent of the fatty acid found in the meat could be classified as being desirable for human consumption. Analysis of the levels of Vitamin E and other anti-oxidants will be of value to see how prone towards rancidity the meat is. Similarly, the effect of the low lipid content on consumer acceptability (flavour, juiciness, etc) requires elucidation. Further research is also required on the amino acid and mineral profile of this meat.

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COMPARISON OF IMPORTED VS. DOMESTIC BEEF CUTS FOR RESTAURANT USE IN VENEZUELA. II. MARBLING LEVELS, PROXIMATE AND MINERAL COMPOSITION.

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Background

Ante mortem and *post mortem* factors influence nutritive value of beef (Seideman *et al*., 1989). Production patterns and the grading system in Venezuela differ from those applied in the U.S.A. Therefore, notorious differences in beef chemical composition and quality might be expected. Based on the wide variation in palatability of Venezuelan beef, the hotels, restaurants and other food service institutions (HRI) have been purchasing imported U.S. Choice or Select beef. Beef imports from U.S.A. have been banned since January 2004 because of the BSE issue. If import resumes, it is anticipated that consumer preference towards U.S. beef will continue. Top quality, well-marbled U.S. beef generally has a thick cover of fat that an increasing number of consumers find repelling. These consumers are either concerned with diet/health issues or are simply typical, price-oriented Venezuelans, who always look for lean beef, with no plate waste. The Venezuelan diet-health oriented, high-income market niche, could be satisfied by a national product if nutrient compositional advantages were guaranteed. To our knowledge, differences between Venezuelan and American beef in proximate and mineral composition have not been scientifically proven. The only available report (Huerta-Leidenz, 1998) compared beef *longissimus* from both origins indicating nutrient reference values from indirect sources; this is, without performing simultaneous analyses of samples under the same laboratory conditions.

Objectives

a) To compare marbling levels, proximate and mineral composition of top-graded Venezuelan and American beef samples commonly used by restaurants of Venezuela; and
b) To examine the variation in marbling levels and chemical composition due to muscle.

Materials and methods

Procurement of high-quality, imported U.S. beef samples

Twenty wholesale Beef Loin, Top Sirloin Cap (Coulottes) and 10 Beef Rib, Ribeye, Lip-On (Ribeyes) cuts were procured frozen in boxes labeled as "Choice or higher" (CH-or-Higher), and transported to Universidad del Zulia (LUZ). Upon arrival, marbling scores were individually assigned. One 2.54cm thick steak was fabricated and kept frozen (-20ºC). To be prepared for chemical analyses, partially thawed steaks were trimmed to zero fat cover (and other surrounding muscles in the case of ribeye). *Longissimus dorsi thoracis* (LDT) and *biceps femoris* (BF) muscles, from ribeyes and coulottes, respectively, were ground for homogenization with a Black & Decker™ food manual processor. Ground muscles were packed by duplicates in plastic Zip-lock™, bags and immediately stored at -20ºC until chemical analyses.

Venezuelan samples of known production history.

A first group of Venezuelan samples were derived from 18 steers of known genetic history (9 F1 Angus and 9 ¾ Brahman) semi-intensively fed during 60d. Animals were slaughtered and hot carcasses were graded (Decreto Presidencial 1896, 1997). In each breed type two carcasses were graded as Optima ("AA") and seven carcasses were graded as Excelente ("A"). At 72 h *post-mortem* ribeyes and coulottes were removed. Steak fabrication and preparation for chemical analyses was similar to the U.S. beef steaks.

Venezuelan samples of unknown production history

Twenty coulottes (10 "A" and 10 "AA") and 20 Ribeye roasts (10 "A" and 10 "AA") were purchased fresh from different butcher stores and supermarkets of Maracaibo city. Both types of retail cuts were reduced to 2.54cm thick steaks and the marbling level was determined with marbling photographs. Steak fabrication and preparation for chemical analyses was similar to the U.S. beef steaks.

Chemical analyses

Except for total lipids (by the Folch *et al.,* 1957 method), proximate analysis was performed according to the A.O.A.C. (1997). Except for phosphorus (by the A.O.A.C., 1997 method), mineral analyses were conducted by atomic absorption and/or atomic emission with ashing procedure (A.O.A.C, 1997), following the analytical methods described by Perkin-Elmer (1994).

Statistical analyses

Marbling scores and proximate compositional data were subjected to a simple one-way analysis of variance (ANOVA) to test differences due to grade (SAS, 1996). ANOVA of mineral contents included the effects of muscle, grade and the two-way interaction. The least squares means (LSMEANS) were separated by Tukey-Kramer´s test (SAS, 1996).

Results and discussion

Marbling levels

ANOVA detected effect (P=0.0001) of carcass grading on marbling level of BF and LDT samples (mean values are not shown in tabular form). Mean marbling levels in domestic BF samples were described as "Traces", while IMPORTED counterparts averaged a higher (P<0.01) "Small" amount of marbling. Top quality ("AA") domestic LDT exhibited "Slight" amounts of marbling, higher (P<0.05) than the "Traces" level described for the second-quality Venezuelan ("A") LDT, but still inferior (P<0.05) to the "Moderate" level found in CH-or-Higher LDT.

Proximate composition

Highly significant effects (P<0.001) of carcass grade on moisture and total lipid contents of BF and LDT were detected. Variation of protein content in BF samples was detected at P<0.10. CH-or-higher BF and LDT samples had lower (P<0.0001) moisture and higher lipid contents when compared to A and AA domestic samples (Table 1 and 2). To our knowledge there are no available reports in Venezuela regarding to the effect of carcass grade on proximate composition of the retail cuts under study.

Table 1. Least square means ± standard error for proximate composition of *biceps femoris*

Component (g/100g of fresh tissue)	Grades[a]		
	A (n=24)	AA (n=14)	Choice-or-higher (n=20)
Moisture	74.35 ± 0.30 [b]	74.27 ± 0.40 [b]	71.71± 0.34 [c]
Ash	1.22 ± 0.02	1.18 ± 0.03	1.20 ± 0.02
Protein	20.46 ± 0.15	20.36 ± 0.21	19.94 ± 0.15
Total lipids	3.46 ± 0.43 [b]	4.18 ± 0.57 [b]	6.01 ± 0.48 [c]

[a] Abbreviations of commercial terminology used to designate quality grades for beef carcasses in Venezuela and U.S.A. Letters A and AA corresponds respectively, to the second ("Excelente") and first ("Optima") quality grades in Venezuela (Decreto 1896, 1997); CH-or-Higher corresponds to imported U.S. boxed beef labeled as "Choice or higher".
[b,c] different letters in the same row indicates significant differences (P<0,05)

Table 2. Least square means ± standard error for proximate composition of longissimus dorsii thoracis

	Grade[a]		
Component (g/100g of fresh tissue)	A (n=24)	AA (n=14)	Choice-or-higher (n=10)
Moisture	74.68 ± 0.32 [b]	74.03 ± 0.42 [b]	68.17 ± 0.52 [c]
Ash	1.13 ± 0.01	1.14 ± 0.02	1.10 ± 0.02
Protein	21.87 ± 0.12	21.48 ± 0.16	21.94 ± 0.19
Total lipids	1.96 ± 0.38 [b]	2.99 ± 0.50 [b]	7.97 ± 0.60 [c]

[a] Abbreviations of commercial terminology used to designate quality grades for beef carcasses in Venezuela and U.S.A. Letters A and AA corresponds respectively, to the second ("Excelente") and first ("Optima") quality grades in Venezuela (Decreto 1896, 1997); CH-or-Higher corresponds to imported U.S. boxed beef labeled as "Choice or higher".
[b,c:] LSMEANS bearing different letters in the same row indicate statistical difference (P<0.05).

Mineral composition

ANOVA detected significant effects (P<0.05) of muscle and carcass grade on the mineral content.

Muscle effects

Except for Mg, P and Na, ANOVA detected the effect of muscle (P<0.05) on variation of most individual minerals under study (Table 3).

Table 3. Least Square means ± standard error for the mineral content of fresh, lean muscles.

	Muscle				
	Biceps Femoris		*Longissimus dorsi thoracis*		
Mineral (mg/100g of fresh tissue)	n		n		P value
Ca	58	6.8 ± 0.18	48	7.4 ± 0.21	0.026
Fe	58	2.4 ± 0.03	48	1.9 ± 0.04	0.001
Mg	54	23.3 ± 0.22	47	23.4 ±0.26	NS
P	58	197.0 ± 1.00	48	198.1 ± 1.14	NS
K	56	347.0 ± 2.02	45	354.3 ± 2.33	0.021
Na	58	61.3 ± 0.71	48	59.4 ± 0.81	0.09
Zn	58	3.8 ± 0.06	48	3.5 ± 0.06	0.002
Cu	58	0.04 ± 0.004	48	0.02 ± 0.004	0.001

NS = Non significant (P> 0.1)

Grade effects

Table 4 shows adjusted LSMEANS for the mineral content of muscles according to grade. "Choice or higher" samples showed higher Ca, Fe and Zn and lower P and K as compared to domestic samples. Mineral content did not vary (P>0.05) across Venezuelan grades. Mineral concentrations for domestic samples coincide with that presented in a previous report (Huerta-Leidenz *et al*., 2003).

Conclusions

Both imported and domestic beef muscles are highly nutritious foods and good sources of protein, Fe, P and Zn; Venezuelan beef steaks should have comparative advantages in the diet/health market niche due to their exceptional leanness.

Table 4. Least Square means ± standard error for the mineral content of 100g of fresh, lean sample, according to carcass grade.

Mineral (mg/100g of fresh tissue)	Grade[a]					
	A		AA		CH-or-higher	
	n		n		n	
Ca	48	6.0 ± 0.20 [b]	28	6.6 ± 0.26 [b]	30	8.6 ± 0.26 [c]
Fe	48	2.1 ± 0.04 [b]	28	2.0 ± 0.05 [b]	30	2.4 ± 0.05 [c]
Mg	43	23.9 ± 0.25 [b]	28	23.2 ± 0.31 [bc]	30	22.9 ± 0.32 [c]
P	48	201.4 ± 1.07 [b]	28	199.1 ± 1.40 [b]	30	192.0 ± 1.44 [c]
K	44	360.7 ± 2.24 [b]	27	349.9 ± 2.86 [b]	30	341.3 ± 2.87 [c]
Na	48	60.3 ± 0.76 [b]	28	60.8 ± 0.99 [b]	30	59.9 ± 1.02 [b]
Zn	48	3.3 ± 0.06 [b]	28	3.5 ± 0.08 [b]	30	4.2 ± 0.08 [c]
Cu	48	0.030 ± 0.004 [bc]	28	0.034 ± 0.005 [b]	30	0.021 ± 0.005 [c]

[a] Abbreviations of commercial terminology used to designate quality grades for beef carcasses in Venezuela and U.S.A. Letters A and AA corresponds respectively, to the second ("Excelente") and first ("Optima") quality grades in Venezuela (Decreto 1896, 1997); CH-or-Higher corresponds to imported U.S. boxed beef labeled as "Choice or higher". [b,c]: different letters in the same row, indicate significant differences ($p<0.05$).

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COMPARISON OF IMPORTED VS. DOMESTIC BEEF CUTS FOR RESTAURANT USE IN VENEZUELA. I. COOKERY TRAITS, CONSUMER IMPRESSIONS AND SHEAR FORCE.

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Background

According to the U.S Meat Export Federation, in 2002 Venezuela took up the third position among the leading markets for U.S. beef in Central and South America. Currently, Venezuela has kept a ban on imports of U.S. beef due to its BSE status. Substitution of high quality U.S. beef in the Venezuelan HRI trade is a clear opportunity but a serious challenge for national beef producers. Some Venezuelan cattlemen, particularly from the Zulia State regions, have been modifying traditional practices (genetics, gender, feeding, and management) to offer competitively, young steer carcasses that grade in the high-quality Venezuelan standards (Decreto 1896, 1997). Despite all these particular efforts, there is a lack of information about how well this new concept of "high-quality" Venezuelan beef will compare in consumer acceptance to imported U.S. beef of the top USDA grades.

Objectives

The purpose of this study was to compare particular samples of high-valued HRI beef cuts of different background (domestic vs. imported) in cookery traits, consumer sensory ratings, and shear force values, according to grade.

Materials and methods

Procurement of high-quality, imported U.S. beef samples. A total of 12 wholesale Beef Loin, Top Sirloin Cap (IMPS/NAMP 184D, commonly referred as Coulottes) and 10 Beef Rib, Ribeye, Lip-On (IMPS/NAMP 112A, commonly referred as Ribeyes) cuts (NAMP, 1997), were procured from the two major food trading companies of Venezuela. The U.S. wholesale cuts were individually vacuum packaged, imported in boxes labeled as "Choice or higher" (CH-or-Higher) and kept in frozen storage (-20ºC). U.S. wholesale cuts were transported to Universidad del Zulia and three 2.54 cm thick steaks were fabricated and kept frozen (-20ºC) until further analyses.

Procurement of high quality, domestic samples. Twenty steers of known genetics (10 F1 Red Angus and 10 ¾ Brahman) were selected to represent a unique "high-quality" domestic beef sample produced in a particular zone of Zulia State. The steer group was placed on pastures during the stocking phase. The semi-intensive fattening (concentrate supplementation) phase lasted 60 d. The group was subjected to typical industry procedures. At 24 h post-mortem, chilled carcasses were evaluated and graded according to Venezuelan (Decreto Presidencial 1896, 1997) and USDA standards (USDA, 1989). Ribeyes and coulottes were removed from the right side of each carcass 48h postmortem. Three 2.54cm-thick steaks were identified, vacuum packaged, and immediately stored at 4ºC during 15 d for aging. When the aging period was completed, samples were blast frozen at -20ºC. Frozen samples were transported in a refrigerated truck to LUZ. Upon arrival, the samples were stored in home freezers during 20 d until further analyses.

Cookery, sensory evaluation and shear force determinations. Sample preparation, cooking procedures and cooking equipment followed those guidelines described by AMSA (1995). Sensory evaluation of the LDT samples was conducted with 69 consumers. Sensory evaluation of the BF samples was conducted two days after with 77 consumers. Overall first impression, flavor, and tenderness were surveyed by using hedonic, non-descriptive, 1-to-9 rating scales (1= Dislike very much; 9= Like very much). Additionally, flavor intensity was evaluated by a descriptive, non-structured hedonic scale (1= Insipid; 9= Strong). Warner-Bratzler shear force determinations (WBS) of LDT and BF samples were conducted simultaneously during two days.

Statistical Analyses.
Carcass data. A simple one-way analysis of variance (F-test) to test differences due to breed type was conducted (SAS, 1996).
Cookery and sensory data. Muscle data were subjected to analysis of variance (ANOVA) by SAS (1996) using cold carcass grade as the main effect, and consumer panelist as a block. The least squares means (LSMEAN) were separated by Tukey-Kramer´s (SAS, 1996). The proportion (%) of consumers indicating rating values of 6 or higher for the overall first impression was used as a measure of acceptability.
WBS data. The effects of grade, muscle and the two-way interaction were analyzed and LSMEANS were separated by Tukey-Kramer´s test (SAS, 1996). The proportion of Tender (WBS value = <3.88 kg), Intermediate (WBS value = 3.88 to 4.98 kg) and tough (WBS value = >4.98 kg) steaks, were determined using tenderness thresholds values (Huerta-Leidenz and Rodas-Gonzalez, 1998).

Results and discussion

Carcass quality of the samples. F1 Red Angus steer carcasses were younger (A^{84} ± 2.91 vs. B^{01} ± 2.91; P=0.0006) according to their skeletal maturity, and trended to exhibit an overall better fleshing as compared to the ¾ Brahman steers. While 70% of the F1 Red Angus carcasses fell in the top quality Venezuelan grade, none of the cold ¾ Brahman carcasses did. Ribeye marbling scores of F1 Red Angus (Practically devoid^{94} ± 27.1) and ¾ Brahman steers (Traces^{70} ± 27.1) were not different (P> 0.05). Average marbling levels for the samples of U.S. "Choice or higher" beef used in the present study, were Small^{98} ± 26.24 and Moderate^{59} ± 32.39, for BF and LDT muscles, respectively.
Cookery traits. Table 1 shows LSMEAN values for cookery traits according to grade and muscle. BF Choice-or higher samples required less (P<0.05) time to reach the endpoint temperature. LDT samples showed similar cooking times to longissimus samples derived from Venezuelan "A" bulls (Malaver et al., 2000). Percentage of cooking loss did not vary across breed types or grades. The greater (P<0.05) weight losses of the U.S. beef steaks are attributed to their larger sizes.
Consumer Sensory Evaluation. Table 2 shows LSMEANS for consumer ratings of sensory traits of LDT and BF according to grade. Consumers assigned higher (P<0.01) ratings to the U.S. imported LDT in all sensorial traits under study. The fact that U.S. CH-or-Higher LDT samples obtained the highest (P<0.001) consumer ratings for flavor and flavor intensity was unexpected. It is commonly believed that many Latin American consumers; accustomed to the strong flavor of relatively old, grass-fed beef, are not pleased with the taste of U.S. beef, due to its bland flavor. In regard to BF muscles, non-significant differences in tenderness were found between the BF steaks graded AA and their Choice-or-higher counterparts.
Level of consumer acceptability. Table 3 shows percentages of consumer acceptability of LDT and BF according to grade. CH-or-higher LDT exhibited the highest level of acceptability (80%). Level of acceptability for each grade, calculated by pooling sensory data from both muscles showed (not presented in tabular form) the highest acceptability level --approximately 67% of 146 evaluations-- was reached by the CH-or-Higher beef. No previous consumer preference studies had been conducted in Venezuela to compare beef from different origins.
Shear force determinations and tenderness classes. No significant variation (P> 0.05) in WBS values due to muscle, or to muscle x grade was detected; therefore, muscle data were pooled. LSMEANS values for WBS by grade, were decreasing in the sequence A >AA> CH-or-Higher, with a significant difference between the top-quality, Venezuelan AA grade and its U.S. counterpart (Table 4). It is noteworthy to indicate that no tough steaks were detected for any sample of the three grades (Table 4). An outstanding proportion (94%) of tender steaks was exhibited by the CH-or-Higher samples.

Conclusions

The use of imported Choice-or-higher BF and LDT cuts is highly justified based on the high consumer acceptability level observed herein. The inferior and unacceptable tenderness impression ratings of Venezuelan grade A BF and LDT steaks, prevents its use in gourmet-type restaurants.
Similar tenderness ratings between the Venezuelan top quality AA and Choice-and-higher, BF steaks, does not necessarily indicate that they can be used interchangeably in the HRI trade, because the first impression and flavor of AA steaks were rated as unacceptable (<6 points). The shorter cooking time of "Choice and higher" BF steaks is another important consideration for food service operators.
Due to the low number of observations and the very specific nature of the samples under study, the present findings must be taken as preliminary.

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TABLE 1. Least squares means ± standard error for cookery traits by grade and muscle[a].

	Venezuelan grade				U.S. grade	
	A		AA		CH-or-higher	
Trait	BF (n=11)	LDT (n=8)	BF (n=5)	LDT (n=5)	BF (n=10)	LDT (n=6)
Cooking time, min	72.3 ± 3.17[d]	84.4 ± 5.08	61.1 ± 4.16[de]	85.5 ± 6.22	52.5 ± 3.87[e]	79.00 ± 8.20
Cooking losses, g	65.2 ± 4.40	97.8 ± 8.90[f]	59.1 ± 5.76	98.5 ± 10.9[f]	54.6 ± 5.33	137.5 ± 14.1[g]
Cooking losses, %	24.3 ± 1.36	26.3 ± 1.41	20.3 ± 1.78	23.4 ± 1.73	25.5 ± 1.62	25.9 ± 2.09

[a] Abbreviations of commercial terminology used to designate quality grades for beef carcasses in Venezuela and U.S.A. Letters A and AA corresponds respectively, to the second ("Excelente") and first ("Optima") quality grades in Venezuela (Decreto 1896, 1997); CH-or-Higher corresponds to imported U.S. boxed beef labeled as "Choice or higher".
[d,e] LSMEANS bearing different letters among *biceps femoris* (BF) samples in the same row indicate statistical difference (P<0.05).
[f,g] LSMEANS bearing different letters among *longissimus dorsii thoracis* (LDT) samples in the same row indicate statistical difference (P<0.05).

Table 2. Least squares means ± standard error for sensory traits[a] of cooked muscles, by grade.

	A		AA		CHOICE OR HIGHER	
Trait	BF (n=12)	LD (n=12)	BF (n=7)	LD (n=7)	BF (n=10)	LD (n=6)
First impression	5.15 ± 0.28	4.56 ± 0.32[e]	5.66 ± 0.37	6.04 ± 0.24[f]	5.14 ± 0.29	7.07 ± 0.22[g]
Flavour	5.25 ± 0.28	4.79 ± 0.31[e]	5.81 ± 0.38	6.11 ± 0.24[f]	5.17 ± 0.29	7.36 ± 0.22[g]
Flavour intensity	5.10 ± 0.26	4.22 ± 0.32[e]	4.42 ± 0.35	4.79 ± 0.24[e]	4.37 ± 0.27	6.01 ± 0.22[f]
Tenderness	5.52 ± 0.27[c]	5.42 ± 0.38[e]	6.41 ± 0.37[cd]	6.28 ± 0.29[e]	7.17 ± 0.28[d]	7.24 ± 0.26[f]

[a] A and AA corresponds respectively, to the second and first quality grades in Venezuela (Decreto 1896, 1997); CHOICE OR HIGHER corresponds to imported U.S. boxed beef
[b] Sensory traits were rated by non-trained panelists using hedonic, 1-to-9 rating scales (1= Dislike very much, bland; 9= Like very much, strong).
[c,d] Within *biceps femoris* comparisons, values bearing different letters in the same row indicate statistical difference (P<0.05).
[e,f,g] Within *longissimus dorsi thoracis* comparisons, values bearing different letters in the same row indicate statistical difference (P<0.05).

TABLE 3. Level of consumer acceptability for cooked muscles according to grade

	Grade[a]					
	A		**AA**		**CH-or-higher**	
Muscle[b]	BF	LDT	BF	LDT	BF	LDT
(n[c])	**(37)**	**(17)**	**(27)**	**(46)**	**(43)**	**(55)**
Acceptability[d], %	44.1	37.8	52.9	66.7	55.8	79.7

[a] Abbreviations of quality grades for beef carcasses in Venezuela and U.S.A. Letters A and AA corresponds respectively, to the second ("Excelente") and first ("Optima") quality grades in Venezuela (Decreto 1896, 1997); CH-or-Higher corresponds to imported U.S. boxed beef labeled as "Choice or higher".
[b] *biceps femoris* (BF) , *longissimus dorsi thoracis* (LDT) .
[c] n= number of samples rated by consumers with a first overall impression rating of 6 or higher by using a hedonic, non-descriptive, 1-to-9 rating scale (1= Dislike very much; 9= Like very much).
[d] Percentage of steaks from the total tastings of any muscle, declared as "Acceptable" based on reaching a first overall impression rating of 6 or higher by using a hedonic, non-descriptive, 1-to-9 rating scale (1= Dislike very much; 9= Like very much).

TABLE 4. Least squares means ± standard error for shear force values of cooked lean beef, samples by grade[a] and tenderness classification

	Venezuelan grade		**Unknown U.S. type**
	A	**AA**	**CH-or-higher**
Trait	**(n=13)**	**(n=9)**	**(n=18)**
Warner Bratzler shear force, kg	3.53 ± 0.19 [c]	3.15 ± 0.22 [c]	2.67 ± 0.15 [d]
Tenderness class [b] , n (%)			
Tender	8 (61.5)	7 (77.8)	17 (94.4)
Intermediate	5 (38.5)	2 (22.2)	1 (5.6)
Tough	0 (0.0)	0 (0.0)	0 (0.0)

[a] Abbreviations of commercial terminology used to designate quality grades for beef carcasses in Venezuela and U.S.A. Letters A and AA corresponds respectively, to the second ("Excelente") and first ("Optima") quality grades in Venezuela (Decreto 1896, 1997); CH-or-Higher corresponds to imported U.S. boxed beef labeled as "Choice or higher".
[b] n = number and percentage (in parenthesis) of steaks (pooled muscled data) classified as follows:
Tender: Warner-Bratzler shear force value less than 3.88 kg.
Intermediate: Warner-Bratzler shear force value between 3.88 kg and 4.98 kg.
Tough: Warner-Bratzler shear force value higher than 4.98 kg.
[c,d] LSMEANS bearing different letters within a grade comparison in the same row indicate statistical difference ($P<0.05$).

BIOLOGICAL ACTIVITIES OF ACE INHIBITORY PEPTIDES DERIVED FROM BEEF HYDROLYSATES

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Background

Recently, the interest in the composition of food has been increased because potential anti-carcinogens and other therapeutic agents of food have been reported (Messina et al, 1994; Gibbs et al., 2004). Of particular interests in nutrition and food science are bioactive peptides that are present in the amino acid sequence of food proteins. These peptides are inactive within the sequence of the parent proteins but can be released by enzymatic proteolysis, for example, during gastrointestinal digestion or during food processing. Also, they are produced partly by the hydrolytic action of commercial enzymes (Gibbs et al., 2004). Once they are liberated in the body, the peptides may act like regulatory compounds. Thus, these peptides could be the potential health enhancing nutraceuticals for food and pharmaceutical applications. (Meisel, H.1997). From the nutritional point of view, meat contains many valuable components and biologically active substances (Korhonen et al., 1998). Arihara et al.(2001) reported that ACE inhibitory peptides were purified from porcine skeletal muscle proteins. However, very few studies have been done on beef protein (Jang et al., 2000). In this respect, we separated ACE inhibitory peptides from beef protein hydrolysates by enzyme hydrolysis and suspected that these peptides may have other biological activities.

Objectives

The aim of this study was to separate ACE inhibitory peptides from beef protein hydrolysates using seven commercial enzymes and investigate whether these ACE inhibition active peptides have any other biological activities, such as cytotoxic activity, antimicrobial activity, and machrophage stimulating activity.

Methods

Protein digestion was carried out with 7 enzymes, thermolysin at pH7.5 and 37°C, proteinase K at pH7.5 and 37°C, pepsin at pH3.0 and 37°C, protease at pH7.5 and 37°C, trypsin at pH 7.6-8.0 and 25°C, tyrosinase at pH6.5 and 25°C, and papain at pH6.2 and 25°C for 8 hours. The enzymatic hydrolysates were purified further with ultrafiltration and gel filtration. Of each hydrolysate, the fraction with the highest inhibitory activity was selected and identified by RP-HPLC. The amino acid sequence of those peptides was revealed by protein sequencer and the biological activities were determined. The ACE inhibitory activity was measured by a method described in our previous report (Jang et al.,2001). Antimicrobial activity was examined against six pathogens, *Escherichia coli, Staphylococuus aureus, Salmonella thyphimurium, Pseudomonas aeruginosa, Bacilus cereus,* and *Listeria monocytogenes* with tryptic soy agar (Difco. U.S.A). The sensitivity of pathogen to the peptides was determined by the disk diffusion method (Ponce et al., 2003) The diameter of the inhibition halos were as: not detected for diameters less than 8 mm; sensitive (+) for diameters 9-12 mm; very sensitive (++) for diameters 13-18 mm and extremely sensitive (+++) for diameters larger than 19mm. The cytotoxic effect of the peptides was assayed in vitro against MCF-7(breast cancer cell), AGS(stomach cancer cell), and LLC(lung cancer cell) by using the MTT assay (Carystinos, et al.,2001). Percent cell viability of test samples was determined as : % Cell Viability = (average OD for test group/average OD for control group) x 100. To acquire tumor cytotoxicity, machrophages should be activated by stimulants such as lipopolysaccharide. The activated macrophage secreted tumor necrosis factor, interleukin-1 (IL-1), and nitric oxide as soluble effectors. In this respect, the nitrite formation was taken as an index of the macrophage (RAW 264.7) stimulating activity (Miwa et al., 1990). Nitric oxide production was estimated by measuring the accumulation of the stable NO metabolite, nitrite, in the culture supernatants using the Griess assay (Green et al., 1982).

Results and discussion

Four peptides were separated from the hydrolysates for 8hrs incubation with 7 enzymes. The ACE inhibition activities of the separated peptides 1155, 325, 1152, 714, and 1134 were 14.64%, 34.77%, 25.09%, 31.66%, and 53.36%, respectively (Tab. 1). Their IC_{50} values were 117.27, 64.27, 52.92, 50.35, and 75ug/ml. The peptide 714 showed that the smallest concentration needed to inhibit 50% of ACE. Although the structure-activity relationships of many bioactive peptides have not yet been fully established, peptides with a defined bioactivity are known to have common structural features. Structure activity correlations among different peptide inhibitors of ACE indicate that binding to ACE is strongly influenced by the C-terminal tripeptide

sequence of the substrate.(Meisel, H.1997). These peptides characterized were studied for biological activity. A wide variety of organisms produce antimicrobial peptides as a primary innate immune strategy. Typically, peptides that are relatively short(less than 100 amino acids), positively charged, and amphiphillic acid are reported to be active against bacteria, fungi, viruses and protozoa(Farnaud, et al., 2004).
Table. 2 shows the effects of peptide 1155 on 6 pathogens. All the treated concentrations of peptide 1155 showed a sensitive inhibition on *S.typh., B.cer., E.coli, and L.mono* but not *S.aureus and P.aeru*. Peptide 325 showed very sensitive inhibition activity, especially on *E.coli* and *P.aeru* with concentration of 200 and 400ug/ml (Tab.3). However, peptide 1152 was very sensitive to *P.aeru*., only with 200ug/ml and 400ug/ml. Peptide 714 showed sensitive activity to *E.coli* at all treated concentrations(Tab.4). From this result, small peptides (3-4 amino acids), 1152 and 325 in particular, resulted in very sensitive activity to *P.aeru.* but the reason was not clear. Bellamy et al.(1993) suggested that the antimicrobial activity of lactoferricin and synthetic analogs seems to be correlated with the net positive charge of the peptides. These cationic peptides kill sensitive microorganisms by increasing cell membrane permeability.
The cytotoxic effect of separated peptides on breast cancer, lung cancer, and stomach cancer was measured by MTT assay. Peptide 325 showed a slight decrease of MCF-7 cell viability dose dependently(Fig. 1). The peptide 325 reduced cell viability by 85% at 400ug/ml of concentration. However, the rest of peptides showed no significant decrease of cell viability. Fig. 2. shows the effect of separated peptides on stomach cancer(AGS) cell. When 400ug/ml of peptide 325 and peptide 1155 were added into the AGS cell, the viability was decreased by 75% and 25%. The peptide 1155 showed significant reduction by up to 20%. However, peptide 714 seemed to act as a nutrient to AGS cell where it increased viability of AGS cell. Also, peptide 325 showed 4% reduction, while the other peptides had no cytotoxic effect on LLC(Fig. 3). Peptide 325 inhibited viability of those 3 tumor cells. Thus, this peptide was selected to investigate NO production by stimulation of machrophage. To investigate machrophage stimulation activity, 100, 300, and 1000ug/ml of peptide 325 were treated at machrophage. However, nitric oxide was not produced in all treatments(data not shown). Miwa et al.(1997) examined if nitric oxide formation by macrophage was stimulated with water extracts from meats and offal. They suggested that a water extract of beef didn't produce nitric oxide. Low concentrations of NO from activated macrophages are beneficial as, along with other reactive nitrogen intermediates, they are responsible for cytostatic and cytotoxic activity against infectious organisms and tumor cells (Komutarin, et al., 2004). However, overproduction of NO has been found to be associated with various disease such as septic shock, autoimmune disease, chronic inflammation by increasing vascular permeability, and the extravasations of fluid and proteins at the inflammatory site (Moncada, et al., 1991; Komutarin, et al., 2004).

Conclusions

ACE inhibitory peptides Separated from beef hydrolysates by enzymes were found to have cytotoxic activity on tumor cells and antimicrobial activity on 6 pathogens while macrophage stimulating activity was not shown.

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Tab. 1. ACE inhibition activity and IC_{50} value of separated peptides

Peptide	1155	325	1152	714	1134
ACE inhibition activity(%)	14.64	34.77	25.09	31.66	53.36
IC_{50} (ug/ml)	117.27	64.27	52.92	50.53	75.0

Tab.2. Antimicrobial activity of peptide 1155 (octomer)

	S. typh.	*B. cer.*	*E. coli*	*L. mon.*	*S. aureus*	*P. aeru.*
100	+	+	+	+	ND	ND
200	+	+	+	+	ND	ND
400	+	+	+	+	ND	ND
DMSO	ND	ND	ND	ND	ND	ND
2% CA*	++	+++	++	++	ND	+++

Tab. 3. Antimicrobial activity of peptide 325 (tetramer)

	S. typh.	*B. cer.*	*E. coli*	*L. mon.*	*S. aureus*	*P. aeru.*
100	ND	ND	+	ND	ND	++
200	ND	ND	++	ND	ND	++
400	ND	ND	++	ND	ND	++
DMSO	ND	ND	ND	ND	ND	ND
2% CA*	++	++	+	ND	ND	++

Tab. 4. Antimicrobial activity of peptide 1152 (trimer)

	S. typh.	*B. cer.*	*E. coli*	*L. mon.*	*S. aureus*	*P. aeru.*
100	ND	ND	ND	ND	ND	+
200	ND	ND	ND	ND	ND	++
400	ND	ND	ND	ND	ND	++
DMSO	ND	ND	ND	ND	ND	ND
2% CA*	++	++	+	ND	ND	++

Tab. 5. Antimicrobial activity of peptide 714 (hexamer)

	S. typh.	*B. cer.*	*E. coli*	*L. mon.*	*S. aureus*	*P. aeru.*
100	ND	ND	+	ND	ND	ND
200	ND	ND	+	ND	ND	ND
400	ND	+	+	ND	ND	+
DMSO	ND	ND	ND	ND	ND	ND
2% CA*	++	++	+	ND	ND	++

*2% CA: citric acid, +++: extremely sensitive, ++:very sensitive, +: sensitive, ND: not detected

Fig. 1. Cytotoxicity of peptides on MCF-7 cell

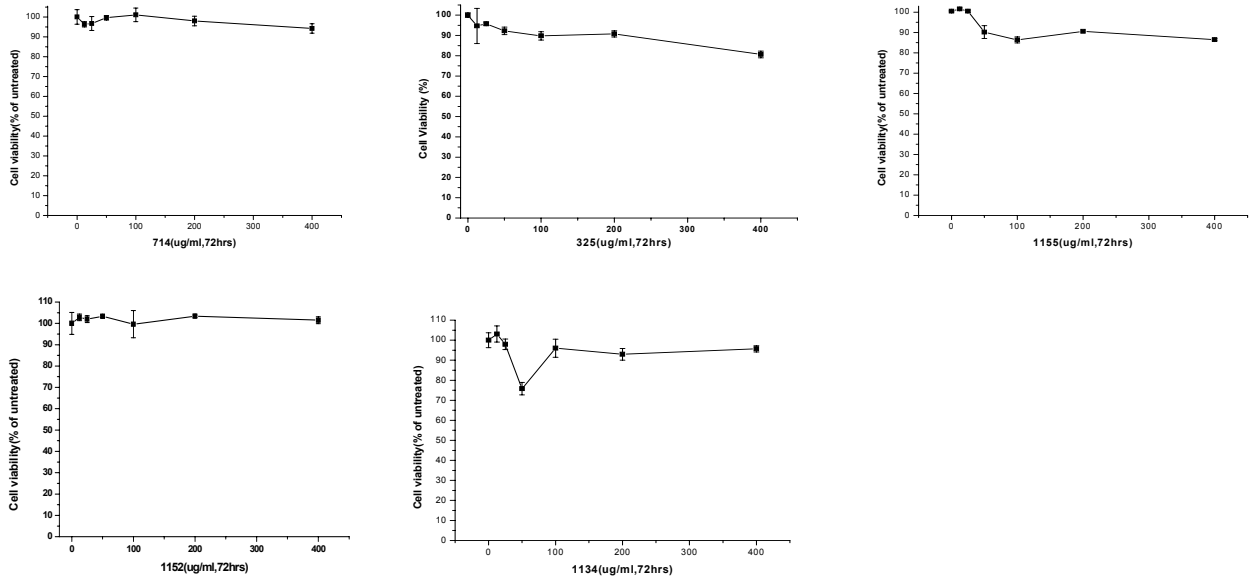


Fig.2. Cytotoxicity of peptides on AGS cell

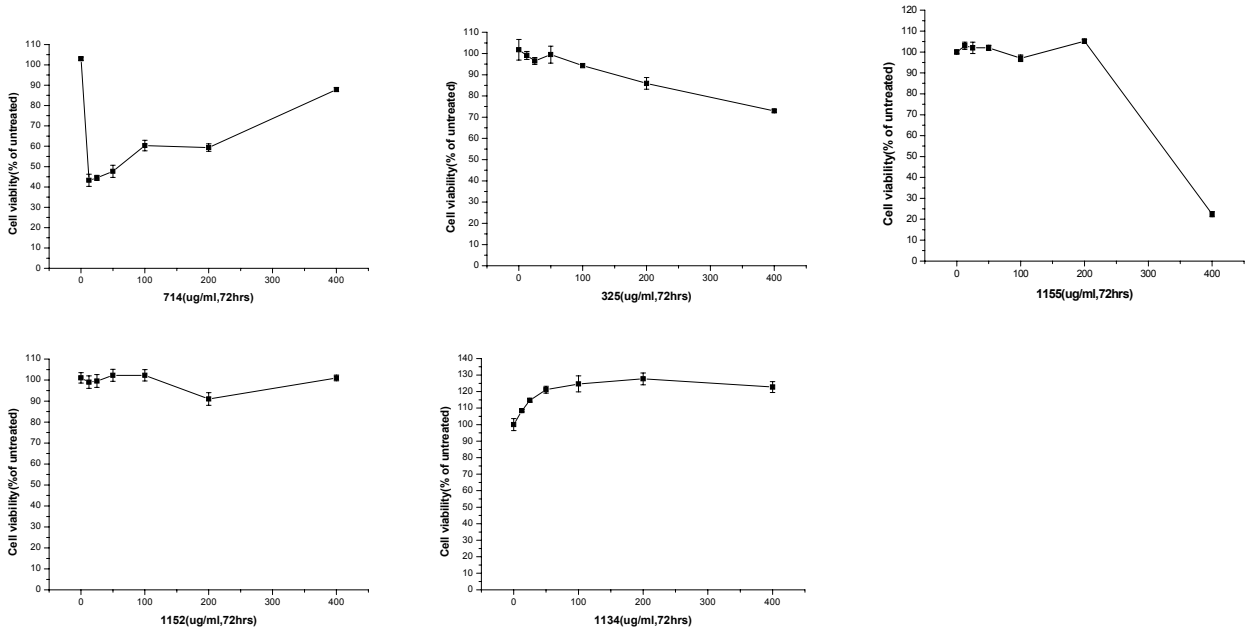
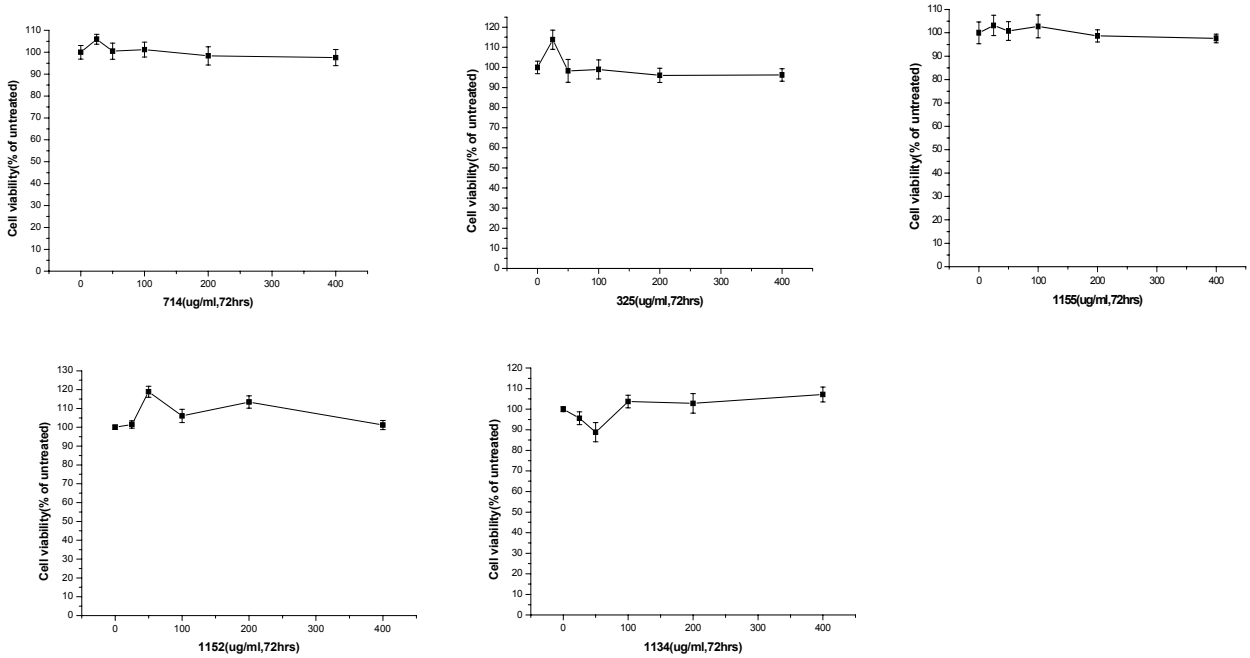


Fig. 3. Cytotoxicity of peptides on LLC cell



* Peptide 1155(octomer), 325(tetramer), 1152(trimer), 714(hexamer), 1134(hexamer)

A NEW METHOD FOR QUANTIFICATION OF APOLAR HETEROCYCLIC AROMATIC AMINES WITH PLANAR CHROMATOGRAPHY

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Background

Heterocyclic Aromatic Amines (HAA) are among the most potent mutagenic substances [1]. Around 20 different HAA at low µg/kg level can be identified in food. During the heating process at high temperatures, these substances are formed in meat from the precursors creatine, creatinine, amino acids and sugars. The concentrations depend on the way of preparation, heating time and temperature [2].

Objectives

HAA are typically quantified by liquid or gas chromatography with detection by UV-absorbance, fluorescence or mass spectrometry [3]. The objective of this study was to develop a new method to determine HAA, which have very similar chemical structures.

Materials and methods

Materials

The standard substances Glu-P-1, Glu-P-2, AαC and MeAαC were purchased from Toronto Research Chemicals, Ontario, Canada; Harman and Norharman from Sigma-Aldrich, Taufkirchen, Germany; HPTLC plates silica gel 60 F_{254} (20 x 10 cm) from Merck, Darmstadt, Germany.

Stock solutions

Standards were dissolved in methanol/NH_3.

Instruments

For sample application, the dissolved substances were sprayed in bands by Automatic TLC sampler 4 (ATS 4, Fig. 1). Afterwards, the plates were repeatedly developed in the same direction by the Automated Multiple Development System (AMD 2, Fig. 2). Drawn by capillary forces the developing solvent (mobile phase) migrated through the layer (stationary phase) over a defined distance. Densitometric evaluation of the developed plate was done by TLC Scanner 3 under UV 366 / > 400 nm. The DigiStore documentation system, i.e. Reprostar 3 with digital camera, made it possible to take a photo of the plate. The software winCATS controls all mechanical and electronic functions of the instruments. All TLC instruments were developed by CAMAG, Muttenz, Switzerland.

Results and discussion

Several affecting parameters had to be investigated to find optimal conditions to separate the six apolar and fluorescent HAA. In trade, various plates are available. These differ in material, thickness, particle size, pH-value, fluorescence indicator and binders. Furthermore, the optimal solvent or solvent mixture had to be found. The separation of the bands depends also on the number of gradient steps and migration distance. Moreover, basic, acid or neutral preconditioning between each gradient step changes the activity and pH-value of the layer. This can influence the separation and the focus of several bands. A five-step development to a migration distance of 40 mm each with the solvent mixture of 98 % diethyl ether and 2 % methanol, turned out to be the optimal condition to separate the substances (Fig. 3).

In Figure 4, pictures of plates after the chromatographic development are shown. Each standard is applicated separately as well as in a mixture of three standards. Mix A consists of Glu-P-1, AαC and Norharman, and mix B of Glu-P-2, MeAαC and Harman. The densitometric scan in Figure 5 shows the separated peaks and the possibility to quantify the substances by peak height or area.

Fluorescence measurement is a very sensitive detection method and has a lower detection limit compared to UV-absorbance. However, the point of time at which the evaluation is done is of vital importance. It was observed that fluorescence emission is not stable. A degradation of fluorescence was detected when plates were not stored in darkness but in daylight or artificial light, respectively. Compared with Figure 4a, Figure 4b shows the decline of fluorescence after 48 hours.

It is also evident that the decrease of fluorescence was different for each substance (Fig. 6a). Fluorescence intensity of AαC and MeAαC decreased faster than those of Glu-P-1 and Glu-P-2. The curves of Harman and Norharman first increased, then decreased. Figure 6b indicates that degradation could be avoided by storing the plates in total darkness.

There were also two great advantages to mention. On one hand, the presented method was very rapid. The multi-step development lasted about 45 minutes, i.e. less than 3 minutes per each determination was necessary, when 16 bands were applied on one plate. On the other hand, the method was cost effective. Only 45 mL of solvent per plate was needed, i.e., less than 3 mL per determination. The cost for the HPTLC plates used was less than 5 euros per plate.

Conclusions

The results show that it was possible to separate six fluorescent HAAs on one HPTLC plate. Moreover, it was found important that quantification by fluorescence took place immediately after developing the plate, because of the degradation fluorescence.

It was shown that modern planar chromatography was suitable for a screening test. Further aims may include the separation of the 15 most frequent HAAs found in meat on one HPTLC plate, as well as their quantification. At the present study, only standard substances were used. To what extent meat matrix may complicate the determination of HAAs, remains, thus, to be investigated.

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Abbreviations

HAA: Heterocyclic Aromatic Amines
Glu-P-1: 2-Amino-6-methyldipyrido[1,2-*a*:3′,2′-*d*]imidazole
Glu-P-2: 2-Aminodipyrido[1,2-*a*:3′,2′-*d*]imidazole
AαC: 2-Amino-9*H*-pyrido[2,3-*b*]indole
MeAαC: 2-Amino-3-methyl-9*H*-pyrido[2,3-*b*]indole
Norharman: 9*H*-pyrido[3,4-*b*]indole
Harman: 1-Methyl-9*H*-pyrido[4,3-*b*]indole

Fig. 1: Sample application with ATS 4

Fig. 2: Automatic multiple development with AMD 2 system

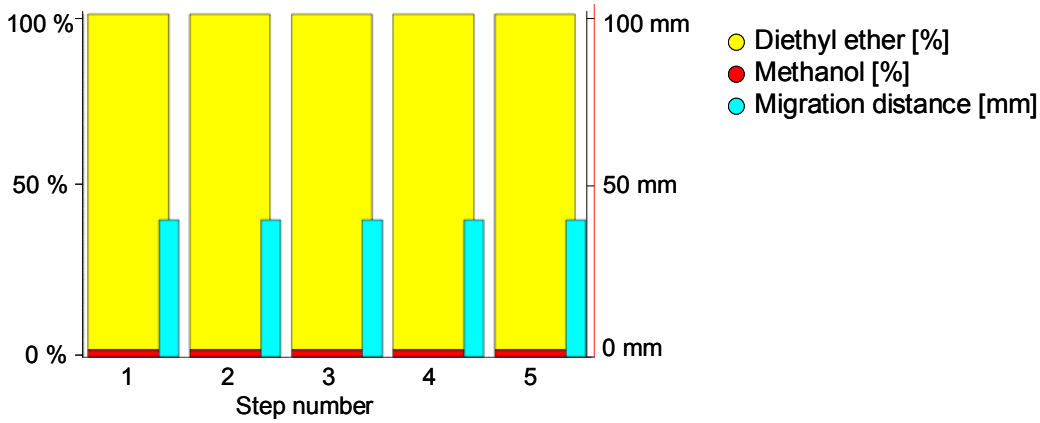


Fig. 3: Gradient scheme to separate the six fluorescent HAA

a)

b)

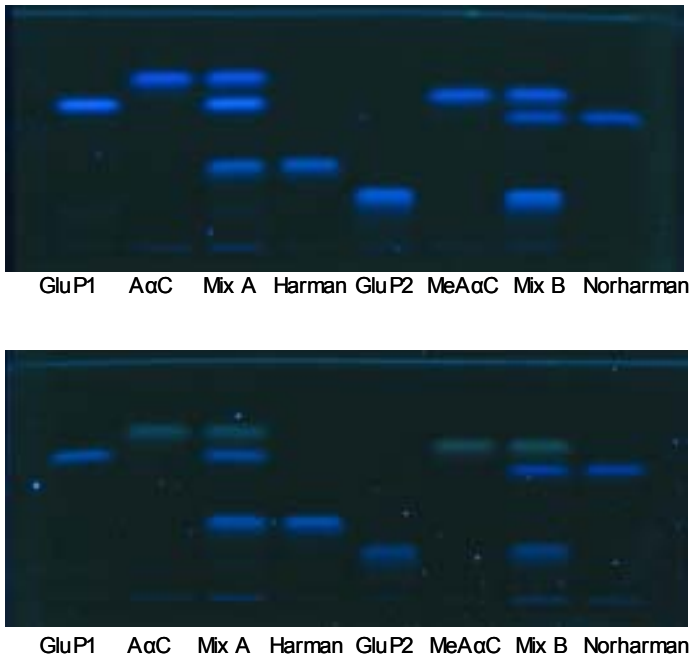


Fig. 4: Plate documented under UV 366 / > 400 nm after 48 hours:
a) stored without light, b) stored in daylight and artificial light

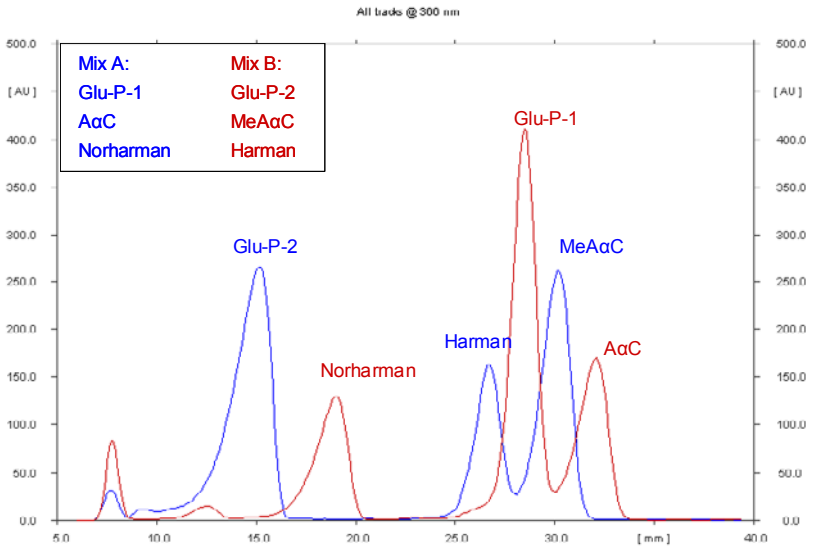
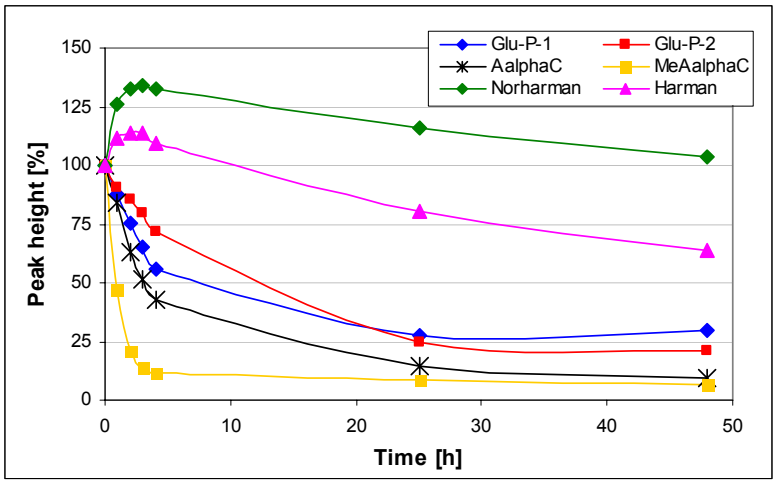


Fig. 5: Densitometric scan of the separated substances under UV 366 / > 400 nm

a)



b)

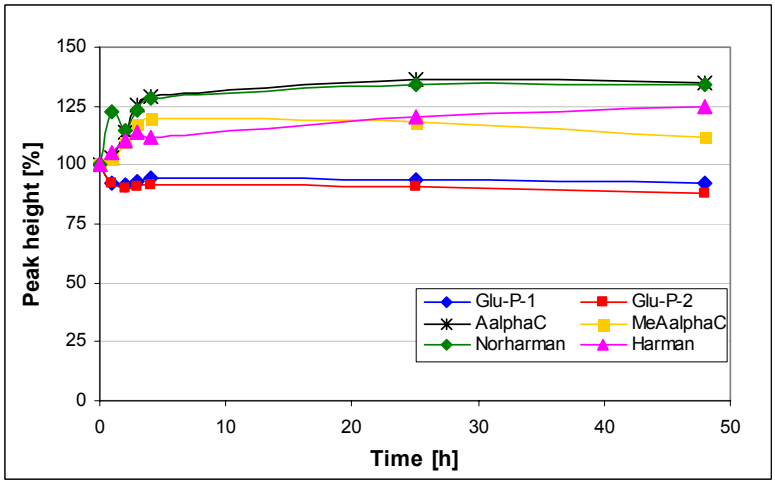


Fig. 6: Fluorescence degradation of 6 HAA during 48 hours: a) by light, b) in darkness

HEME IRON AND B VITAMINS AS QUALITY MARKERS IN COOKED MEATS

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Background

Meat consumption greatly contributes to the daily intakes of both heme iron and B vitamins in the diet (Lombardi-Boccia et al., 2003; Lucarini et al., in press). Meat represents the main source of highly available iron. An accurate knowledge of the levels of heme iron in meats and diets is of great importance because of the significant difference between heme and non-heme iron in bioavailability. As far as the vitamin B complex is concerned, meat is one of the main sources of niacin in the average Italian diet (Lucarini et al., in press), one serving of pork provides the daily thiamine requirement (LARN, 1996). Thermal processes which meat undergoes represent an important factor strongly affecting the nutritional value of meats and meat-based dishes. Losses of both heme iron and B vitamins occur during cooking processes of meat, and therefore, the amount of these micronutrients ingested may vary greatly.

Objectives

The present study focuses on how different cooking procedures (frying, stewing, roasting, "escalope") influence the retention of heme iron and B vitamins (thiamine, riboflavin, niacin) in meat cuts of beef, veal, chicken and turkey. Furthermore, the possibility of using heme iron and /or B vitamins as markers of the nutritional quality of cooked meats was also undertaken.

Materials and methods

The following meat cuts were analysed: beef (sirloin, fillet, roast beef, topside, flank), veal (fillet), chicken (breast, leg-lower part, leg-thigh, wing), turkey (breast, leg-lower part, leg-thigh). The meat cuts were cooked according to preparation protocols of some Italian recipes: poultry legs and wings were roasted in oven at 180°C for 50 min; breast and all the other meat cuts were hand-trimmed of all visible fat and fried (without the addition of fat or salt) in an iron-free pan with medium heat until red colour disappeared (beef fillet and roast beef were underdone); beef flank was stewed and turkey breast was also cooked as "escalope". To calculate weight loss, the cuts and the meat-based dishes were weighed before and after cooking, the latter after resting for 20 min at room temperature. Bones were removed, wherever necessary, and both raw and cooked meats were freeze-dried and then ground in a food blender (equipped with stainless steel blade) to ensure homogeneous and representative samples for analysis. Three samples for each meat cut and two preparations for each recipe (comprehensive of all the raw ingredients) were analysed for total iron, heme iron, thiamine, riboflavin and niacin content.

Iron content

Analysis of total iron were performed by ICP-Plasma on a Perkin-Elmer Optima 3200XL, following liquid ashing of the samples (4ml HNO3+1ml H2O2) in a microwave digestion system. Standard Reference materials: Bovine muscle (BCR 184, Community Bureau of Reference, Brussels) and Bovine liver (NBS 1577a, National Bureau of Standards, Gathersburg, MD 20899) were analysed to verify the accuracy of the analysis. Heme iron was determined following the analytical conditions described by Lombardi-Boccia et al. (2002a).

B vitamins

Thiamine and riboflavin were separated and quantified by HPLC after acidic and enzymatic (Takadiastase) hydrolysis of the samples, following the procedure described by Arella et al. (1996). Niacin was extracted following the method of Lahély et al. (1999) and quantified by HPLC following the method of Ward and Trenerry (1997). Identification of the correct peaks was performed by comparison with retention times of external standards: thiamine hydrocloride and riboflavin were obtained from Sigma Chemical Co. (St Louis, NO, USA), niacin was obtained from Fluka Chemie (Buchs, Switzerlands).

Results and discussion

The effect of heat treatments on total iron and heme iron content are presented in table 1. Cooked meats showed a higher iron concentration compared to the raw samples, this was due to the moisture losses occurred upon cooking. A previous study carried out on heme iron concentration in meats (Lombardi-Boccia et al., 2002b) showed that heat treatments did not cause losses in total iron content but modified the heme:non-heme iron ratio. Our present findings showed that frying caused losses in heme iron ranging from 3% (roast beef) to 24% (topside) (Tab.1). Less severe losses in heme iron content were found in poultry breast (both fried and cooked as escalope) (Tab.1). However, when meat was roasted (poultry meat), a substantial reduction in heme iron content was induced with losses ranging from 22% (turkey) to 43% (chicken wing) (Tab 1). The concentrations of B vitamins in raw and cooked meat cuts are presented in Table 2. Thiamine concentration varied greatly among cuts of the same species ($p<0.05$), in raw beef ranging from 0.02 mg/100g (sirloin) to a maximum of 0.14 mg/100g (flank). In the case of chicken and turkey, only breast samples were analysed, and the thiamine concentration was very low in both (Tab.2). Riboflavin content among cuts of beef varied from 0.09 to 0.17 mg/100g, fillet showing the highest concentration (Tab.2). Chicken breast showed the lowest riboflavin concentrations. Niacin content was almost identical in the various meat cuts of the same species. The highest amounts of niacin were found in poultry. The effect of cooking on the content of B vitamins in meat is also presented in Table 2. Heat treatments decreased the content of B vitamins. Among the vitamins analysed, thiamine was the most susceptible to thermal degradation, and ended up undetectable in most samples (Tab.2). Riboflavin, generally more resistant to heat, showed losses ranging from 37 to 60%. Comparable losses of riboflavin have been reported by Bodwell et al.(1986) and by Chan (1995). Complete retention of riboflavin was found in roast beef, a meat cut subjected to only a short cooking. Cooking induced less severe losses in niacin content, varying from 26% (turkey breast) to 42% (beef fillet). The values of thiamine and riboflavin found in literature (Bodwell et al., 1986; Chan et al., 1995, Leonhardt et al., 1997) are very similar to ours in both raw and cooked red meats, but markedly higher than ours in chicken and turkey breast.

Table 1. Total iron, heme iron and % of heme iron to total iron in raw and cooked meats (f.w.).

	Total Fe mg/100g	Heme Fe mg/100g	Heme %		Total Fe mg/100g	Heme Fe mg/100g	Heme %
Beef				**Chicken**			
Sirloin, raw	2.07±0.1	1.72±0.1	83	Breast, raw	0.40±0.1	0.12±0.1	30
Sirloin, fried	3.59±0.1	2.64±0.2	74	Breast, fried	0.58±0.1	0.16±0.1	28
Fillet, raw	2.35±0.2	2.11±0.3	90	Leg, raw	0.63±0.1	0.29±0.1	46
Fillet, fried	3.38±0.2	2.86±0.2	85	Leg, roasted	1.20±0.2	0.42±0.1	35
Roastbeef, raw	2.04±0.1	1.77±0.1	87	Thigh, raw	0.70±0.1	0.20±0.1	30
Roastbeef, fried	3.74±0.1	3.14±0.1	84	Thigh, roasted	1.34±0.1	0.30±0.1	22
Topside, raw	1.93±0.1	1.68±0.2	87	Wing, raw	0.63±0.2	0.28±0.1	44
Topside, fried	2.88±0.2	1.89±0.2	66	Wing, roasted	0.92±0.2	0.23±0.1	25
Thick flank, raw	1.81±0.2	1.61±0.1	88				
Thick flank, stewed	2.45±0.2	1.34±0.1	55				
Veal				**Turkey**			
Fillet, raw	0.85±0.3	0.71±0.3	84	Breast, raw	0.50±0.1	0.14±0.1	28
Fillet, fried	1.58±0.4	1.33±0.6	83	Breast, fried	0.79±0.1	0.21±0.1	27
				Breast, raw	0.51±0.1	0.20±0.1	39
				Breast, escalope	0.74±0.1	0.36±0.2	48
				Leg, raw	0.88±0.2	0.43±0.1	49
				Leg, roasted	1.51±0.2	0.58±0.2	38
				Thigh, raw	0.99±0.3	0.49±0.1	50
				Thigh, roasted	1.46±0.2	0.57±0.1	39

Values are Mean ± STD of three replicates.

Table 2. Thiamine, riboflavin and niacin content in raw and cooked meat cuts (f.w.).

	Thiamine	Riboflavin	Niacin		Thiamine	Riboflavin	Niacin
		(mg/100g)				(mg/100g)	
Beef				**Veal**			
Sirloin, raw	0.02±0.01	0.12±0.01	5.0±0.35	Fillet, raw	0.11±0.02	0.08±0.01	6.9±0.35
Sirloin, fried	tr	0.07±0.01	3.2±0.09	Fillet, fried	tr	0.05±0.01	4.3±0.18
Fillet, raw	0.08±0.01	0.17±0.01	5.7±0.25				
Fillet, fried	tr	0.07±0.01	3.3±0.09	**Chicken**			
Roastbeef, raw	0.05±0.01	0.10±0.01	5.5±0.21	Breast, raw	0.04±0.01	0.03±0.01	8.0±0.30
Roastbeef, fried	tr	0.10±0.02	3.3±0.08	Breast, fried	tr	0.01±0.01	5.0±0.28
Topside, raw	0.08±0.01	0.09±0.01	6.5±0.14				
Topside, fried	tr	0.05±0.01	4.2±0,12	**Turkey**			
Flank, raw	0.14±0.02	0.13±0.01	5.3±0.16	Breast, raw	0.02±0.01	0.06±0.01	7.2±0.28
Flank, stewed	0.12±0.02	0.07±0.01	2.8±0.16	Breast, fried	tr	0.03±0.01	5.3±0.90
				Escalope, raw	0.09±0.01	0.08±0.01	7.5±0.13
				Escalope, fried	0.06±0.01	0.07±0.01	5.9±0.44

Values are Mean±STD of 3 replicates.

Conclusions

This study updated some data concerning heme iron and vitamin B content in cuts of beef, veal and poultry and evaluated how cooking procedures affect their concentration. The knowledge of variations in the concentration of both heme iron and some B vitamins among raw and cooked meats is of utmost importance for two reasons: firstly, it allows the correct calculation of the actual nutrient intake at consumer level, and secondly, some of the micronutrients can be used as biochemical indicators of the nutritional quality of cooked meats. Our findings show in particular, that heme iron concentration vary greatly among the procedures analysed because its retention was strictly dependent on the severity of the heat treatment utilized. Therefore, heme iron concentration might serve as a useful index of the nutritional quality of cooked meats.

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EFFECT OF SUPRANUTRITIONAL AND ORGANICALLY-BOUND DIETARY SELENIUM ON THE NUTRITIVE VALUE AND CASE LONGEVITY OF BEEF

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Background

Elevated dietary selenium (Se) confers positive health benefits. Although some molecular forms are more potent than others (Ip et al., 2000; Finley and Davis, 2001), total Se intake must be three to ten fold the recommended dietary requirement for advantages, such as tumor inhibition, to be observed (Ip, 1998; Combs, 1999). As such, much effort has been devoted to identifying foods naturally high in or that can be readily enriched with Se (Finley et al., 1996; Lawler et al., 2004). Recently, we reported a 3.4-fold increase in the Se content of muscle from beef steers finished on a supranutritional organically-bound Se (2.7 mg·kg diet^{-1}) diets (Lawler et al., 2004). These diets did not negatively influence steer performance or carcass characteristics. Although some studies have documented the affects of inorganic versus organically-bound forms of dietary Se on the case life characteristics of meat, none have been conducted in beef describing the influence of supranutritional dietary Se. We hypothesized that skeletal muscle, from steers fed supranutritional Se as high Se grain, will have increased Se content, and not differ in nutritive quality and case longevity than muscle from steers fed adequate Se.

Objectives

Assess the nutritive value of *M. gastrocnemius*, and case longevity characteristics of *M. longissimus* from beef steers fed supranutritional dietary Se in the form of high Se wheat.

Materials and methods

Twenty crossbred beef steers (351 ± 24 kg initial body weight [BW]), individually fed (120 days) either 0.35 (n = 11; adequate; NRC, 1996) or 2.70 (n = 9; supranutritional) mg Se·kg BW^{-1}·day^{-1}, were utilized to assess the affects of supranutritional organically-bound Se on the nutritive value of the *M. gastrocnemius* (rear shank), and case longevity of the *M. longissimus* (strip loin; NAMP #180). The Se content of the adequate and supranutritional Se diets provided 9.5 and 65 mg·kg^{-1} BW·d^{-1}, respectively. Diets were similar in feed composition and nutrient content (25 % wheat, 39 % corn, 25 % grass hay, 5 % desugared molasses, and 6 % wheat middling based supplement; 14.0 % crude protein, 2.12 Mcal NEm·kg^{-1} DM, and 1.26 Mcal NEg·kg^{-1}; values expressed on a dry matter basis). High Se wheat (10.3 mg·kg^{-1}) directly replaced the adequate Se wheat (0.40 mg·kg^{-1}) to deliver the supranutritional Se treatment.

Immediately following slaughter (day 121), carcasses were placed in a cooler (4°C) for 48 hours. *M. gastrocnemius* samples (~3 g wet basis) were collected a 0, 6, 12, 24, 36 and 48 hours of the chilling period, wrapped in foil, snap frozen in liquid nitrogen and stored (-80°C) for subsequent analyses. The hour 0 *M. gastrocnemius* samples were analyzed for dry matter, ash, nitrogen, pH, lipids (AOAC, 1990), expressible moisture (Jauregui et al., 1981), and Se. Hydride generation atomic absorption spectrometry was used to analyze for Se following digestion of samples with nitric acid (Finley et al., 1996). All samples collected over time were analyzed for glutathione peroxidase (GSH-Px) activity. The coupled enzyme method of Paglia and Valentine (1967) was used to measure GSH-Px activity using H_2O_2 as the substrate and NADPH as the source of reducing equivalents. A BioRad assay kit (Hercules, CA) was used to determine protein concentrations (Quant microplate reader, Bio-Tek Instruments, Inc., Winooski, VT).

The *M. longissimus lumborum* was removed at the completion of the chilling period (48 hours), and two steaks (2.5 cm) were cut from the anterior end and weighed. Each steak was placed on a styrofoam tray, wrapped with a clear oxygen-permeable film, and displayed in a retail cooler (2.78ºC) for 12 days under fluorescent lighting with 150 lumens of light at the meat surface. Hunter L, a and b were measured daily with a Minolta CR-310 colorimeter (Minolta Corp., Ramsey, NJ) using a 50-mm diameter measurement

area, D65 light source with zero angle of reflectance. Additional subjective measurements were estimated using a scale from 1 (purplish red) to 8 (very dark red); a score of 3 was considered the ideal oxymyoglobin state. To measure drip loss, steaks were removed from their package and exudates on days 2, 4, 8, and 12, weighed, re-wrapped, and returned to the cooler. The amount of drip loss was determined as a percentage of initial weight. At the conclusion of the case life study, steaks were removed from the retail cooler, weighed, and cooked on flat grills to an endpoint temperature of 71 ± 3ºC. Internal temperature was evaluated using a digital thermometer placed in the approximate geometric centre of each steak. The steaks were weighed again after cooking, and cooking loss was calculated as a percentage of initial raw weight.

Nutrient values, moisture measurements and pH were analyzed as a completely randomized design, and drip loss, colour evaluations and GSH-Px data were analyzed as repeated measures using the mixed models procedure of SAS (v8.0; SAS, Cary, NC). The autoregressive order one and the spatial power law covariance structures were used for drip loss and colour evaluations, and GSH-Px activity analyses, respectively.

Results and discussion

M. gastrocnemius samples from steers fed high Se wheat diets had greater Se concentration and GSH-Px activity than the muscle from steers fed adequate Se (Table 1; $p < 0.05$). No sampling hour x treatment interaction was detected for GSH-Px activity throughout the chilling period ($p > 0.05$). This increase in muscle Se content was expected and consistent with other studies where organically-bound forms of Se were fed to barrows (Kim and Mahan, 2001) and steers (Hientz et al., 2001). The predominant form of selenium in high selenium grains and forages is selenomethionine (Olson et al., 1970; Djujic et al., 2000). This selenoamino acid is interchangeable with methionine during translation (Waschulewski and Sunde, 1988; Butler et al., 1989), resulting in Se being temporarily sequestered in the muscle and away from selenoprotein synthesis. However, once the rate of selenomethionine incorporation into protein has been saturated, Se would be released for selenoprotein synthesis at a rate equivalent to methionine/selenomethionine catabolisM. Compared to the 3.4-fold increase in Se concentration, the lack of proportional increase in GSH-Px activity confirms that the maximal responsiveness of GSH-Px synthesis to dietary Se is near or only slightly greater than adequate dietary requirements (Berggen et al., 1999). Although supranutritional Se had no influence on colour change ($p > 0.16$), steaks (*M. longissimus*) from steers fed the high Se wheat diets had less moisture lost (drip loss) over the 12-day case life study (Table 2; $p < 0.01$). This indicates that a supranutritional Se diet results in the mitigation of protein breakdown and subsequent release of water.

Conclusions

Supranutritional dietary selenium, from high selenium wheat, enhanced selenium concentration and glutathionine peroxidase activty of the *M. gastrocnemius*, and decreased drip loss from the *M. longissimus*. Caution should be used when assuming that enhanced Se concentration of a meat product will result in greater product stability during case display. This study clearly demonstrates the nutritive value of beef muscle products can be enhanced using feeds naturally high in selenium without negatively affecting the quality or case longevity of beef muscle.

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Table 1. Post-slaughter (48 hours) attributes of the *M. gastrocnemius* from steers fed either an adequate or supranutritional selenium diet formulated from high selenium wheat.

	Dietary Selenium[1]			
Item	Adequate (0.35 mg·kg diet^{-1})	Supranutritional (2.7 mg·kg diet^{-1})	Standard Error	*P* Value[2]
Dry matter, %	28.51	28.64	0.40	0.81
Selenium[3], µg·g^{-1}	0.37	1.24	0.05	<0.01
Ash[3], %	1.08	1.07	0.01	0.89
Crude protein[3], %	23.21	22.83	0.19	0.16
Lipid[3], %	5.06	5.38	0.46	0.61
pH	5.47	5.48	0.03	0.68
Expressible moisture[4], %	39.25	40.04	1.46	0.70
Glutathione peroxidase[5]	262.06[a]	326.27[b]	19.32	0.04

[1]Dietary selenium expressed on a dry matter basis.
[2]*F* test probability.
[3]Expressed on an "as is" basis.
[4]Expressed as a percentage of initial weight.
[5]Activity = *m*U·mg protein^{-1}·minute^{-1}. Multiple samples were collected during the chilling period, and data were analyzed as repeated measures. No time x treatment interactions were detected; as such, the main effect least squares means are reported.
[ab]Differing superscript within row indicates difference ($P < 0.05$).

Table 2. Case-life[1] characteristics of the *M. longissimus* from steers fed either an adequate or supranutritional selenium diet formulated from high selenium wheat.

Item	Dietary Selenium[2]		Standard Error	*P* Value[3]
	Adequate (0.35 mg·kg diet^{-1})	Supranutritional (2.7 mg·kg diet^{-1})		
Drip Loss, %	3.18a	2.22b	0.22	0.01
Cooking loss[4], %	17.83	18.69	1.15	0.59
Color Estimates[5]				
Hunter a	15.81	16.46	0.32	0.17
Hunter b	6.02	6.23	0.11	0.20
Hunter L	41.03	40.63	0.44	0.53
Subjective[6]	4.64	4.58	0.08	0.59

[1]The case-life was conducted over 12 days and data were analyzed as repeated measures. No time x treatment interactions ($p > 0.05$) were detected; as such, the main effect least squares means are reported.
[2]Dietary selenium expressed on a dry matter basis.
[3]*F* test probability.
[4]Expressed as a percentage of initial weight.
[5]Minolta CR-310 colorimeter, Minolta Corp., Ramsey, NJ.
[6]1 = purplish red and 8 = very dark red; a score of 3 was considered the ideal oxymyoglobin state.
[ab]Differing superscript within row indicates difference ($P < 0.05$).

UP-TO-DATE KNOWLEDGE ON THE NUTRITIONAL COMPOSITION OF POULTRY MEAT

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Background

Poultry, especially chicken broilers and turkey, is one of the most widely consumed muscle foods in the world. Poultry meat is an important source of dietary energy and nutrients, providing high quality protein, essential fatty acids, vitamins, and highly bio-available minerals (D'Amicis and Turrini, 2002). Updated information concerning nutrient contents of poultry muscles is needed for the establishment of the nutritional value of both fresh and processed products, including ready-to-eat items. The amount, type and proportion of fatty acids as well as cholesterol in muscles are of particular health concern. These data are necessary for poultry processors in developing strategies to manufacture healthy products with desirable nutritional composition. Based on the wealth of literature on poultry muscle quality, one would assume that the nutritional value of poultry meat has been well established. In reality, this is a false assumption, because the results are in some cases based on whole poultry carcasses, and in other cases on specific muscles, and many times the data are incomplete or out-of-date.

Objectives

The aim of the paper is to present the nutritional value of poultry meat on the basis of the most up-to-date literature available and to point out the areas where more research is needed to advance out understanding of the subject.

Results and discussion

Chemical composition

The chemical composition of poultry breast (B) and thigh/leg (T/L) muscles was recently reviewed by Lesiow (2004) and is presented in Table 1. The richest in protein are B muscle of turkeys, broilers, geese and ducks, followed by T/L muscles of ducks, geese, turkeys and broilers. The fat content of B muscle, in an ascending order, is: turkey female, Pekin and Muscovy ducks, hens, broilers, turkey males, Mule ducks and geese, and for T/L muscle, the order is: Pekin, Mule and Muscovy ducks, hens, turkey, geese and broilers. B muscle contains more protein (0.4 to 3.8%) and less fat (0.29 to 5.07%) than T/L muscles. Moisture content of Pekin and Muscovy ducks is greater than of other kinds of poultry. Broilers, hens and Mule ducks have intermediate moisture content, while turkey and geese have substantially lower values. The B muscle of broiler, Pekin duck and goose has a higher percentage of moisture than their T/L muscles. The differences in chemical composition between males and females are also well documented (Lesiów, 2004, Table 1). Male broiler muscles have a higher moisture and ash content than do females. A lower moisture content and higher fat content are observed in turkey and Mule duck male B muscle when compared with females. A reverse trend, i.e., higher moisture and lower lipid contents, holds true for male and female B muscle of Pekin ducks. Muscovy duck and goose male B muscle has a higher fat content than the female counterpart. Turkey and Pekin duck male T muscle contains less fat than female T muscle. There is a lack of literature report on the chemical composition of Mule duck and goose T/L muscles.

The content of collagen influences nutritional and dietetic values of meat. Poultry B muscle contains less collagen (except for Pekin ducks) than T muscle (Table 1). As a protein of lower nutritive value, collagen is the lowest in turkey and goose and higher in broiler and Pekin duck muscles. Information is scarce about the collagen content in Mule and Muscovy duck muscles, and data available for geese were published in 1983-1984 (Bielińska et. al., 1984). The collagen content is sex dependent. Male broiler B and T muscles and turkey and goose T muscle contain more collagen than female muscles. The reverse is true for turkey and goose female B muscle.

Vitamins and Minerals

Broiler meat is an important source of niacin (PP) and vitamin B6, and duck and goose meat are rich in thiamine (B1) and riboflavin (B2). Broiler and turkey T/L muscles contain more vitamin B2 and less vitamins PP and B6 than B muscle. However, literature about vitamins in poultry meat dates back over 20 years and the content of vitamins in duck and geese is not presented for particular muscles but instead for the whole flesh (total amount of musculature in a carcass) (Hamm and Ang, 1984; Posati, 1979).

Mineral content of broiler and turkey B and T/L muscles has been reviewed (Lesiow, 2004). However, data for duck and goose meat are typically presented in relation to whole carcass flesh and are very often incomplete. Broiler and turkey T muscles are richer than B muscle in Fe (10.70 vs. 6.81 μg/g, and 11.87 vs. 5.01 μg/g) and Zn (15.27 vs. 6.65 μg/g, and 26.03 vs. 9.08 μg/g). This is due to the higher myoglobin and haemoglobin content and greater metabolic activity associated with the red muscle fibres. Turkey and broiler muscles are generally also rich in phosphorus and potassium. The mineral content of broiler B and L muscles is identical between sexes. There is no information about the possible influence of sex on the mineral content of the muscles of other poultry species. There is an interesting discrepancy (from 7.1% to 105%) in the mineral content of duck and goose flesh presented by Kunachowicz et. al. (2002) and Posati (1979), respectively.

Cholesterol and FFA

From the health and nutrition standpoint, it is important not to simply reduce the fatness and cholesterol content of carcasses but rather, to improve the FFA make-up, such as the proportion of n-3 fatty acids, in poultry muscles. The modern dietetics recommend the decrease of polyunsaturated PUFA n-6/n-3 ratio in human diet and to separately evaluate the content of linolenic acid, arachidonic acid, eicosapentaenoic acid (EPA), and docosahexaenoic acid (DHA) in foods (Okuyama et al., 1997).

The cholesterol content of poultry B muscle, in an ascending order, is as follows: broilers, hens and turkeys, Muscovy ducks, geese, Pekin and Mule ducks. The corresponding cholesterol content of broiler, turkey and goose T/L muscles is substantially less than in hens (Lesiów, 2004). The cholesterol content of B muscle is lower in broilers (by 56.5%) and turkeys (by 44.4-50.0%) than in their corresponding T/L muscles. On the contrary, the cholesterol level in goose B muscle is higher (by 20.5%) than in its T muscle. No data can be found on the cholesterol content of duck T/L muscles. B muscles of male turkey, goose and Mule duck show higher cholesterol content than their female counterparts.

The principal fatty acids in poultry muscles are oleic, palmitic and linoleic (Lesiów, 2004). In broiler, hen, Pekin and Muscovy ducks as well as goose, the lipids of B muscle are comprised of more saturated fatty acids (SFA), PUFA and less monounsaturated fatty acids (MUFA) than T/L muscle lipids (Table 1). Komprda et al. (2001) observed no differences in FFA composition between B and T muscles of turkey. However, the same research group reported later that turkey B muscle contained lower amounts of SFA, PUFA but more MUFA than T muscle (contrary to broilers) (Komprda et al., 2002). The PUFA n-6/n-3 ratio of broiler and turkey B muscle is lower in comparison with T muscle, whereas in hens, Muscovy ducks and geese the ratio is slightly higher in B than in T/L muscles. Pekin and Muscovy duck B muscle contains more SFA than other species of poultry (Lesiów, 2004). The percentage of MUFA is generally higher in goose B and T muscles and in duck T muscle compared to muscles from other poultry species. The most favourable ratio of PUFA n-6/n-3 is within broiler and turkey muscles, and then within hens, ducks and geese. Literature data within the duck species is difficult to compare due to variations in the age of the birds used in different studies as well as different production conditions employed. Turkey male B muscle has a higher MUFA content but lower SFA and PUFA, and turkey male T muscle has a higher SFA content but lower PUFA content than turkey female corresponding muscles. Goose male B muscle possesses a more favourable FFA profile compared to female B muscle as evidenced by the higher percentage of MUFA and lower level of SFA (Batura et. al., 1999).

Conclusions

It should be pointed out that the chemical composition and thus, the nutritional value of poultry meat is influenced by many production factors, such as diet (nutritional composition of the feed), the conditions in which the birds are grown, and the specific strains. For example, our previous studies (Xiong et al., 1993a,b) showed that broiler meat varied slightly in basic composition (protein, lipid, mineral and water) between

strain crosses. Therefore, evaluations of nutritional value of different poultry species should be done in the context of the ever-changing breeding technology that is likely to underscore strain-dependent variations in the meat composition. Results published by individual research laboratories are generally dealing with the whole poultry carcasses (e.g., mineral content in hen, duck and geese) or with specific muscles, and in some cases, the data are not complete (e.g., cholesterol and FFA content for different duck species) or up-to-date (e.g., vitamin content in turkey, duck and goose thigh/leg muscles). Further research is needed to fill in the gaps in the knowledge of the nutritional value of poultry muscles.

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Table 1. Chemical composition (%), relative collagen content (%), and cholesterol concentration (mg/100g) in poultry breast (B) and thigh/leg (T/L) muscles (Lesiów, 2004).

	Moisture		Protein		Fat		Collagen		Cholesterol	
	B	T/L	B	T/L	B	T/L	B	T	B	T/L
Broilers	74.36	73.21	22.80	19.14	1.58	6.65	0.54	1.05	47.42	74.20
Hens	73.21	74.04	-	-	1.51	4.49	-	-	50.07	100.61
Turkey (M)	72.74[22*]	72.24	23.36	19.54	1.63	4.84	0.39	0.57	50.85[20]	73.45
Turkey (F)	73.51[15]	72.91	23.29	19.52	1.25	6.28	0.43[16]	0.48	48.20[14]	72.30
Ducks:										
Pekin	76.82[7]	75.80	21.20	20.90	1.31	2.00	1.15	0.70	111.06	-
Muscovy	76.43[12]	76.06	20.89	20.31	1.43	3.43	-	-	67.00	-
Mule	74.55[12]	74.50	21.78	21.40	2.41	2.70	-	-	105.0	-
Geese	72.36[17]	71.55	22.48	20.38	3.11	6.51	0.40	0.60	84.35	70.00

*age (weeks)

Table 2. Proportions of FFA (%) in poultry breast (B) and thigh/leg (T/L) muscles (Lesiów, 2004).

	SFA		MUFA		PUFA		n-6/n-3	
	B	T/L	B	T/L	B	T/L	B	T/L
Broilers	33.12	31.07	36.95	41.08	28.41	26.41	5.35	6.33
Hens	34.01	31.79	35.13	43.40	25.57	22.38	8.01	7.69
Turkey (M)	30.10	32.00	36.60	32.70	27.10	29.70	5.16	5.46
Turkey (F)	32.10	29.10	32.10	32.60	29.20	32.10	4.62	5.55
Ducks:								
Pekin	45.46[9]	30.99	34.61	51.11	17.03	12.44	-	12.23
Muscovy	38.70[10.5]	34.75	30.80	43.92	30.50	18.08	22.46[12]	19.55
Mule	-	29.07[14]	-	46.27	-	19.87	-	24.81
Geese	27.99[15]	27.35	62.80	65.00	9.10	7.58	112.7	107.3

INTRODUCTION INTO FOODS COMBINATORICS

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Background

The Food Combinatorics combines ideas of modern informatics, trophology, biophysical and chemical bases of food technology and particular technologies of multicomponent food products for special groups of population classified by age and metabolic state. Combinatorics is a science studying combinations of various objects (Encycloperia). ("Combination" is the term from Latin "combinatio"; it means combination – mutual arrangement of something.).

Objects of mathematical combinatorics are: all kinds of permutations, arrangements, and combinations. As applied to food processing industry, the notion of combinatorics is identified with arrangement, rearrangement, and combination of dominant characteristics of the objects of foods processing. Such objects are: raw materials; functional components; formulation mixtures formed from raw materials and functional components; half-finished products obtained at different stages of formulation mixtures processing; finished products which are the final purpose of the food protophysiological technology; diets which predetermine the effectiveness of its physiological technology including consumption, digestion, assimilation, and evacuation; technological processing, equipment for implementation of technological processes, parameters ensuring effective functioning of technological processes and equipment.

In addition, using the term "food combinatorics", the authors mean that it combines principles and methods of realization of results of designing of food products as material objects with predetermined characteristics created from separate elements, not providing these characteristics individually.

Objectives

The aim of the present work is to summarize personal notions of the author about an interdisciplinary field of knowledge - FOOD COMBINATORICS developed by them in recent years.

Materials and methods

All above-stated allows us to formulate the following definition:

THE FOOD COMBINATORICS is an interdisciplinary field of knowledge directed for studying and practical application of regular features of mutual influence of combinations of objects in the food production process.

Taking into account the experience and specifics of scientific and practical interests of the authors, one can state that the greatest attention is paid to the complex of problems connected with the quality improvement of baby foods prepared on the basis or with the use of components obtained in processing of meat raw materials, eggs, and milk.

Results and discussion

Medical and biological aspects of food combinatorics

On the whole, these aspects determine the complex of necessary and appropriate conditions providing possibility of quantitatively determined improvement of baby food quality through combinatory changes forming their alimentary adequacy of macro- and micronutrients and predetermine rightfulness of their assignment to so-called products of new generation.

The authors believe that the content of the paper, which follows hereinafter, will be better understood as logically tied links of the common methodological chain, if the following definition is given: Polycomponent baby foods of new generation are the safe products whose formulations comprise non-traditional for products

on meat, milk, cereals or fruit-vegetable bases some macro- or microfractionated components of these kinds of raw materials, as well as physiological functionally- methabolic ingredients added in amounts and ratios, assisting in the presence, apart from nutrient adequecy, of predetermined level of methabolic adequacy or special properties, and providing the absence of negative organoleptical perceptions in children, who are combined by determinated age, homeostasis or other signs.

As far as biological value of protein components contained in new baby foods is concerned, it should be noted, that apart from the conventional amino acid balance predetermined by the combinations of indispensable amino acids and in special cases dispensable amino-acids, the individual protein digestion is taken into account in the proposed methodology. In addition, while creating products, guaranteeing absence or minimum probability of occurrence of protein allergenic reaction, the most important are combinations of molecular-mass characteristics of almost all macromolecular and polypeptide components of total protein. At the same time, special attention is to be paid to the fact that these combinations depend on similar combinations in protein ingredients of the total protein and on the complex of processing technologies and parameters of their implementation.

In full measure, medical and biological aspects of the food combinatorics embrace the analysis, evaluation, and purposeful use of combinations and arrangements of factors, which predetermine nutrient and metabolic adequacy of such macronutrients of baby foods as fatty acids and carbohydrates, which have dominant influence on the food energetic value.

One of the most important part of medical and biological aspects is the study and practical realization of combinatory factors forming macro- and microelement adequacy of food products and diets containing them. Quantitatively the analysis of combinations not concerning the process factors and forming the food value on the whole can be accomplished with the help of mathematical models, developed by researchers headed by N.N.Lipatov (Jr.) (Academician of the Russian Academy of Agriculture).

In realization of these models by computers as applied to designing of foods for babies we used the rated values of the alimentary reference summarized in Table 1.

Table 1. Rated indices of the alimentary reference for babies up to one year

Nutrients	Content	Nutrients	Content
Indispensable amino acids:	g/100 g of prot.	Na	20.00
Isoleucine	4.60	S	11.20
Leucine	9.80	P	24.00
Lysine	7.50	Cl	54.4
Methionine+cystine	4.00	*Microelements:*	µg/1 g of protein
Phenylalanine+tyrosine	8.60	Fe	800.00
Threonine	4.60	J	2.80
Tryptophan	1.50	Co	-
Valine	5.20	Mn	2.40
Histidine	-	Cu	32.00
Fatty acids:	g/100 g of fat	F	_
∑ UFA	41.78	Zn	240.00
∑ MUFA	43.03		
∑ PUFA including:	12.42	*Vitamins:*	mg/1g of protein
Linoleic	10.85	B_1	$3.2x10^{-2}$
Linolenic	0.62	B_2	$4.8x10^{-2}$
Arachidonic	0.95	B_6	$3.2x10^{-2}$
Macroelements:	mg/1 g of protein	B_{12}	$3.2x10^{-3}$
K	48.00	PP	$3.2x10^{-1}$
Ca	40.00	A	$4.8x10^{-2}$
Mg	4.00	E	$4.8.10^{-1}$

Conclusions

The authors hope that the International Association of scientists in the field of food technology (including the meat technology) would pay due attention to the present information and take an active part in discussions and further development of theoretical, experimental, and practical aspects of new interdisciplinary scientific direction - THE FOOD COMBINATORICS.

NON-TRADITIONAL IDEAS ABOUT ECOLOGICAL SAFETY AND NUTRITIONAL ADEQUACY OF FOODSTUFFS (INCLUDING MEAT-BASED ONES)

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Background

Analysis of available scientific literature about modern trends and prospects for quantitative evaluation of negative effects of indirect anthropogenic activities on ecology suggests that at the present time there is no appropriate informational-algorithmic support, sufficient for carrying out this evaluation, both on the whole and as applied to separate links of exotrophic chain "production of foods → storage → marketing → consumption → digestion → evacuation of catabolism products → utilization of all wastes".

As applied to foods and food processing, inadequate filling of this scientifically-intensive niche is largely caused by incorrectness, and as a consequence, "discrepancies" in terminology, phenomenological and noumenological notions and definitions, being the base for creation of computer-implemented versions of information-algorithmic support for calculation and analysis of dominant quantitative indices associated with ecology of foods.

Objectives

To fill this gap we offer herein for food scientists and food industry specialists the terms, definitions and criteria, being essentially our innovations.

Materials and methods

Terms and definitions

*Ecology** (definition from encyclopedia) – (from Greek oĭkos – house, dwelling, place of living and … logia) science about relations of plant and animal organisms and associations formed by them between each other and the environment.

*Ecology** (interpretation of the authors) – interdisciplinary science studying direct and (or) indirect mutual influence of a human organism in the process of his activities in places of living or location and different forms of existence of being there: animals, plants, micro organisms and organic, mineralorganic, mineral and field objects of natural or anthropogenic origin.

*Ecological safety** - notion as applied to food raw materials, technologies, devices, foodstuffs and their packaging combining necessary and adequate conditions providing absence or minimization of negative effects on the environment during production, processing marketing and use of listed objects.

*Food safety** - a group of indices which quantitatively characterize necessary and adequate conditions, eliminating probability of occurrence of pathological violations of physical, psychical and intellectual status of human organism, caused by components of raw materials and (or) ready products.

*Ecological purity** - (as applied to foodstuffs) = good ecological condition & food safety - is a total property of a particular food or products from the same assortment group characterizing their ability to have minimum effect on the environment during their production, storage, marketing and consumption and provide the absence of substances negatively influencing physical, psychical and intellectual status of a human organism.

*Nutrient (alimentary) adequacy** - a group of indices qualitatively and quantitatively characterizing contents and mutual balance of macro as well as micronutrients and their components in raw materials and final products.

*Metabolic adequacy** - a group of indices characterizing potential efficiency of use by the organism of nutrients as supplied to it by foodstuffs during their consumption, digestion and assimilation.

*Food value (adequacy)** - this is a potential ability, suggesting safety, of foodstuffs or raw materials used in their production to provide in their totality a material and energetic balance of the organism taking into consideration physiological and psychological requirements of the individual consumer or a group of consumers who are combined by their regional, national, age, professional or other signs.

Results and discussion

We have created the following set (1-6) of criteria:

Criterion of **relative ecological safety** of food processing

$$E_{П} = \left[\prod_{\alpha} \left(\frac{\Delta \mathrm{Ds}_{\alpha}}{\Delta \mathrm{Ds}_{\alpha 0}} \right) \cdot \prod_{\beta} \left(\frac{\Delta \mathrm{DA}_{\beta}}{\Delta \mathrm{DA}_{\beta 0}} \right) \cdot \frac{m_0}{m} \right]^{\frac{1}{\alpha + \beta + 1}} \qquad (1)$$

Where: $E_{П}$ - relative ecological safety of production of a particular food, units (with regards to base, reference); $_{\Delta}Ds_a$ – difference between high critical limit of the share of α-th contaminant and its actual pre-limit share in production effluents, corresponding to compared complexes of technological processes, % of ПДУ (Limit value, LV); $_{\Delta}DA_{\beta}$ - difference between top critical limit of the share of β-th contaminant and its actual pre-limit share in atmospheric emissions, corresponding to compared complexes of technological processes, % of LV; *m* - mass of unutilized production garbage, as accumulated in equal time periods in the implementation of compared versions of production processes, t. Index $_0$ corresponds to base (reference) version.

Rated criterion **of food safety**

$$S_{П} = \prod_{i}^{4} \left(1 - \prod_{ij} \frac{X_{ij}}{X_{ПДУij}} \right) * \left(1 - \max \prod_{ij} \frac{X_{ij}}{X_{ПДУij}} \right) \qquad (2)$$

Where: $S_{п}$ – rated food safety, share units; *i* = 1 = corresponds to a group of multiplicative indices of physical contamination of the product, $1 \le j \le k$; *i* =2 – corresponds to a group of multiplicative indices of chemical contamination of the product, $k + 1 \le j \le l$; *i* = 3 – corresponds to a group of multiplicative indices of radiological contamination of the product, $l + 1 \le j \le m$; *i* =4 – corresponds to a group of multiplicative indices of microbiological contamination of the product, $m + 1 \le j \le n$; X_{ij} corresponds to actual value of the unit ij-th index of contamination of the comparable product (corresponding unit of measurement). $X_{ПДУij}$ corresponds to limit value of the ij-th index of contamination. In the case when $S_{п} \le 0$ – the product **is not allowed** for consumption!

Criterion of **ecological purity**

$$e_{П} = (E_{П} \& S_{П})^{0,5} \qquad (3)$$

Rated criterion of **nutrient adequacy**

$$N_{П} = \left[\prod_{i=1}^{n} \mathrm{N}_i^{sign(1-N_i)\cdot\alpha_i} \right]^{0,5/n} \cdot \left[\mathrm{N}_{\min}^{\alpha_{\min}} \right]^{0,5} \qquad (4)$$

Where: $N_{П}$ - rated criterion of nutrient adequacy, share of units; N_i – determined rated *i*-th index of nutrient adequacy, share of units; $N_i = P/P_0$ – correlation of mass fractions of protein in comparable and reference objects; $N_2 = R_p$ - coefficient of rationality of amino acid composition (as $R_{p0} = 1$); $N_3 = L/L_0$ – ratio of mass fractions of fat in comparable and reference objects; $N_4 = \sum SFA/\sum SFA_0$; $N_5 \sum MUFA/\sum MUFA_0$; $N_6 = \sum PUFA/\sum PUFA_0$ -ratios of sums of saturated, monounsaturated and polyunsaturated fatty acids in the

compared and reference objects; $N_7 = C/C_0$ – ratio of mass fractions of carbohydrates in compared and reference objects; $N_8 = macr/macr_0$ – correlation of mass fractions of essential macroelements in compared and reference objects; $N_9 = micr/micr_0$ - ratio of mass fractions of essential microelements in compared and reference objects; $N_{10} = vit_A/vit_{A0}$ - ratio of mass fractions of essential water-soluble vitamins in compared and reference objects; $N_{11} = vit_L/vit_{L0}$ - ratio of mass fractions of essential fat-soluble vitamins in compared and reference objects; *Sign (1-Ni)* – function of sign; *Sign (*1-*Ni)* = +1, if *Ni* ≤*1* and *Sign (*1 – Ni) = -1, if *Ni >1;* N_{min} - index of nutrient adequacy, possessing minimum value; α_i – determined by expertise coefficients of weightiness of the *i th* index of nutrient adequacy, taking the values 0; 25; 0.5, 0.75; 1.0 (the less is the coefficient of weightiness, the less individual influence has the corresponding to it determined index of nutrient adequacy on its multiplicative value); α_{min} - coefficient of weightiness corresponding to $N_{min.}$

Rated criterion **of metabolic adequacy**

$$M_{II} = \left[\prod_i^n m_i^{sign(1-m_i)\cdot\beta_i}\right]^{0,5/n} \cdot \left[m_{min}^{\beta_{min}}\right]^{0,5} \quad (5)$$

where: M_{Π} – rated criterion of metabolic adequacy, share units; M_i – determined rated *i*-th index of metabolic adequacy; M_i –index of relative fractional adequacy of protein composition, share units; $M_2 = \pi$ – digestibility of protein in vitro, share units from initial tyrosine; M_3 …M_5 –ratio of: linoleic, linolenic and arachidonic polyunsaturated fatty acids in comparable and reference objects, shares of units; M_6 – ratio of assimilation of fat of comparable and reference objects, share of units; $M_7 = C_1/C_{10}$ – ratio of mass shares of metabolically hydrolyzed carbohydrates in comparable and reference objects, share of units; $M_8 = C_2/C_{20}$ - ratio of mass shares of metabolically non-hydrolyzed carbohydrates in comparable and reference objects, shares of units; $M_9 = Q/Q_0$ – ratio of energy value of comparable and reference objects, share of units; M_{10}…$M_{14} = S_1$ – relative organoleptic indices, share units; M_{min} - –index of nutrient adequacy, possessing minimum value; β_{min} - coefficient of weightiness, corresponding to M_{min}; β_i – determined by expertise coefficients of weightiness, taking up the values 0; 0.25, 0.5; 0.75; 1.0.

Criterion of **food adequacy**

$$K_{II} = (S_{II} \& N_{II} \& M_{II})^{1/3} \leq 1, \text{ share un.} \quad (6)$$

Conclusions

The authors of this paper hope that the above-stated ideas and criteria will allow to scientists and specialists, involved in the problems connected with ecological aspects of supply of people with high quality products to quantitatively interpret the obtained results.

References

ROLE OF THE CHOSEN DIETARY FIBRE PREPARATIONS IN SHAPING OF STRUCTURE AND QUALITY OF MODEL MEAT PRODUCT

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Background

The interest of the aware consumers towards food products with specific health-promoting properties, is not decreasing (Bacers, Noll 1998). Food products, containing such components as dietary fibre (Cludesdale 1997, Cieślik, Topolska 2002) satisfy some of these expectations.

Wheat and oat fibre preparations of the newest generation are characterized by a neutral flavour (taste and smell) and a high water binding capacity. Owing to its chemical structure, dietary fibre may absorb water to its capillary system and distribute it throughout the whole volume of the product via a generated three-dimensional network. In this study, the network of fibres, created with added dietary fibre is introduced in the matrix of finely comminuted sausage batter.

Objectives

The aim of the undertaken work was to determine the role of the chosen dietary fibre preparations, applied as a fat replacer and as a functional additive in shaping of structure and quality of a model meat product.

Materials and methods

The studied dietary fibre preparations were: wheat (WF 600-30) and oat (HF 600-30), which were applied to finely comminuted cooked meat product. The evaluation of the application of the chosen dietary fibre preparations as fat replacers was carried out, exchanging 30 % of the formulation fat. Hydrated preparations were introduced to the batter at a preparation to water ratio of 1 to 3.5. The functional role of the dietary fibre preparations in the system of finely comminuted meat product was examined, introducing them as a functional additives at a quantity of ca 1.5 % of total batter weight.

The raw material of the model meat product consisted of the tendinous pork meat (21.4 %), lean beef meat (28.6 %), fine pork fat (21.4 %) and water (28.6 %). The control product had the same composition, but without added dietary fibre (variant K). The products were manufactured according to typical production technology of bologna sausage. The tins were filled with 400 g of sausage batter, cooked in water (75^{o}C) until core temperature of 72^{o}C was reached. After cooking, the tins were chilled in cold water and stored refrigerated at +4 – 6^{o}C until the test samples were collected.

After removal of fat and jelly, basic chemical composition of the products was analysed, ie., water, protein, fat, and sodium chloride. The energy value of the product, expressed in kcal/100 g, was also calculated. The binding capacity of the product was evaluated as cooking losses, as well as slice strengths determined mechanically with the Zwick apparatus, model 1445 MOPS (Tyszkiewicz, Olkiewicz 1991).

The texture profile was tested by the instrumental TPA method (Crystall et al. 1994) and hardness, springiness, gumminess, cohesiveness and chewiness were characterized. The parameters of the testing instrument were as follows: deformation – 80 %, speed of the test – 60 mm/min, thickness of the sample – 20 mm and diameter – 25.4 mm.

The rheological characteristics ie., plasticity, elasticity and fluidity, were determined by Continuously Stress-Relax Analysis (CASRA) method, with the application of Universal TestinG Machine (UTM) Zwick, model 1445 MOPS (Tyszkiewicz, Olkiewicz 1997).

The desirability of colour, taste and texture and overall palatability of the product were evaluated as well. The sensory evaluation was performed in a specific laboratory by a professional panel of 8 members. The facility meets the requirements of standard ISO 8589, and uses the computer system ANALSENS.

Two experimental series of production for all variants were carried out. Chemical composition, textural profile, and sensory evaluation were repeated twice for each experiment. The values of the remaining variables are the means of either 5 (cooking loss) or 10 (slice strength) repetitions. The results of the tests were subjected to one-way analysis of variance using Statgraphics for Windows ver. 3.1.

Results and discussion

The effect of the added dietary fibre (wheat - W, oat - O) preparation used as fat replacers (R) and as functional additives (F) on basic chemical composition of the model meat product is presented in Table I.

Table I. Effect of dietary fibre used as a fat replacer (R) and as a functional additive (F) on the basic chemical composition of model meat products

Application of dietary fibre preparation:	Variant	Water [%]	Fat [%]	Total protein [%]	Energy value [kcal/100g]	NaCl [%]
fat replacer (R)	K	63.9 ^{a}	23.7 ^{b}	10.3 ^{a}	254.6 ^{b}	1.45 ^{c}
	WR	66.7 ^{b}	19.5 ^{a}	10.5 ^{ab}	220.5 ^{a}	1.39 ^{b}
	OR	65.5 ^{ab}	20.2 ^{a}	10.6 ^{b}	226.0 ^{a}	1.33 ^{a}
	NIR	*1.8*	*2.1*	*0.2*	*19.1*	*0.03*
functional additive (F)	K	63.9 ^{c}	23.7	10.3	254.6	1.45
	WF	62.8 ^{b}	23.4	10.1	252.2	1.46
	OF	62.0 ^{a}	23.8	10.1	256.6	1.43
	NIR	*0.8*	*0.9*	*0.3*	*8.8*	*0.05*

Means in the same column with different superscript are significantly different (P≤0,05)

The dietary fibre preparations used as fat replacers (WR, OR) had a significant effect on lowering of fat content, energy value and NaCl content as well as on the increasing of the contents of water and protein of the product. The dietary fibre preparations, when introduced as functional additives (WF, OF), did not have any other significant effect on the basic chemical composition than lowering the water content of the product, as compared to the control variant.

Table II. Effect of the dietary fibres, used as a fat replacer (R) and as functional additive (F), on the binding capacity and textural parameters of the sausage.

Application of dietary fibre preparation:	Variant	Cooking loss [%]	Slice strength [N/cm^2]	Hardness [N]	Gumminess [N]	Chewiness [Nmm]
fat replacer (R)	K	3.84 ^{a}	3.08	71.4 ^{a}	11.9 ^{a}	53.14 ^{a}
	WR	6.91 ^{b}	3.07	84.6 ^{b}	14.6 ^{b}	65.1 ^{b}
	OR	8.83 ^{c}	3.22	80.6 ^{b}	13.8 ^{b}	63.1 ^{b}
	NIR	*1.57*	*0.52*	*6.5*	*1.4*	*8.6*
functional additive (F)	K	3.84	3.08	71.4 ^{a}	11.9 ^{a}	53.1
	WF	3.43	3.33	79.7 ^{b}	13.4 ^{b}	59.3
	OF	4.05	3.64	83.0 ^{b}	13.6 ^{b}	58.2
	NIR	*0.63*	*0.70*	*5.1*	*1.4*	*10.2*

Means in the same column of with different superscript are significantly different (P≤0,05)

Water binding capacity of the model product was evaluated in terms of cooking loss and slice strength (Tab. II). When used as fat replacers (WR, OR), the dietary fibre preparations caused a significant increase of cooking loss, yet a good slice strength not differing from the control product. The highest cooking loss was found in the product, in which dietary oat fibre replaced 30 % of the fat in the formulation. As functional additives (WF, OF), the studied preparations did not have any significant effect on water binding of the product.

When used as fat replacers (WR, OR), the dietary fibre preparations increased the textural measures of hardness, gumminess and chewiness significantly (Tab. II). Except for chewiness, this was also the case when fibre was used as a functional additive (WF, OF), they had a significant influence on the increase of hardness and gumminess. Products with added fibre (WR, OR, WF, OF) did not differ significantly from the controls for the remaining texture parameters, not found in the table.

Table III. Effect of dietary fibre as a fat replacer (R) and as functional additive (F) on the rheological characteristic

Application of dietary fibre preparation	Variant	Plasticity [x10^{5}N/m^2]	Elasticity [x10 $^{-6}$m^2/N]	Fluidity [x10^{-8}Nm^2/Ns]
fat replacer (R)	K	2.36	1.14	3.25
	WR	2.70	1.06	3.08
	OR	2.57	1.04	3.16
	NIR	*0.39*	*0.16*	*0.57*
functional additive (F)	K	2.36 ^{a}	1.14 ^{b}	3.25
	WF	2.60 ^{b}	1.01 ^{a}	3.26
	OF	2.66 ^{b}	0.99 ^{a}	3.07
	NIR	*0.16*	*0.12*	*0.50*

Means in the same column with different superscript are significantly different (P≤0,05)

The substitution of fat with dietary fibre did not have any significant effect on rheological characteristic of the products (Tab. III). The dietary fibre preparations, employed as functional additives (WF, OF) caused a significant increase of plasticity, with the simultaneous significant decrease of elasticity, in comparison to the control products.

Table IV. Effect of dietary fibre as a fat replacer (R) and as functional additive (F) on sensory parameters

Application of dietary fibre preparation	Variant	Colour desirability [c.u.]	Taste desirability [c.u.]	Texture desirability [c.u.]	Overall palatability [c.u.]
fat replacer (R)	K	5.0	5.3	5.2	5.6
	WR	5.0	5.3	5.4	5.5
	OR	5.2	5.0	5.1	5.2
	NIR	*0.7*	*0.5*	*0.4*	*0.6*
functional additive (F)	K	5.0	5.3^{ab}	5.2	5.6
	WF	4.8	5.0^{a}	5.3	5.3
	OF	5.2	5.4^{b}	5.4	5.5
	NIR	*0.8*	*0.3*	*0.4*	*0.5*

Means in the same part of column with different superscript are significantly different (P≤0,05)

In spite of their role in texture, the employed dietary fibre preparations did not play any significant role in sensory desirability (Tab. IV). The taste of the product containing oat fibre (OF) was preferred over that containing wheat fibre.

Meat products with a different type of dietary fibre preparation (wheat or oat) did not differ significantly from each other in terms of physiochemical parameters, texture, rheological or sensory properties.

For most characteristics evaluated, the products with added fibre did not differ from the control samples in quality. Also in earlier studies, in which wheat fibre of earlier generation was used as a fat replacer, Makała (2002a,b) found that the examined products had characteristics, such as structure, binding capacity and sensory desirability, similar to those of the controls. Dietary fibre used as a fat replacer produced better sensory quality than reported in the earlier protein - polysaccharide systems (Olkiewicz, Kostyra, Adamik 1998). A significant increase in cooking losses is a drawback of using dietary fibre preparations as fat substitutes, as also reported in earlier studies of Egbert (1991) and Keeton (1992).

To receive all benefits of added dietary fibre in meat products ie., desirable nutritional function as well as their favourable role in sensory quality, further studies are needed to optimize their application in fat substitution or functional administration.

Conclusions

1. Dietary fibre preparations used to replace 30% of fat caused a significant increase of cooking loss, whereas the slice strength and rheological quality did not differ from the control products.
2. The products containing 1.5 % of wheat or oat fibre were characterized by better binding capacity, sensory palatability and hardness than the control product. At the same time the product received nutritional value in the form of dietary fibre, which is known to promote the health of human gastro-intestinal tract.
3. Wheat and oat fibre may be used as fat replacers and functional additives without compromising the sensory and textural quality of finely comminuted cooked meat products.

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EFFECT OF ADDITION OF TEA CATECHINS AND VITAMIN C ON SENSORY EVALUATION, COLOUR AND LIPID STABILITY IN COOKED OR RAW BEEF AND CHICKEN PATTIES

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Background

Colour and lipid stability in retail meats are very important quality characteristics, which influence consumer acceptability. Minced meats undergo oxidative changes and develop rancidity more quickly than intact muscle since grinding exposes more of the muscle surface to air and microbial contamination. Many attempts have been made to reduce pigment and lipid oxidation in meats through endogenous and exogenous treatment with antioxidants, in particular, vitamin E and vitamin C. Mitsumoto (2000) reported that dietary vitamin E supplementation for several weeks decreased oxidation of meat or fat in poultry, pork and beef and that exogenous vitamin C addition retarded pigment and lipid stability in ground pork and beef. In an increasingly competitive market, meat processors constantly strive to produce more healthy meat products. Tea catechins are polyphenolic antioxidants found in green tea, which possess a range of health promoting properties. Meat products containing natural antioxidants, as opposed to synthetic derivatives, are more desirable from a consumer viewpoint.

Tang et al. (2000) found that supplementation of poultry diets with tea catechins (300 mg/kg feed) retarded lipid oxidation in chicken meat compared to controls and dietary vitamin E (200 mg/kg feed) supplemented meat. To date, it is unknown whether tea catechin addition to meat products is as effective in maintaining meat quality as vitamin C.

Objectives

The aim of this study was to compare the effects of addition of tea catechins and vitamin C on the colous, lipid stability and sensory quality in cooked and raw beef as well as chicken patties.

Materials and methods

Fresh beef striploin and chicken breast muscles (~10 kg of each) were obtained from a local meat processing unit. Meat samples were vacuum packaged and stored at 0ºC for approximately 3 days prior to commencement of each trial. Tea catechins (TC) (80.15%) were obtained from Kinglong Natural Plant Products Industry Ltd., Changsha, Hunan, China. Sodium ascorbate (Vitamin C: VC) (99.0-101.0%) was obtained from Pfizer Ireland Pharmaceuticals, Ringaskiddy, Co. Cork.

Cooking and packaging

Beef and chicken samples were minced twice through a plate with 4 mm holes (mincer model P114L, Talsa, Valencia, Spain) following removal of all external fat and connective tissue. Following mincing, beef and chicken were assigned to one of the following five treatments: CNTRL, control meat; TC200, meat plus 200mg TC/kg muscle; TC400, meat plus 400mg TC/kg muscle; VC200, meat plus 200mg VC/kg muscle, VC400, meat plus 400mg VC/kg muscle. Sodium chloride (1%) was added to all samples.

Minced beef and chicken (125 g portions) were formed into patties using a meat former (Ministeak burger maker, O. L. Smith Co. Ltd., Italy) and stored overnight at 0ºC. Patties were cooked in a fan-assisted oven (model 10GN1/1, Zanussi Professional, Conegliano, Italy) at 180ºC until an internal meat temperature of 72ºC was reached, and subsequently held at 180ºC for a further 15 minutes. After chilling, cooked patties were placed in low oxygen permeable (<1cm^3/m^2/24hr/atm) polystyrene/ethylvinylalcohol (EVOH)/ polyethylene (PE) trays and flushed with 30% CO_2 : 70% N_2 using a vacuum sealing unit (VS 100, Gustav Müller & Co. KG, Homburg, Germany) equipped with a gas mixer (Witt-Gasetechnik GmbH & Co. KG,

Witten, Germany). Trays were covered and heat-sealed with a low oxygen permeable ($3cm^3/m^2/24hr$) laminated barrier film with a polyolefin heat sealable layer. Fresh raw beef and chicken patties were stored in 80% O_2 : 20% CO_2. All samples were stored for up to 7 days under fluorescent lighting (approximately 700 lux) at 4°C.

Colour determination

Surface colour measurements were determined using a CR-300 Chroma Meter (Minolta Co., Osaka, Japan) which consisted of a measuring head (CR-300), with an 8 mm diameter measuring area, and a data processor (DP-301). The chroma meter was calibrated on the Hunterlab colour space system using a white tile (D_{65}: Y = 94.4, x = 0.3172, y = 0.3339). Hunter *L** (lightness), *a** (redness) and *b** (yellowness) values were measured on days 1, 3 and 6 for cooked meats and on days 2 and 7 for raw beef and chicken.

Measurement of lipid oxidation

Lipid oxidation was measured by the 2-thiobarbituric acid distillation method of Tarladgis et al. (1960) as modified by Ke et al. (1977) and results were expressed as 2-thiobarbituric acid reactive substances (TBARS) in mg malonaldehyde (MDA)/kg meat.

Sensory evaluation

An untrained sensory panel of 8 to 11 people evaluated cooked beef and chicken patties after 1, 3 and 6 days of storage. Patties were placed on paper plates, reheated using a microwave oven at high power (800 W) for 30 sec and served to the panellists individually. Panellists were asked to evaluate sample colour, flavour, taste and tenderness on an 8-point scale ranging from extremely desirable (8) to extremely undesirable (1). In addition, panellists were asked to rank samples, in order of preference from best (1) to worst (5) separately for beef and chicken, in terms of overall acceptability.

Statistical analysis

Data were analyzed by the General Linear Models procedure of SAS (1988).

Results and discussion

Cooked patties

Both tea catechins treatments (TC200 and TC400) showed low ($P<0.05$) sensory colour scores (Fig. 1) and Hunter *b** values (10.2 and 9.9, respectively) in cooked meat patties compared to controls (*b** values: 13.2) and both vitamin C treatments (VC200 and VC400) (*b** values: 12.6 and 12.5, respectively). Tea catechins treatment (TC200) had low ($P<0.01$) Hunter *a** values (1.2) in cooked meat patties compared to controls (2.9) and both vitamin C treatments (VC200 and VC400) (3.3 and 3.0, respectively). Tea catechins treatments resulted in no significant differences ($P>0.10$) in the sensory flavour, taste, tenderness and Hunter *L** value in cooked meat compared to controls and both vitamin C treatments. Tea catechins treatments had no effects ($P>0.10$) on overall acceptability in cooked beef patties, but decreased ($P<0.001$) acceptability in cooked chicken meat during display compared to controls and vitamin C treatments. Neither of the vitamin C treatments significantly affected ($P>0.05$) sensory traits and Hunter colour values in cooked meat compared to controls. Tea catechins and vitamin C treatments effectively reduced ($P<0.001$) lipid oxidation in cooked meat patties compared to controls (Fig. 2).

Raw patties

Tea catechins treatment, TC400, showed lower ($P<0.01$) Hunter *b* values (6.6) in raw meat patties compared to controls (7.4). In contrast, the vitamin C treatment (VC200) resulted in the highest Hunter *b* values (7.7). Both tea catechins treatments (TC200 and TC400) greatly suppressed ($P<0.01$) lipid oxidation in raw meat patties compared to controls, and TC400 resulted in the lowest TBARS values (Fig. 3). Vitamin C treatments (VC200 and VC400) did not significantly reduce ($P>0.05$) lipid oxidation in raw meat patties compared to controls (Fig. 3).

Tea catechins have been recognized as efficient antioxidants by scavenging free radicals and chelating metal ions (Shahidi and Wanasundara, 1992; Tang et al., 2002). In the current study, tea catechins caused discoloration possibly by binding with the iron component of myoglobin, and delayed lipid oxidation by reacting with free radicals. Discoloration caused by tea catechins clearly reduced the visual appearance and overall acceptability of the meat patties by panellists. Maher et al. (2002) reported that addition of tea catechins (1000 mg/kg muscle) and rosemary (1000 mg/kg muscle) to minced beef greatly improved the colour and lipid stability under aerobic and modified atmosphere conditions compared controls. Tang et al.

(2001) reported that lipid oxidation in cooked chicken meat was more effectively controlled by the addition of tea catechins (300 mg/kg meat) than that in cooked beef, and that inhibition of lipid oxidation resulting from tea catechins was greater than that for vitamin E. In this study, tea catechins caused meat discoloration but inhibited lipid oxidation to a greater extent than vitamin C.

Meat

Beef was more susceptible ($P<0.01$) to oxidation as either cooked or raw than chicken meat. Raw meat stored in high oxygen conditions (80% O_2 : 20% CO_2) was more prone to lipid oxidation than cooked meat stored in anaerobic conditions (30% CO_2 : 70% N_2). Tea catechins and vitamin C treatments were effective ($P<0.001$) for lipid oxidation in cooked beef but not ($P>0.10$) in cooked chicken meat. Tea catechins treatments were effective ($P<0.05$) for lipid oxidation in raw beef compared to the control and vitamin C treatments but not ($P>0.10$) in raw chicken meat. Since chicken meat was very stable for lipid oxidation compared to beef in this study, neither tea catechins nor vitamin C could act well as antioxidants for lipid oxidation in cooked or raw chicken meat patties.

Conclusions

Addition of tea catechins caused discoloration in cooked beef and chicken patties, but effectively reduced lipid oxidation in cooked and raw beef patties. Further studies are necessary to elucidate the antioxidant mechanisms of tea catechins in muscle systems.

Acknowledgements

Thank you to researchers of the Packaging Laboratory, Department of Food and Nutritional Sciences, University College Cork for their participation in the sensory evaluation panels. The author is grateful for the assistance of Dr. Keisuke Sasaki, National Institute of Livestock and Grassland Science, Tsukuba, Japan.

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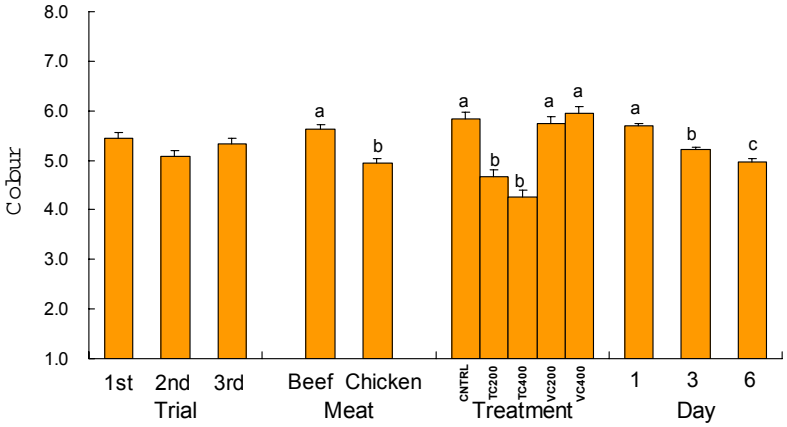

Fig. 1 Effect of meat, treatment and day on sensory colour scores in cooked beef and chicken meat patties. Least-squares means and standard error bars are shown. a,b,c: within main effects, means with no common letters differ (P<0.05).

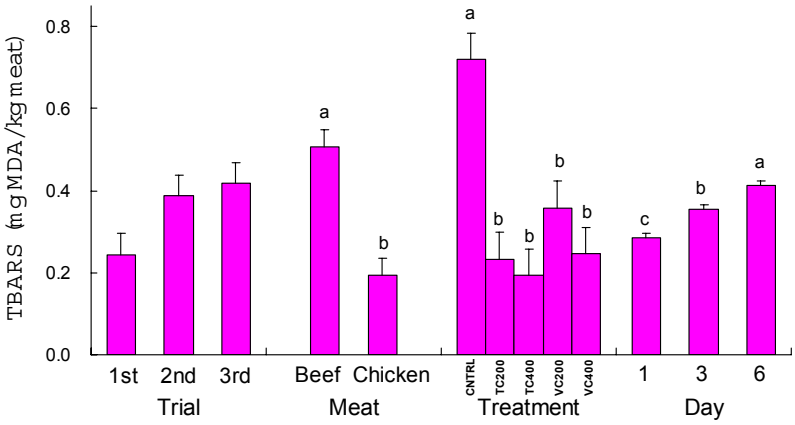

Fig. 2 Effect of meat, treatment and day on TBARS value in cooked beef and chicken meat patties. Least-squares means and standard error bars are shown. a,b,c: within main effects, means with no common letters differ (P<0.05).

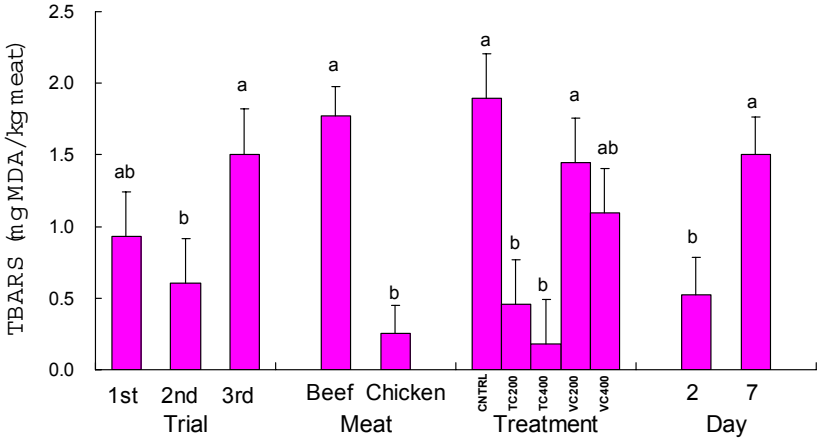

Fig. 3 Effect of meat, treatment and day on TBARS value in raw beef and chicken meat patties. Least-squares means and standard error bars are shown. a, b: within main effects, means with no common letters differ (P<0.05).

THE COMPOSITION AND OXIDATIVE STABILITY OF LIPIDS IN LONGISSIMUS MUSCLE FROM GRAZING CATTLE SUPPLEMENTED WITH SUNFLOWER OIL OR LINSEED OIL

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Background

Manipulation of ruminant ration composition has been employed to enhance the concentrations of conjugated linoleic acid (CLA) and omega-3 polyunsaturated fatty acids (PUFA) in milk and meat. Compared with conventional indoor rations, consumption of grass, rich in n-3 PUFA, led to an improvement in the fatty acid profile of beef, by increasing PUFA and CLA concentrations, decreasing the n-6:n-3 PUFA ratio and decreasing the saturated fatty acid concentration (French et al., 2000). Enrichment of concentrate rations with plant oils, such as linseed oil or sunflower oil, also resulted in an increase in the concentration of CLA in bovine muscle (Enser et al., 1999, Noci et al., 2002). No information is available on the efficacy of such supplementation strategies for grazing beef cattle.

Strategies that improve the fatty acid composition must not impair other quality characteristics of beef. Appearance, specifically colour, is an important quality attribute influencing the consumer's decision to purchase. Increasing the PUFA concentration *per se* and/or increasing the concentration of longer carbon chain PUFA, predispose lipids to oxidation (Rey et al., 2001). Lipid oxidation is believed to be linked to muscle pigment oxidation and consequently to colour stability (O'Grady et al., 2001).

Objectives

The first objective of this study was to investigate the effect of plant oil supplementation of grazing cattle on the fatty acid profile of muscle, in particular the n-3 PUFA and CLA concentrations. The second objective was to determine the effect of alterations in the fatty acid composition on colour and lipid stability of beef.

Materials and methods

Forty-five Charolais crossbred heifers (mean initial bodyweight = 330 kg, s.d. 39.90) were blocked by initial bodyweight and, within block, randomly assigned to one of three dietary treatments (n = 15): unsupplemented grazing (GO); restricted grazing plus 2 kg/head/day of linseed oil-enriched meal (LO) or restricted grazing plus 2 kg/head/day of sunflower oil-enriched meal (SO). Concentrate and grass allowances were monitored at three-week intervals during a 5-month experimental period to achieve similar carcass weights across the treatments. Animals were slaughtered at a commercial facility, carcasses were chilled for 48 h at 4^{o}C, and the *M. Longissimus dorsi* (LD) was excised from each carcass. Intramuscular fat was extracted from muscle samples using chloroform and methanol (2:1 v/v), methylated at 50^{o}C for 20 minutes in alkaline and then acidic conditions and the fatty acid methyl esters obtained were analysed by gas chromatography (Supelcowax 100 m CP-Sil 88, Varian 3800). Vitamin E concentrations were measured as described by O'Sullivan et al. (2003).

Samples of LD collected 48h *post mortem* were vacuum packaged and stored at 4^{o}C for a further 24h prior to analysis. Samples were cut into steaks (25.4 mm thickness) and placed in retail display trays. Trays were over-wrapped with oxygen permeable film for aerobic storage or flushed with 80% O_2: 20% CO_2 for storage under modified atmosphere conditions. All samples were stored for up to 10 days at 4^{o}C under simulated retailed display conditions (616 lux fluorescent lighting). Colour measurements were made at 2 day intervals using a Cr-300 Chromameter (Minolta Co. Ltd., Japan) set on the CIE colour scale and reported as the 'a' redness value. Lipid oxidation was measured by the distillation method of Tarladgis et al. (1960) as modified by Ke et al. (1977) and results were expressed as 2-thiobarbituric acid reactive

substances (TBARS) in mg malondialdehyde/kg muscle. The data were analysed as a randomized block design using Genstat 6.0.

Results and discussion

Fatty acid data are summarised in Table 1. In general, the fatty acid composition of GO-fed cattle was similar to that previously reported by French et al. (2000). Compared to GO, SO-fed cattle had a higher concentration of C18:1 trans-11, C18:2, cis 9, trans-11 CLA, C20:4, total PUFA and n-6 PUFA but a lower concentration of C12:0, C18:3 and C22:5 and higher P:S and n-6:n-3 PUFA ratios. Compared to GO, LO-fed cattle had a higher concentration of C18:1 trans-11, cis 9, trans-11 CLA and n-3 PUFA and n-6:n-3 ratio but a lower concentration of C12:0, C20:4, C22:5 and C22:6. Compared to LO, SO-fed cattle had a higher concentration of C18:1 trans-11, C18:2, cis 9, trans 11 CLA, C20:4, C22:6 and n-6 PUFA, a lower concentration of C18:3 and a higher n-6:n-3 ratio.

Table 1. Fatty acid and Vitamin E concentrations in *M. Longissimus dorsi* from grazing cattle, either unsupplemented (GO) or supplemented with sunflower oil (SO) or linseed oil (LO)

Fatty acids (mg/100 g muscle)	GO	SO	LO	s.e.d.	Significance[1]
C12:0	1.52b	0.88a	0.88a	0.157	***
C14:0	53.36	47.77	49.81	6.926	NS
C16:0	542.2	520.1	525.6	69.23	NS
C18:0	435.6	431.6	406.1	56.45	NS
C18:1 cis-9	843.0	847.1	780.1	125.3	NS
C18:1 trans-11	76.63^{a}	227.0^{c}	157.8^{b}	24.60	***
C18:2n6 cis	58.80^{a}	78.39^{b}	62.50^{a}	4.816	***
CLA c9,t11	18.37^{a}	47.43^{c}	32.00^{b}	5.976	***
CLA t10, c12	1.73	0.93	1.51	0.479	NS
C18:3n3	34.34^{b}	22.14^{a}	31.72^{b}	2.929	***
C20:4n6	11.75^{a}	12.47^{c}	9.57^{b}	0.803	***
C20:5n3	7.63	6.40	6.40	0.561	0.06
C22:5n3	12.69^{b}	10.40^{a}	9.69^{a}	0.688	***
C22:6n3	2.71^{b}	2.34^{b}	1.65^{a}	0.269	**
SFA[2]	1089	1058	1037	134.5	NS
MUFA[2]	1032	1186	1037	143.3	NS
PUFA[2]	158.0^{a}	203.0^{b}	181.1^{ab}	14.93	*
P:S Ratio	0.15^{a}	0.21^{b}	0.18^{ab}	0.015	**
n-6 PUFA[2]	86.92^{a}	106.5^{c}	92.51^{c}	6.405	***
n-3 PUFA[2]	59.49	48.18	55.03	4.725	0.07
n-6:n-3 Ratio	1.46^{a}	2.24^{c}	1.72^{b}	0.096	***
Total fatty acids	2513	2688	2513	329.1	NS
Vitamin E (ug/g)	2.70^{a}	3.16^{b}	1.99^{c}	0.224	**

[1]NS = not significant; *,** and *** = P<0.05, P<0.01 and P<0.001, respectively; [2]SFA=total saturated fatty acids; MUFA = total monounsaturated fatty acids; PUFA = total polyunsaturated fatty acids; n-6 PUFA = sum of C18:2, C18:3n-6, C20:2, C20:3n-6, C20:4 and C22:2; n-3 PUFA = sum of C18:3n-3, C20:3n-3, C20:5, C22:5 and C22:6.

Vitamin E concentration was lowest in LO-fed cattle and highest in SO-fed cattle (Table 1). Since similar amounts were supplied by the concentrates this suggests greater metabolism and a possible greater requirement for Vitamin E in the diet that supplied the greatest amount of n-3 PUFA.

There was no effect of diet on colour stability of beef (Table 2). Muscle lipids tended to be more susceptible to oxidation in MAP (higher TBARS) than in aerobic packaging with muscle from SO-fed animals more stable than LO-fed animals (Table 2). Muscles from LO-fed animals had lower lipid stability compared to GO-fed animals on day 2 and 6 of display in MAP.

Table 2. Surface redness ('a' value) and lipid oxidation (TBARS) in *M. longissimus dorsi* from grazing cattle either unsupplemented (GO) or supplemented with sunflower oil (SO) or linseed oil (LO) stored in aerobic or in modified atmosphere packs (MAP).

		Storage Time (days)					
	Packaging	0	2	4	6	8	10
Redness							
GO	Aerobic	17.99	13.46	9.36	8.10	8.83	8.74
SO	Aerobic	17.79	12.78	10.11	8.63	9.15	9.13
LO	Aerobic	18.53	13.23	9.87	7.61	7.97	8.79
s.e.d.		1.067	0.900	0.639	0.528	0.551	0.607
Significance[1]		NS	NS	NS	NS	NS	NS
GO	MAP	17.99	17.33	14.84	13.03	10.20	9.09
SO	MAP	17.79	17.45	15.41	12.56	10.63	8.52
LO	MAP	18.53	18.30	16.56	14.60	10.81	9.50
s.e.d.		1.067	0.703	0.960	1.351	0.973	1.004
Significance[1]		NS	NS	NS	NS	NS	NS
Lipid oxidation							
GO	Aerobic	0.83	0.82	0.46^{b}	0.39^{a}	0.65^{b}	0.86^{b}
SO	Aerobic	0.37	0.37	0.26^{a}	0.28^{a}	0.31^{a}	0.41^{a}
LO	Aerobic	0.52	0.67	0.27^{a}	0.62^{b}	0.63^{b}	1.01^{b}
s.e.d.		0.200	0.258	0.088	0.065	0.135	0.169
Significance[1]		NS	NS	*	**	*	**
GO	MAP	0.83	0.77^{a}	1.27^{b}	0.97^{a}	3.14	4.83^{b}
SO	MAP	0.37	0.58^{a}	0.53^{a}	0.80^{a}	2.37	3.05^{a}
LO	MAP	0.52	1.16^{b}	0.93^{b}	1.82^{b}	3.48	4.82^{b}
s.e.d.		0.200	0.122	0.215	0.430	0.571	0.654
Significance[1]		NS	**	**	*	NS	*

[1]NS = not significant, * and ** = P<0.05 and P<0.01, respectively. Within packaging type and day, means with a common superscript do not differ significantly.

Conclusions

Supplementing grazing animals with plant oil-enriched concentrates resulted in a further beneficial effect on the fatty acid composition of muscle compared to grazing alone. Sunflower oil was more effective than linseed oil in increasing the concentration of CLA and TVA, but had a negative effect on the n-6:n-3 PUFA ratio. Linseed oil had a less pronounced effect on the CLA concentration than sunflower oil, but it also had a less negative effect on the n-6:n-3 PUFA ratio. While linseed oil supplementation caused a transient increase in lipid oxidation, this was not reflected in a loss in colour stability.

Acknowledgement

Funded by the Irish Government under the National Development Plan 2000-2006.

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ISOLATION AND PURIFICATION OF ACE INHIBITORY PEPTIDE DERIVED FROM CHICKEN BONE EXTRACT

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Background

The angiotensin I-converting enzyme (ACE) catalyses the formation of angiotensin II, a strong vasopresser, from angiotensin I, together with inactivation of bradykinin possessing hypotensive activity (Ondetti et al. 1977). Such dual effects of ACE inhibitors are contributory to their potent antihypertensive activity. Commercially available antihypertensive drugs such as captopril and enarapril are very potent ACE inhibitors. Recently, many ACE inhibitory peptides have been isolated from casein (Maruyama et al. 1985), sardine muscle (Matsui et al. 2000), dried bonito (Yokoyama et al. 1992), and porcine skeletal muscle (Arihara et al. 2001, Katayama et al. 2003 & 2004). However, there are few reports on deriving ACE inhibitory peptides derived from chicken bone extract.

Objectives

The objective of this study is to isolate and purify ACE inhibitory peptide derived from chicken bone extract.

Materials and methods

Materials and reagents

Chicken bone was obtained from ITOHAM FOODS Inc. Hippuryl-L-histidyl-L-leucine (HHL) was purchased from Nacalai Tesque (Kyoto, Japan). Commercial ACE (from rabbit lung) and trypsin were purchased from Sigma Chemical Co. (St. Louis, MO. U.S.A).

Preparation and hydrolysis of chicken bone extract

One kg of chicken bone was cut into about 3 cubic cm fragments using a saw, and heated under pressure at 121 ^{o}C for 30 min in an autoclave. The treated bone was suspended in 4 L of distilled water and boiled for 4 hours at 100 ^{o}C. The heated solution was filtered through a paper towel, and the filtrate was collected. Thereafter, the filtrate was centrifuged for 60 min at 10,000 x g to remove fat. The remaining solution was filtered again through a No. 5A filter paper (Toyo Roshi Kaisya Ltd., Tokyo, Japan), then the filtrate was lyophilized and used as chicken bone extract. Trypsin was used for hydrolysis of chicken bone extract. Five mg/ml of chicken bone extract was used as substrate. The ratio of enzyme to substrate was 1/100 (based on protein content). After 6 hours of hydrolysation at 37 ^{o}C (pH7.5), the solution was heated at 95 ^{o}C for 10 min to inactivate the protease. The solution was centrifuged for 10 min at 10,000g, and the supernatant was collected for experiments.

Purification of ACE inhibitory peptide

The chicken bone extract hydrolysate was fractionated by high-performance liquid chromatography (HPLC) with gel-filtration (column:TSK gel G2000SW$_{XL}$, 7.8 x 300 mm, Tosoh Co., Tokyo, Japan). Elution was performed with an isocratic elution of 0.2 M PBS (pH7.0) at a flow rate of 1.0 ml/min, after which the absorbance was detected at 225 nm (first HPLC run). The active fraction was collected, and rechromatographed under reversed-phase mode (column:Inertsil ODS-2 (octadecyl silica column), GL-Science, Inc., Tokyo, Japan). Elution was performed with a linear gradient of 0-35 % CH_3CN in 0.1 % TFA at a flow rate of 1.0 ml/min., after which the absorbance was detected at 225 nm (second HPLC run). The active fraction was again collected and rechromatographed under the same conditions except for the eluting solution. This time the elution was performed with a linear gradient of 8-14 % CH_3CN in 0.1 % TFA at a flow rate of 0.5 ml/min. Absorbance was again detected at 225 nm (third HPLC run). The active fraction was once again collected and rechromatographed under reverse-phase mode (column:Cosmosil 5PE-MS (phenyl

column), GL-Science, Inc., Tokyo, Japan). Elution was performed with an isocratic elution of 10 % CH_3CN (fourth HPLC run) and 5 % CH_3CN (final HPLC run) at a flow rate of 0.5 ml/min. This time the absorbance was detected at 215 nm.

Assay for ACE inhibitory activity

The ACE inhibitory activity was measured according to the method of Cushman and Cheung (1971) with slight modifications. This assay is based on the liberation of hippuric acid from hippuryl-L-histidyl-L-leucine (HHL) catalyzed by ACE. Six µl of sample solution was mixed with 20 µl of ACE (60 mU/ml) which dissolved in borate buffer (pH8.3), and with 50 µl of 7.6 mM HHL dissolved in 0.01 M borate buffer and 0.608 M NaCl. The mixture was incubated for 30 min at 37 ^{o}C. To terminate the enzyme activity, 554 µl of 0.1N HCl was added. To extract hippuric acid liberated from HHL by ACE, 1.5 ml of ethyl acetate was added and the tubes were vigorously shaken, and then centrifuged for 15 min at 1,000 x g. One ml of the ethyl acetate layer was dried for 10 min at 100 ^{o}C. Hippuric acid liberated by ACE was photometrically measured at 228 nm after ethyl acetate extraction. IC_{50} value was defined as the concentration of ACE inhibitors needed to inhibit 50 % of ACE activity.

Analysis of peptide

The amino acid sequence of the purified peptide was analyzed by a protein sequencer (Procise 492, Applied Biosystems, Foster City, CA, USA).

Synthesis of peptide

The peptide was synthesized by a solid phase method with a peptide synthesizer (Symphony, RAININ Instrument Co. Inc., Woburn, MA, USA).

Results and discussion

The plain chicken bone extract did not show ACE inhibitory activity, but its hydrolysate showed relatively strong activity with an IC_{50} value of 0.781 mg/ml. This level of activity is comparable to that of carnosine, which is a well-known substance with ACE inhibitory activity. The hydrolysate of chicken bone extracted by trypsin was fractionated by gel-filtration HPLC and the findings are shown in Fig. 1 (first HPLC run). Three fractions with the highest inhibitory activity (12-15 min) were further purified by reversed-phase HPLC (second and third HPLC run). Then the fraction with the highest inhibitory activity (26-27 min) was further purified by reversed-phase HPLC (fourth ie., the final HPLC run). From the single active fraction shown in Fig. 2 (final HPLC run), one peptide with a high ACE inhibitory activity was purified. The single purified peptide was sequenced by a protein sequencer. The amino acid sequence of the one ACE inhibitory peptide was determined, and the structure of the peptide was YYRA (Tyr-Tyr-Arg-Ala, MW 571.67). The IC_{50} value of this single isolated peptide was 33.9 µg/ml, and it showed a relatively high level of activity as do other ACE inhibitory peptides derived from foods. A search for sequence homology in certain databases (FASTA WWW service) demonstrated that the same sequence exists in the primary structure of Ig heavy chain V region (27-30 position) as shown in Fig. 3. Therefore, a novel ACE-inhibitory peptide not previously reported, was now introduced.

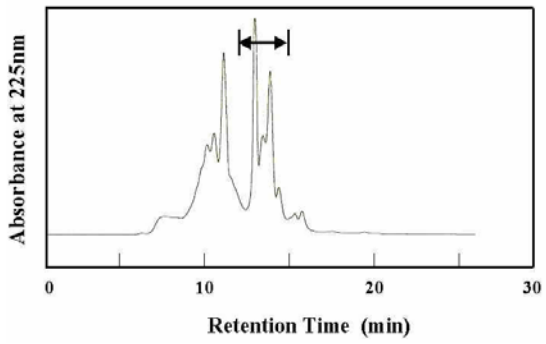

Fig. 1 Gel-filtration HPLC chromatograph (first HPLC run) Arrow indicate active fraction.

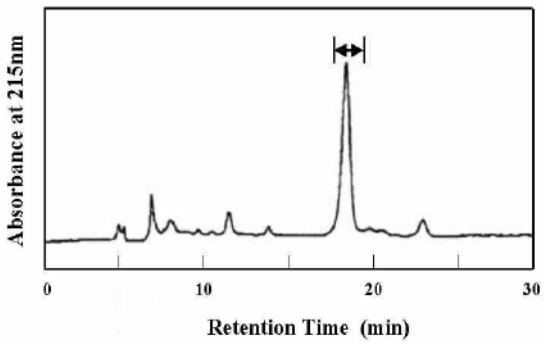

Fig. 2 Reversed-phase HPLC chromatograph (final HPLC run) Arrow indicate active fraction.

Fig. 3 Position of the peptide in the sequence (YYRA) of Ig heavy chain V region.

There are some peptides with potent inhibitory activity in vitro or when intravenously administered that are not active in vivo, when administered orally. It is thought that these peptides are digested into inactive peptide fragments or amino acids after oral administration. It is of great importance that the peptides have sufficient antihypertensive activities in vivo. Further studies are, thus, needed to measure the ACE activity using spontaneously hypertensive rats (SHR).

Conclusions

A novel ACE inhibitory peptide was isolated and purified from chicken bone extract. The amino acid sequence of the peptide was YYRA (Tyr-Tyr-Arg-Ala), which was of the origin of the Ig heavy chain V region (27-30 position). The result of this study suggests that ACE inhibitory peptides derived from chicken bone extract could be utilized in developing physiologically functional foods.

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IDENTIFICATION OF SOURNESS-SUPPRESSING PEPTIDES IN COOKED PORK LOINS

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Background

It is well known that meat becomes more palatable with postmortem conditioning, which brings about not only tenderization but also improvement of flavor. The free amino acids and peptides that increase during postmortem aging play an important role in the improvement of meat taste (Nishimura, T., 1998). Some reports have indicated that there are peptides that change taste perception. It has been shown that dipeptides such as Gly-Glu, Pro-Glu and Val-Glu have a buffering action and improve the taste of Japanese sake. Glutamic acid-rich oligopeptides and casein hydrolysate have been reported to mask a bitter taste (Noguchi et al., 1975; Tamura et al, 1990). It has been reported that the addition of hydrolyzed wheat gluten to an umami taste solution enhanced its strength. A peptide fraction of MW 1,000-10,000 from beef vacuum-cooked at 60 ^{o}C for 6 hours has been reported to improve the taste of un-aged beef soup by suppressing its sourness（Ishii et al, 1995. Peptides enhancing umami in the presence of inosinic acids were also isolated from chicken hydrolysate by protease (Maehashi, et al, 1998). However, in the case of pork, there is little information on peptides that improve meat taste.

Objectives

The present work was conducted to identify the peptides that suppressed the sourness and improved the taste of vacuum-cooked pork loins after *post mortem* conditioning of 20 days at +4^{o}C. This study was also carried out to clarify the mechanism of sour taste suppression by the peptides.

Materials and methods

Preparation of peptide fractions

Pork loins were stored in the vacuum-packages at +4^{o}C for 20 days after slaughter, and the packages were heated in a water bath at 60^{o}C for 360 min. The ground pork loin was freeze-dried and the lipids were extracted with n-hexane. The ground lean muscle was then homogenized with a ten-fold volume of de-ionized water. The homogenate was centrifuged at 10,000×g for 20 min and the supernatant was collected. Ethanol was added (final concentration, 80%) to this supernatant, followed by centrifugation and then filtration. After evaporation and further freeze-drying of the filtrate, the LD muscle powder was dissolved in de-ionized water. The solution was ultrafiltrated through MW500 and 1,000 cut-off membranes (Amicon Co., Beverly, USA), and then freeze-dried. Each peptide fraction, Fraction Ⅰ (MW < 500), Fraction Ⅱ (MW 500-1,000), or Fraction Ⅲ (MW > 1,000), was obtained.

Sensory evaluation

Using a modification of the paired-preference tests, the changes in the strength of basic tastes on the addition of the peptide fractions were evaluated by a well-trained panel. The relative difference was expressed within a scale of -5 to 5 points, the point in the absence of a peptide fraction being taken as zero. Solutions of 0.2% lactic acid (sourness), 0.04% monosodium glutamate (umami), 0.001% quinine hydrochloride (bitterness), 0.5% sodium chloride (saltiness), and 1.0% sucrose (sweetness) were used as basic taste solutions for sensory evaluation.

Peptide analysis

Peptides were analyzed by HPLC on a reversed-phase column (Senshupack VP-318, Senshu Sci. co., Tokyo, Japan). The filtrate was applied on the column and eluted with a linear concentration (0-40%) gradient of acetonitrile containing 0.1% trifluoroacetic acid at 40 ^{o}C for 80 min. The flow rate was 1.0 ml/min and peptides were detected at 220 nm. The amino acid sequences of the peptides were determined with an ABI amino acid sequencer (Model 477A, Applied Biosystem Japan, Tokyo, Japan), and the molecular weights were measured with a mass spectrometer (TSQ 7000, Thermo Finnigan, San Jose, USA).

Assaying of binding to epithelial tissue of porcine tongues

The epithelial tissue containing foliate papillae was removed from the underlying dermis of fresh porcine tongue with a scalpel and fine forceps. After mincing with scissors, the epithelial tissue was homogenized with a ten-fold volume of 10mM Tris-HCl buffer (pH7.4) containing 10 mM $CaCl_2$ and 30 mM mannitol, and the homogenate was centrifuged at 30,000×g for 20 min. The precipitate was further homogenized with 10mM Tris-HCl buffer (pH 7.4) containing 100 mM mannitol, and the homogenate was centrifuged at 40,000×g for 20 min. This precipitate then served as the epithelium of porcine tongues.

The binding of radioactively labeled L-lactic acid (L-[U-^{14}C] lactic acid, sodium salt (120 Ci /m mol)) to the epithelium was measured. Sodium lactate solution (0.15 ml; 0.5%) containing labeled L-lactic acid and K-phosphate buffer (pH 7.2; 0.04 ml; 0.5M) was then added to 0.15 ml of epithelium solution (approximately 0.17mg protein/ml). Then, 0.16 ml of a peptide fraction solution or de-ionized water was added to this mixture, the final total assay volume being 0.4 ml. The same volume of 10 mM Tris-HCl buffer (pH 7.4) instead of the epithelium solutions was used as a blank. Each sample was incubated at room temperature for 10 min and then filtered rapidly through a 0.45μm cellulose acetate filter (Nihon Millipore Ltd., Tokyo, Japan). After filtration, the filter was immediately rinsed with 10 ml buffer 20 mM Tris-HCl buffer, pH） and the filter disc then placed into a bottle containing 5 ml of scintillation fluid (Aquasol^{TM}, New England Nuclear Co., Montpellier, USA). Then the radioactivity (C-DPM) of the samples was counted with a liquid scintillation counter (LSC-5100, Aloka Co., Mitaka, Japan). Each binding value was calculated by subtraction of the C-DPM value for the blank without epithelium from that with epithelium.

Assaying of binding to a synthetic lipid membrane with a taste sensor

The binding of sour taste substances to a synthetic lipid membrane composed of two types of lipids, dioctylphosphate and triocthylmethyl ammonium chloride, was measured with a taste sensor (SA401, Anritsu Co., Atsugi, Japan). A tartaric acid solution (1-10mM) containing 10 mM KCl was used as the sour taste solution. Using the taste sensor system, binding of tartaric acid to the membrane was relatively well reflected by the electric potential (mV) of the taste sensor. The electric potential of the tartaric acid solution with or without the peptide fraction was measured.

Results and discussion

Recovery of peptide fractions of pork

The amount of Peptide fraction I (MW < 500) in pork loins gradually increased as the storage period became longer, as did that of Fraction II (MW 500-1,000) up to 20 days. However, the amount of Peptide fraction III (MW > 1,000) remained unchanged throughout storage for 30 days. The result that peptides increased during storage for 20 days is in accordance with our previous study (Okumura, 1996). The amounts of peptides in fractions I, II and III from pork loins stored for 20 days were 30.2, 7.5 and 1.6 mg/g meat, respectively. With heating at 60°C for 6 hours after *post mortem* conditioning of two days, the amount of all three peptide fractions increased. However, in the pork stored for 20 days the fractions remained almost unchanged. Although we did not examine the reason for this, the fact that the activities of endogenous proteases in porcine muscle were lost during *post mortem* aging for 20 days may be responsible for the phenomenon.

Effect of addition of peptides fraction on basic tastes

The effect of added peptide fractions on the basic tastes were examined by sensory evaluation. Figure 1 shows that the sourness of a lactic acid solution by addition of Fraction II (0.074%) from pork loins stored at 4°C for 20 days was weaker than that without peptide fraction, while the addition of Fraction Ⅰ (0.37%) or III (0.016%) had little effect on sourness suppression. This indicated that some peptides in Fraction II suppressed sourness. The increase of these peptides during conditioning seemed to play an important role in the change of the sourness of unaged pork. Although the taste of beef taken from the carcass immediately after slaughter is sour and bloody, meat after conditioning at a low temperature has no such taste. Mild taste created during conditioning may be related to an increase in peptides. Therefore, it seemed that Fraction II masked the sourness or metallic taste of immediately *post mortem* pork meat and improved the taste during conditioning.

Purification and identification of the peptides suppressing sourness

In order to identify peptides suppressing sourness, the analysis of peptides in Fraction II of pork loins stored for 2 and 20 days at 4°C was performed. Figure 2 showed that the main peaks indicated by arrows presenting peptides (1), (2) and (3), were greatly increased during storage. On amino acid analyses of the peptides with

an amino acid sequencer and a mass spectrometer, peptides (1), (2), and (3) were identified as APPPPAEVHEVV, APPPPAEVHEVVE and APPPPAEVHEVHEEVH. These peptides were homologous to the peptide, APPPPAEVHEVHEEVH that was generated from troponin T in pork during *post mortem* conditioning. The structure of these peptides was similar to that of the peptide increasing during *post mortem* aging of beef. Many researchers have reported that troponin T was degraded by the activity of proteases during *post mortem* aging of chicken, porcine and bovine muscles at low temperature. The purified troponin T has also been shown to be degraded by calpain and cathepsin, indicating that the peptide APPPPAEVHEVHEEVH seems to be produced by the action of calpain and / or cathepsin. The clarification of the mechanism is the next problem to be resolved.

Using synthetic peptide, APPPP or APPPPAEVHEV, which are common amino acid sequences in the three peptides, the effect of these peptides on sourness was examined by sensory evaluation. The addition of APPPP to a lactic acid solution did not change the sour taste, whereas the addition of APPPPAEVHEV suppressed the sour taste. From these results, it was concluded that the three peptides containing common sequences in Fraction II suppressed the sour taste, and the increases in these peptides during conditioning delivered the mildness to the taste of meat. This is the first report that discovered the peptide suppressing sourness.

Mechanism of suppression of sourness on the addition of peptides

i) Binding assay of peptide using the epithelium of porcine tongue: The effect of concentration of peptide on the lactate binding to the epithelium was examined. The higher the concentration of Fraction Ⅱ added to the sample solution at pH 5 was, the lower was the amount of lactic acid binding to the epithelium (Fig. 3).

ii) Binding of tartaric acid to a synthetic lipid membrane: The binding of tartaric acid to a synthetic lipid membrane was also inhibited by the addition of peptide (3) in Fraction II, suggesting that Fraction II inhibited the binding of sour taste substances to the membranes of tongues. Furthermore, this inhibitory action depended on the concentration of Fraction II. It was concluded that the peptides of Fraction II derived from pork extract suppressed sourness through its interaction with sour taste channel or sour taste substances. That is, sour taste substances can not reach sour taste ion channel, because the peptide is either binding with sour taste substances or covering the entrance of sour taste ion channel (Fig. 4).

Conclusions

A peptide fraction (Fraction II; molecular weight 500-1000) from pork loins were cooked in vacuum-packages at 60°C for 6 hours suppressed sourness. Three peptides (APPPPAEVHEVV, APPPPAEVHEVVE and APPPPAEVHEVHEEVH) were isolated from Fraction II. These peptides seemed to suppress sourness through their interaction with sour taste channels or sour taste substances.

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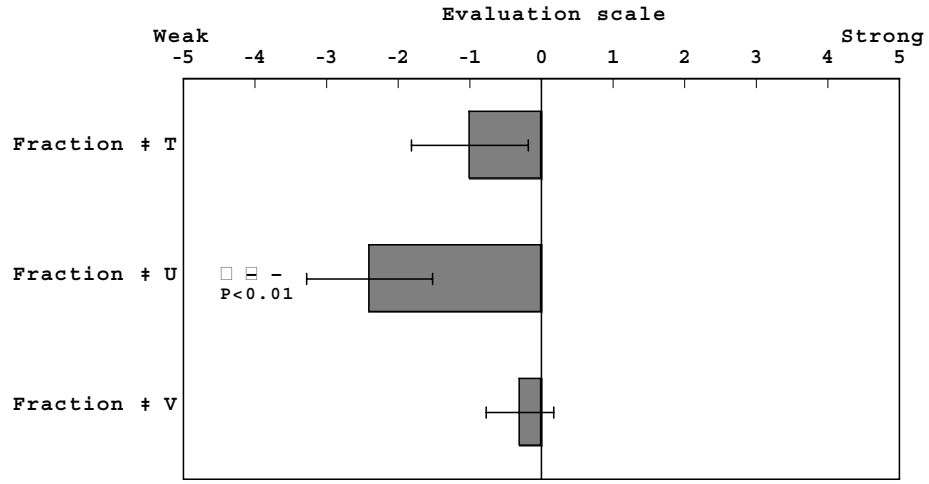

Figure 1. Effects of addition of peptide fraction on sourness intensity

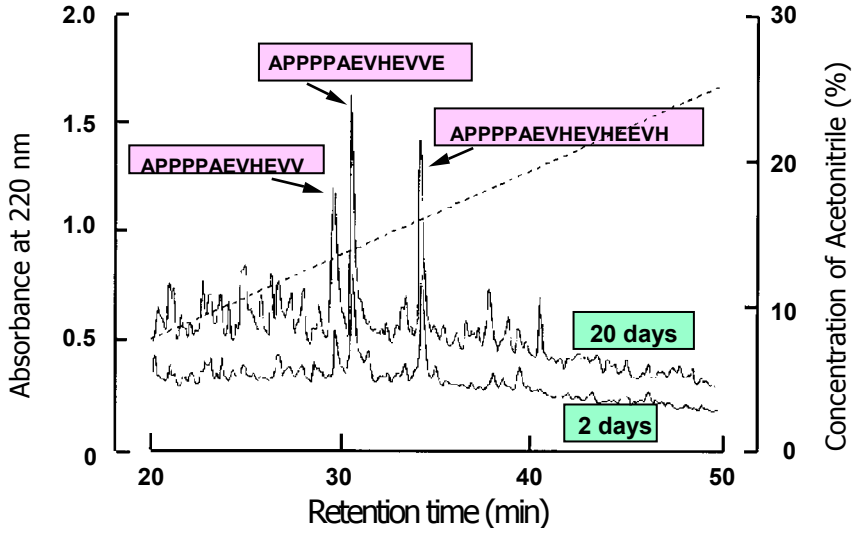

Figure 2. HPLC profile of Fraction II (MW 500-1000) in pork loins stored at 4 °C for 2 or 20 days.

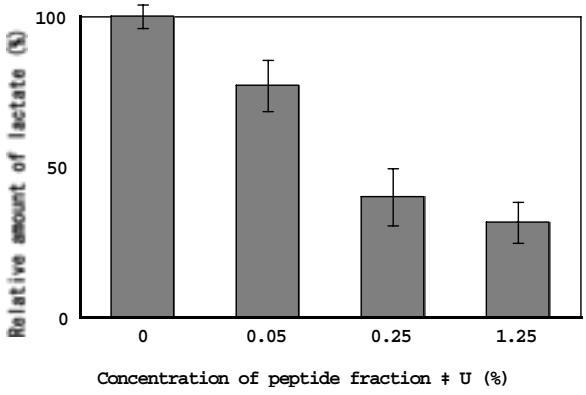

Figure 3. Effect of Fraction II on lactic acid binding to tongue epithelium

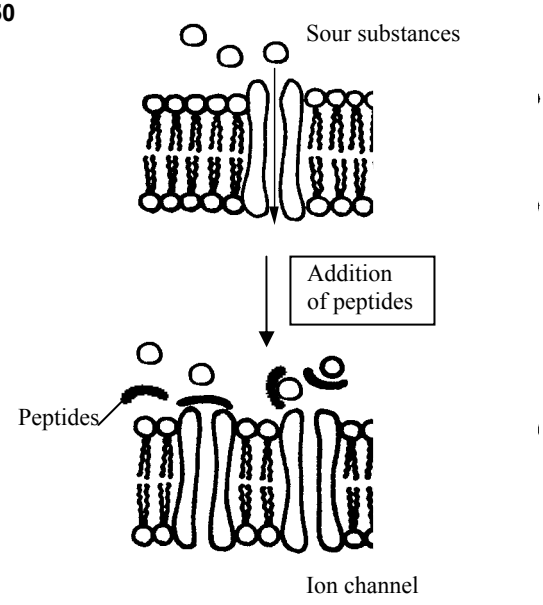

Figure 4. Proposed mechanism of suppression of sourness by addition of peptides

EFFECT OF MILK-REPLACER REARING *VS* NATURAL REARING ON FATTY ACID COMPOSITION OF SUCKLING LAMB LIVER

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Background

"Castilla y León" is the region of Spain with the largest sheep stock –c.a. 6 millions–, from which approximately 2.5 millions are slaughtered annually for human consumption. The mayor part of these are suckling lambs 'lechales' –c.a. 60-70%– with age between 25-45 days and with a carcass weight of less than 7 kg, coming from milk production systems (Sañudo *et al*., 1998). The importance, value and quality of suckling lamb meat in 'Castilla y León' has been recognized and protected by a geographical indication: 'Lechazo de Castilla y León' (Council Regulation 2081/92/EC).

In general, in this region the suckling lambs are reared exclusively either with ewe's milk or with milk-substitute, although the last is being more widely used, especially in milk breeds. It appears that rearing suckling lambs, with one or the other type of milk has effect on several production aspects: economical –i.e. feed conversion, milk's price, cheese making, etc.–, meat quality –i.e. nutritional and organoleptic characteristics–, animal welfare and health (De la Fuente *et al*., 1998; Pérez *et al*., 2001; Napolitano *et al*., 2002), supporting the one or the other of both possibilities.

No studies on the fatty acids content of suckling lamb's liver have been found in the literature; however, there were found references dealing with the fat composition of older lambs (Enser *et al.*, 1998; Mir *et al.*, 2000; Moibi and Christopherson, 2001).

Objectives

The purpose of this study was to evaluate the effects of the type of rearing on the fatty acid composition of the liver of an autochthonous lamb breed from Spain (Churra). It was expected that fatty acid profile of fat depots from unweaned young lambs would really reflect the composition of the ingested milk (Bas and Morand-Fehr, 2000, Napolitano *et al*., 2002) –due to the fact that rumen metabolic development has not occurred at this early stage (Lane *et al*., 2000).

Materials and methods

Samples

Thirteen livers of 'lechales' from the Churra breed which were hand reared with milk-replacer and seventeen livers of Churra 'lechales' reared with ewe's milk were purchased from a local slaughterhouse. All samples were homogenised and kept frozen at -40 ºC prior to all analysis.

Fatty acid profile of liver

For analysis of fatty acids, the fat from aliquots of 35 g of liver was extracted according to the method described by Bligh and Dyer (1959). The methyl esters of fatty acids (FAME) from the fat were obtained by base-catalyzed transesterification with $NaCH_3O$ (Sehata *et al.* 1970). Gas chromatographic (GC) analysis were carried out using a Hewlett Packard 6890 Series GC System Chromatograph equipped with a automatic injector (HP 7683 Series Injector), and a Hewlett Packard 5973 Mass Selective detector. The column used for the separation was a Supelco 2-4136 Omegawax^{TM} 250 fused silica capillary column (30 m × 0.250 mm, 0.25 µm film thickness). The GC conditions were as follows: Initial oven temperature 50 ºC, held for 1 min, then programmed at 10 ºC min^{-1} to 150 ºC and held for 1 min, then 12 ºC min^{-1} to 180ºC, then 2 ºC min^{-1} to188ºC and held for 6 min, then 2 ºC min^{-1} to 220 ºC and held for 2 min, and finally 20 ºC min^{-1} to 260 and held for 7 min. Injector and detector temperature were 200 ºC and 300 ºC, respectively. The flow rate of the carrier gas (He) was 1 ml min^{-1} and 1 µl of solution was injected in the mode split, ratio 30:1, and the pressure was 16 psi.

The FAMEs were identified by using individual standards and by their mass spectrometry data obtained with a HP Mass Spectral Libraries (Hewlett Packard, revision D 01.00, 1998) as well as by their retention time found in literature. Quantities of FAMEs were calculated from the response factor of standards or, in case of those standards that were not available, from the response factors of their respective isomers. Results were expressed as percentage of weight of the identified peaks.

Statistical analysis
Fatty acid composition were analysed using ANOVA with one factor, rearing system.

Results and discussion

The fatty acid compositions of the liver from both types of feeding are shown in table 1. The most abundant fatty acids were C18:1, C18:0 and C16:0 and compared with other fatty depots of meat, a high percentage of polyunsaturated fatty acid was observed. These results are in agreement with those found in liver of older lambs in other studies (Enser *et al*., 1998; Mir *et al*., 2000; Moibi and Christopherson, 2001).

Liver from artificially reared lambs showed a lower content of saturated fatty acids ($P<0.001$) and a higher content of unsaturated fatty acids ($P<0.001$). The percentage of monounsaturated fatty acids was lower in the liver obtained from ewe-reared lambs ($P<0.001$) whereas no difference were observed for polyunsaturated fatty acids.

Saturated capric (C10:0), pentadecanoic (C15:0), palmitic (C16:0), margaric (C17:0) and stearic (C18:0) fatty acid contents were lower in the liver of lambs reared with milk substitute ($P<0.005$, $P<0.001$, $P<0.05$, $P<0.001$, and $P<0.001$, respectively). The amount of branched fatty acids was ten times greater in the samples of liver of lambs raised with ewe's milk ($P<0.001$) which can be attributed to the presence of these fatty acids only in ewe's milk. On the contrary, the monounsaturated oleic (C18:1) fatty acid contents were lower in the liver of ewe-reared lambs ($P<0.001$) whereas the cis-heptadecenoic fatty acid (C17:1) were higher ($P<0.001$), the same as the entire fatty acids with an odd number of carbon atoms.

The content of linoleic (C18:2), eicosadienoic (C20:2) and eicosatrienoic (C20:3) fatty acids were higher ($P<0.005$, $P<0.001$ and $P<0.05$, respectively) in the liver of lambs reared with milk substitute. On the other hand the content of linolenic (C18:3), arachidonic (C20:4) and eicosapentaenoic (C20:5) ($P<0.001$) polyunsaturated fatty acids were lower.

The P/S ratio was higher in lambs reared with milk substitutes ($P<0.001$) while the percentage of ω-3 polyunsaturated fatty acids was higher in lambs reared with ewe's milk ($P<0.001$).

The fatty acid profile of liver rather reflects the composition of the milk ingested by lambs. The rumen of suckling lambs is not functional yet and so the fatty acid composition of liver is similar to the fatty acid composition of ingested milk. According to Napolitano *et al.* (2002) milk substitutes showed a lower content of saturated fatty acids (60.2 *vs* 66.4%) and a higher content of monounsaturated (30.1 vs 20.1%) and polyunsaturated (9.7 vs 6.2%) fatty acids compared to milk from ewe. Most of the fat components of milk substitutes are derived from vegetables oils, which are characterised by a lower level of saturation compared to animal fats.

Conclusions

Substantial differences in fatty acid composition between liver from suckling lambs reared with ewe's milk and from those reared with milk-replacer were observed in this study. The liver of the lambs reared exclusively with ewe's milk showed a higher content of saturated fatty acids and a lower content of monounsaturated fatty acids than liver from artificially reared lambs. The nutritional characteristics of the livers of suckling lambs are influenced by the type of feeding.

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Table 1. Fatty acids profile of liver fat of suckled lambs reared with ewes' milk or milk replacer.

Fatty acids	Ewes' milk	Milk replacer	Significance
C10:0 capric	0,04±0,04	0,01±0,01	P<0,005
C12:0 lauric	0,21±0,15	0,33±0,17	NS
C14:0 myristic	1,57±0,63	1,32±0,40	NS
C15:0 br. isomer	0,04±0,04	0,00±0,00	P<0,001
C15:0 br. isomer'	0,13±0,08	0,00±0,00	P<0,001
C15:0 pentadecanoic	0,46±0,12	0,06±0,05	P<0,001
C16:0 br. isomer	0,12±0,08	0,11±0,18	NS
C16:0 palmitic	19,48±3,39	16,82±2,00	P<0,05
C16:1 isomer	0,20±0,11	0,31±0,33	NS
C16:1 palmitoleic	0,5±0,16	0,71±0,44	NS
C16:1 isomer'	0,02±0,06	0,02±0,05	NS
C17:0 br. isomer	0,37±0,08	0,00±0,00	P<0,001
C17:0 br. isomer'	0,39±0,14	0,00±0,00	P<0,001
C17:0 br. isomer''	0,03±0,07	0,00±0,00	NS
C17:0 margaric	1,33±0,30	0,21±0,18	P<0,001
C17:1 cis-heptadecenoic	0,17±0,07	0,01±0,02	P<0,001
C17:1 isomer	0,13±0,07	0,00±0,00	P<0,001
C18:0 stearic	29,56±3,53	18,78±3,49	P<0,001
C18:1 isomer	0,00±0,00	0,03±0,06	P<0,05
C18:1 ω9 oleic	17,67±2,90	26,95±1,84	P<0,001
C18:1 isomer'	0,16±0,44	1,70±1,97	P<0,005
C18:1 isomer''	2,09±0,74	3,97±1,87	P<0,001
C18:1 isomer'''	0,52±0,34	2,83±1,29	P<0,001
C18:1 isomer''''	0,81±0,47	2,90±2,10	P<0,001
C18:1 isomer'''''	0,00±0,00	0,39±0,39	P<0,001
C18:2 isomer	0,08±0,13	0,29±0,26	P<0,01
C18:2 isomer'	0,00±0,00	0,50±0,43	P<0,001
C18:2 ω6 linoleic	8,26±1,04	10,91±2,76	P<0,01
C18:2 isomer''	0,00±0,00	0,05±0,10	P<0,05
C19:0 nonadecanoic	0,20±0,13	0,05±0,09	P<0,01
C18:3 ω3 α-linolenic	0,82±0,32	0,10±0,21	P<0,001
C18:2 CLA	0,38±0,22	0,88±0,89	P<0,05
C20:0 arachidic	0,10±0,07	0,06±0,12	NS
C20:1 gadoleic	0,18±0,14	0,30±0,37	NS
C20:2 isomer	0,00±0,00	0,09±0,08	P<0,001
C20:2 eicosadienoic	0,03±0,04	0,33±0,50	P<0,05
C20:3 ω6 eicosatrienoic	0,51±0,20	1,64±1,82	P<0,05
C20:4 ω6 arachidonic	12,32±1,85	7,30±1,48	P<0,001
C20:5 ω3 eicosapentenoic	1,09±0,48	0,05±0,08	P<0,001
C22:0 behenic	0,02±0,03	0,01±0,02	NS
Saturated	54,05±3,85	37,75±3,79	P<0,001
Monounsaturated	22,45±2,80	40,11±6,08	P<0,001
Polyunsaturated	23,50±3,04	22,14±3,95	NS
Branched	1,08±0,31	0,11±0,18	P<0,001
ω3	1,92±0,76	0,15±0,22	P<0,001
ω6	21,12±2,77	20,17±3,81	NS
P/S	0,44±0,08	0,59±0,11	P<0,001

br.: branched
P: Polyunsaturated
S: Saturated
CLA: conjugated linoleic acid
NS: no significance

MEAT PROTEIN-TANNIN INTERACTIONS: OBSERVED ANTIOXIDANT ACTIVITY AND POTENTIAL HEALTH BENEFITS

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Background

A bearberry-leaf (*Arctostaphylos uva-ursi* L. Sprengel) extract was shown to be a good source of natural antioxidants and imparted strong antioxidant activity in model and meat systems (Pegg et al., 2001; Amarowicz et al., 2004). This observed activity stems chiefly from the polyphenolic constituents present in the extract (Amarowicz and Pegg, 2004). Polyphenolic-protein interactions are a matter of continuous research due to their importance in food: nutritional effects centre on the capacity of phenolics to bind and precipitate proteins. Tannins, which are complex secondary metabolites of plants widely distributed in foods and comprised of gallic acid esters or flavan-3-ol polymers, are most commonly responsible for such interactions with proteins. Tannins can form soluble and insoluble protein complexes; the formation depends not only on the size, conformation and charge of the protein molecules, but also on the molecular weight, length and flexibility of the tannins involved (Naczk et al., 2001a). The precipitation of a protein-tannin complex results from the development of a sufficiently hydrophobic surface on the adduct due to polyphenols binding to the protein surface and cross-linking of different protein molecules with the polyphenols. The bearberry extract is rich in tannins (~10%), which are partially responsible for the observed antioxidant activity in meat systems. Until recently, tannins have been basically considered as antinutrients in foods because they decrease the nutritional value of protein; however, it begs the question, "since the tannins function as antioxidants, do the complexes so formed in meat also do the same?"

Objectives

The objectives of this study were to verify the formation of protein-tannin complexes in meat and then to assess whether or not the antioxidant activity of the native tannin persisted in the protein-tannin complex.

Materials and methods

A crude bearberry-leaf extract was prepared according to Amarowicz et al. (2004) and then dechlorophyllized according to Pegg et al. (2003) on a silicic acid column using hexanes and 95% (v/v) ethanol as mobile phases. Dried extracts were stored at 4°C until used. The cooked pork systems were prepared and TBARS of the stored refrigerated products were determined according to the methods described by Pegg et al. (2001).

Isolation of Myosin

Myosin was isolated from chilled post-rigor knuckle muscle of hogs slaughtered at Mitchell's Gourmet Foods (Saskatoon, SK) and then shipped to the University of Saskatchewan 48 h post-mortem. A flow diagram for the isolation and purification of myosin from the muscle tissue is depicted in Fig. 1. All work was carried out in a refrigerated cabinet at 4°C. The modified Hasselbach-Schneider solution comprised 0.6 *M* KCl, 10 m*M* $Na_4P_2O_7 \cdot 10H_2O$ and 1 m*M* $MgCl_2$ in 0.1 *M* KH_2PO_4/K_2HPO_4 buffer, pH 6.4.

Production of Tannin-Protein Complexes from Porcine Myosin

Fifty milligrams of tannins (*i.e.*, obtained by Sephadex LH-20 chromatography of the crude ethanolic extract of bearberry: the first mobile phase used was 95% (v/v) ethanol to remove some polyphenolics {*i.e.*, arbutin and gallic acid} followed by the second, 1:1 (v/v) acetone:water; the acetone fraction collected was called "tannins") were dissolved in 50-mL distilled water. One hundred milligrams of isolated myosin were dissolved in 100 mL of a 0.20 M acetate buffer, pH 5.0, containing 0.17 M NaCl. The two solutions were mixed and allowed to stand at room temperature for 30 min. The reaction mixture was transferred to a 250-mL polypropylene centrifuge tube, centrifuged for 10 min at -2°C at 10,000 × *g* in a Beckman J2-HC centrifuge. The supernatant was carefully decanted. Precipitate adhering to the wall of the centrifuge tube was scrapped and the product was mixed with *ca.* 20-mL distilled water and then transferred to a small 50-

mL beaker. The contents in the beaker were lyophilized. The tannin content bound to proteins was determined by a colorimetric assay described by Hagerman and Butler (1978).

Results and discussion

The bearberry-leaf extract was found to be a rich source of tannins, comprising *ca.* 10% of the crude dechlorophyllized preparation, and possessed marked antioxidant activity in model and meat systems. Previous research in our laboratory has indicated that it contains both hydrolyzable and condensed tannins. When applied to meat systems, the tannins retarded lipid oxidation of the cooked products during storage. The effect was concentration dependent as depicted in Figure 2. At a 25-ppm addition level (sample B) the tannins offered little protection against oxidation of meat lipids (*i.e.*, only 35% inhibition of lipid oxidation by day 7), but when doubled, an efficacy almost equivalent to that of the synthetic antioxidant, *tert*-butylhydroquinone (TBHQ) added at the same level, was observed (*i.e.*, > 98% inhibition). This is quite interesting considering that the tannin constituents have considerably larger molecular masses than that of TBHQ (FW=166), which on a mole basis was added to the meat system in a far greater quantity. The question is, was the free-radical scavenging capability restricted to the free tannins in the meat meatrix or were those bound to protein in the form of protein-tannin complexes also capable of exhibiting antioxidant activity?

Tannins are notorious as protein precipitants and thereby decrease the nutritional value of food proteins. It is generally assumed that the resultant complex has limited or no value; however, is this really so? Tannins from bearberry-leaf extract were found to be strong precipitants of model proteins such as bovine serum albumin and fetuin (Naczk et al., 2001b); however, we wanted to investigate their interaction with meat proteins. As myosin is the dominant protein in muscle tissue, it was isolated from fresh meat used for the TBARS studies. Electrophoretic separation of the protein from the resultant product by SDS-PAGE confirmed that the isolated protein was indeed myosin of good purity, when comparisons of the separated bands were made to commercially-available myosin. Addition of tannins to the isolated myosin dissolved in a dilute salt solution resulted in protein precipitation. The precipitate was recovered, dried and confirmed to be a complex of protein and tannin by Hagerman and Butler's colorimetric assay. It was suspected that the myosin-tannin complex so formed might have biological value as an antioxidant. When added to meat systems, which were then thermal processed, cooled and stored under refrigeration conditions for a period, TBARS data indicated that the myosin-tannin complexes imparted protection to the meat against lipid oxidation. Again, the efficacy was concentration dependent as depicted in Figure 2. At a 200-ppm addition level (sample D), the complex exhibited weak inhibition of lipid oxidation; however, when greater addition levels were incorporated in the system (*i.e.*, 500 and 750 ppm; samples E and F, respectively), an antioxidant efficacy equivalent to that of the free tannins added at 50 ppm was observed. As the myosin-tannin complex is free of tannin residues, the antioxidant activity observed in the cooked meat systems is due to either the complex itself or the release of tannin constituents therefrom. Because tannins can have strong covalent bonding interactions with protein, the latter theory seems to be more likely. The fact that protein-tannin complexes can impart a beneficial biological activity to a food system tends to negate the historical view that the protein-tannin precipitation products only reduce the nutritional value of food. This study demonstrates that the complex formed could be of a more significant value, as protein-tannin complexes consumed in food products may provide persistent antioxidant activity in the gastrointestinal tract against free-radical species.

Conclusions

The tannin constituents of the bearberry-leaf extract offered marked antioxidant activity to meat systems. Their interaction with meat proteins, such as myosin, did not result in a loss of the observed antioxidant activity. In fact, spectral data from TBARS analyses of cooked pork systems indicated that the isolated protein-tannin complexes added to meat systems survive thermal processing and give stability to meat lipids against oxidation. Thus, tannins bound to proteins may provide a sink for persistent antioxidant activity.

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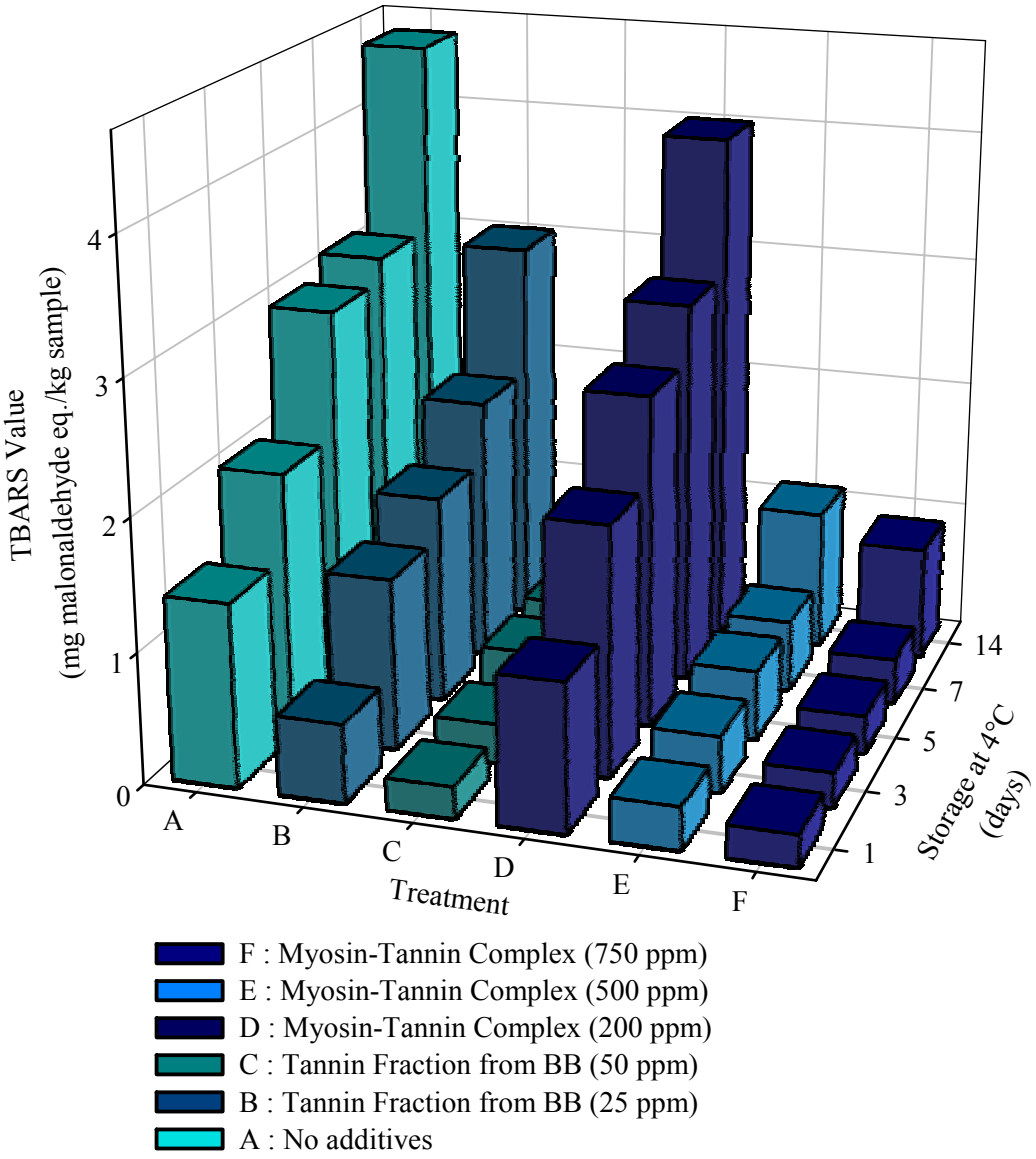

Fig. 2. TBARS values of cooked pork systems as affected by polyphenolics from the bearberry-leaf (BB) extract and a myosin-tannin complex.

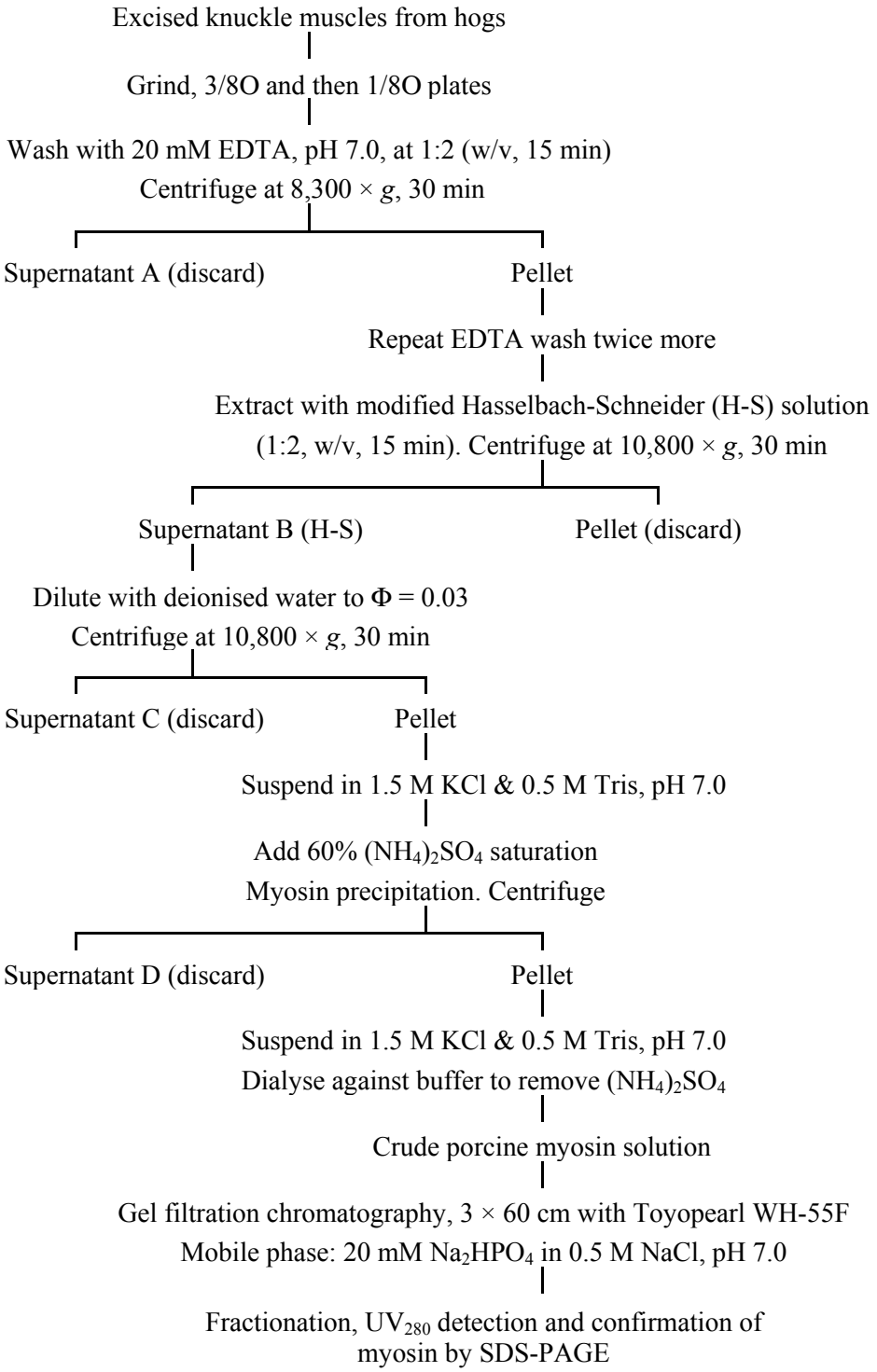

Figure 1. Flow diagram for the isolation and purification of myosin from porcine knuckle muscles.

SUCKLING LAMB FATTY ACIDS AS AFFECTED BY EWES FEEDING SYSTEM

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Background

Milk produced by ewes grazing grass has a different fatty acid composition than milk from ewes given concentrates (Banni *et al*. 1996). In particular, milk from grazing ewes has lower levels of saturated fatty acids (SFA) and higher levels of fatty acids thought to be more beneficial to human health such as polyunsaturated fatty acids (PUFA) and conjugated linoleic acid (CLA) (Banni *et al*. 1996). In suckling ruminants, milk bypasses reticulorumen and is digested directly in the abomasum. Therefore, also milk fat escapes ruminal biohydrogenation. The effect of production system, and in particular of grass feeding, on the intramuscular fatty acids of suckling lamb (raised with their dams at pasture) has been studied (Velasco *et al*., 2001; 2004). However, literature lacks reports on the effect of the production system of ewes (pasture *vs*. stall feeding) on the intramuscular fatty acids of lambs fed exclusively maternal milk.

Objectives

The objective of this trial was to study the fatty acid composition of intramuscular fat in lambs fed exclusively milk from ewes consuming pasture or concentrates.

Materials and methods

Twenty pregnant Comisana ewes were selected about 30 days before lambing and divided into two groups of ten animals. One group of ewes was allowed to graze a vetch pasture (grass) from 07:00 to 18:00 every day. The second group of animals was penned and given hay and concentrate (stall). After lambing, all ewes were allowed to stay with the respective lambs between 18:00 and 07:00 of the following day in two different group pens. The lambs were kept in these pens for the whole trial, and fed exclusively maternal milk. Every ewe gave milk to only one lamb. In case of twin lambing, one was removed.

The lambs were slaughtered at 38 days of age. Twenty-four hours after slaughter samples of *longissimus thoracis* muscle were taken at the level of the 13th thoracic rib, minced, vacuum-packed (50 g for each animal), and stored at –25°C until analysed (10 days on average). Intramuscular fat was extracted, from a 4 g ground meat sample, according to Folch *et al*. (1957) as described by French *et al*. (2000). Fatty acids were quantified as fatty acid methyl esters prepared by acid-catalysed methanolysis (French *et al*. 2000). Separation of fatty acid methyl esters was performed with a capillary column SP-2380 in fused silica (60 m x 0.25 mm i.d.; 0.20 µm film thickness) using He as a carrier gas. The gas-chromatograph used was a Thermo Finnigan, TRACE with the software ChromQuest (Thermo Finnigan, San Jose, CA, USA). The conditions were the following: initial temperature 140°C; initial isotherm 1 min; temperature increasing, 1°C per min up to 165°C with an isotherm of 1 min, then increasing 6°C per min to the final temperature of 225°C; final isotherm 8 min; carrier He, injector temperature 230°C; detector (F.I.D.) temperature, 250°C; injector mode split, flow carrier on column constant (1.2 ml/min). The different fatty acids were identified by the retention time with reference to fatty acid standards. The fatty acid standards were purchased from Larodan Fine Chemicals AB (Malmö, Sweden). Standard mix was prepared in our laboratory, and palmitic acid was used as the reference fatty acid (response factor = 1.00). Fatty acids are expressed as percentage (w/w). Data were analysed by t-test to compare the two maternal feeding regimes (grass *vs*. stall).

Results and discussion

Mean carcass weight of the lambs was unaffected by the feeding treatment of the ewes, and was on average 9.3 kg. Intramuscular fatty acid proportions are reported in Table 1. Palmitic acid (16:0) was more abundant ($P < 0.05$) in the fat from lambs raised by ewes consuming concentrates. At the moment of writing this report, total fatty acid composition of ewe milk has not been finished. However, in a recent trial Scinardo

Tenghi (2003) found that ewes allowed to graze a natural pasture exclusively, showed lower ($P < 0.001$) levels of palmitic acid in their milk compared to ewes given hay and concentrate. Our result on the meat is in accordance with the report of Velasco *et al*. (2001). Trans-vaccenic acid (18:1 11trans) was more abundant ($P = 0.01$) in the fat of the grass-fed lambs. It is well known that milk from cows fed pasture has higher levels of this fatty acid compared to milk from animals given concentrates (Jahreis *et al*., 1997). Linoleic acid (18:2 cis *n*-6) was more abundant ($P < 0.0005$) in the intramuscular fat from lambs raised by ewes given concentrates. In milk, Scinardo Tenghi (2003) reports a concentration of linoleic acid in concentrate-fed ewes being thrice the amount of that in milk of grazing ewes. Linolenic acid (18:3 *n*-3), however, was three times more abundant ($P < 0.0005$) in the fat of the lambs of the grazing ewes compared to the offspring of the stall group. It is well known that grass contains high proportions of linolenic acid (Aurousseau *et al*., 2004). However, in the lambs of the grass group, we found a proportion of this fatty acid much higher than that reported so far in the literature for light lambs (Velasco *et al*. 2001, 2004). On the other hand, compared to lambs of the stalled ewes, conjugated linoleic acid (9cis, 11trans isomer; CLA) was present at about double concentration in the fat of the offspring of the grazing ewes ($P < 0.0005$). This isomer of conjugated linoleic acid in the ruminant tissue may be derived mot only from milk CLA but also from trans-vaccenic acid through the action of Δ^9 desaturase (Barber *et al*., 2000). Indeed, in milk from ewes grazing pasture, both CLA and trans-vaccenic seem to be at higher concentration compared to milk from ewes given concentrates (Jahreis *et al*., 1999). Two long chain *n*-3 fatty acids (EPA; 20:5 and DHA; 22:6) were more abundant (respectively $P < 0.0005$ and $P = 0.01$) in the intramuscular fat of lambs from grass group compared to stall group. These fatty acids are derivates of linolenic acid, and diets rich in this fatty acid result in an increased level of EPA and DHA in meat (Raes *et al*., 2004). Since the *n*-3 fatty acids were more abundant ($P < 0.0005$) and the *n*-6 fatty acids less abundant ($P < 0.01$) in lambs of the grass group compared to the stall group, the former produced a lower ($P < 0.0005$), and therefore more favourable (Wood and Enser, 1997) *n*-6/*n*-3 ratio in their meat than the latter. In both groups of lambs, however, this ratio was below 4.0, which is the recommended minimum for a human diet as a whole (Enser *et al*., 1998).

Conclusions

Intramuscular fatty acid composition of lamb fed exclusively maternal milk was highly affected by the feeding regime of the ewes. In particular, meat from lambs fed by ewes grazing grass showed higher levels of trans-vaccenic acid (18:1 *trans*11), CLA, linolenic acid and its derivates (EPA and DHA). Meat of lambs of the concentrate-fed ewes was higher in linoleic as well as other *n*-6 fatty acids. The *n*-6 to *n*-3 ratio was lower, and therefore more favourable in the meat of lambs of the grazing ewes.

Acknowledgments

This trial was founded by Regione Siciliana – Assessorato Agricoltura e Foreste – Progetto 6/F Assistenza Tecnica in zootecnia.

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Table 1. The intramuscular fatty acid composition of lambs as affected by the diet of the ewe.

	Treatment			
Fatty acid	Grass	Stall	SEM	*P*-value
12:0	0.26	0.33	0.030	0.260
14:0	2.32	2.96	0.247	0.199
14:1	0.17	0.25	0.023	0.078
16:0	12.71	15.50	0.659	**0.030**
16:1	0.76	0.87	0.068	0.434
18:0	8.15	9.52	0.292	**0.014**
18:1 9tr	0.10	0.02	0.026	0.137
18:1 11tr	1.71	1.15	0.119	**0.014**
18:1 cis *n*-9	16.54	16.89	0.556	0.762
18:2 tr	1.67	0.79	0.182	**0.011**
18:2 *n*-6	8.28	12.88	0.632	**<0.0005**
18:3 *n*-6 (γ-linolenic)	0.57	0.89	0.045	**<0.0005**
18:3 cis *n*-3	13.23	4.24	1.080	**<0.0005**
18:2 cis-9, trans-11(CLA)	1.35	0.62	0.103	**<0.0005**
18:2 trans-10, cis-12	0.04	0.03	0.006	0.463
20:2 *n*-6	0.22	0.34	0.024	**0.010**
20:3 *n*-3	0.13	0.15	0.027	0.830
20:4 *n*-6	11.89	19.90	2.010	**0.042**
20:5 *n*-3	7.36	3.39	0.510	**<0.0005**
22:5 *n*-3	6.86	5.11	0.318	**0.003**
22:6 *n*-3	5.67	4.17	0.308	**0.010**
n-3 Fatty acids	26.26	11.81	1.790	**<0.0005**
n-6 Fatty acids	22.06	33.91	2.130	**0.003**
n-6:*n*-3 ratio	0.85	3.07	0.305	**<0.0005**

EFFECT OF A LINSEED OIL SUPPLEMENTATION ON TOTAL FATTY ACIDS OF MUSCLES AND ON COLOUR STABILITY AND LIPID OXIDATION OF BOVINE MEAT

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Background

Recent decline of beef consumption in European countries is mainly due to problems with food safety (e.g. BSE) and the health aspect of eating red meat. Enhancing the polyunsaturated fatty acid, especially n-3 PUFA, and decreasing the saturated fatty acid (SFA) content of meat improve the nutritional value and increase the attractiveness of meat to consumers. In beef, it has been previously described that the PUFA/SFA and n-6/n-3 ratio can be modulated by dietary manipulation with oil. For ruminants, pasture, grass silage, whole linseed and linseed oil are the most important sources of n-3 fatty acid but the dietary PUFA must escape biohydrogenation in the rumen (Raes et al., 2004). In bovine, increasing PUFA of the diet can also increase the production of conjugated linoleic acid (CLA) (Enser et al; 1999 ; Bauchart et al; 2002) which may have health benefits to man. If nutritional value of meat can be improved by increasing dietary PUFA level, its sensorial quality must remain acceptable to consumers. It has been established that animal diet can affect beef colour stability and lipid oxidation during display life (Gatellier et al., in press)) and after cooking.

Objectives

The aim of this study was to assess the effect of increasing dietary PUFA level, especially n-3 PUFA, with linseed added to the diet or linseed oil directly infused in the duodenum, on intramuscular total fatty acids composition and on oxidative stability of bovine meat.

Materials and methods

Experiment was carried out with 12 crossbred Charolais x Salers 15 months old steers. Three diets were given for 70 days: a *control (C)* diet consisting in hay (45%) and concentrate feed (55%); a *linseed* (*L*) diet consisting of the same control diet supplemented with 4% of lipids provided by extruded linseed; a *linseed oil* (*O*) diet consisting of the control diet supplemented with 4% of lipids provided by linseed oil continuously infused into the proximal duodenum in the aim to bypass rumen hydrogenation. Animals were slaughtered in the INRA abattoir and, at 24 h *p.m*., muscle *Semi tendinosous* was removed. Meat portions were placed on a fibre board tray, overwrapped with an oxygen permeable film and exposed in darkness for a maximum of 13 days at 4°C. Total lipids in muscles have been extracted according to the method of Folch et al. (1957). Their fatty acids were converted into methyl esters by transmethylation using borotrifluorure at 14% in methanol according to the method of Sébédio et al; (1999) for their analysis by gas-liquid chromatography using CP Sil 88 glass capillary column. Colour measurement was determined with a spectrophotometer equipped with an integrating sphere (2°-viewing angle, illuminant D65). Colour coordinates were calculated in the CIELAB (1976) system. The results were expressed as lightness (L*), redness (a*) and yellowness (b*). Meat discoloration was determined by difference R_{630}-R_{580} (Renerre, 2000). Lipid oxidation was measured by the TBA-RS method according to Mercier et al. (1998). All values are reported as the mean +/- standard deviation for each animal group. The unpaired Student *t*-test was used to test differences between each group.

Results and discussion

	muscle fatty acids						
Treatments	**Diet C**		**Diet L**		**Diet O**		Significance
	LSM	SEM	LSM	SEM	LSM	SEM	(P≤ 0.05)
Fatty acids (%)							
C16:0	**24.7**	2.8	**23.6**	1.6	**24.4**	3.0	0.6754
C18:0	**17.8**	2.1	**16.2**	3.7	**15.0**	4.3	0.2832
C18:1*cis*9	**31.0**[ab]	3.6	**33.8**[a]	4.5	**27.7**[b]	3.7	**0.0198**
C18:2 *n*-6	**6.3**[a]	1.6	**4.8**[a]	1.6	**8.5**[b]	2.1	**0.0019**
C18:3 *n*-3	**1.0**[a]	0.1	**1.5**[a]	0.5	**8.7**[b]	1.5	**0.0001**
CLA *cis9, tr11*	**0.4**[a]	0.1	**0.7**[b]	0.1	**0.4**[a]	0.1	**0.0001**
Sum SFA[a]	**46.7**	4.1	**44.2**	3.3	**43.6**	5.0	0.3260
PUFA n-6/PUFA n-3 ratio	**2.980**[b]	0.579	**2.581**[b]	0.534	**1.035**[a]	0.168	**0.0001**
P/S ratio[b]	**0.270**[a]	0.087	**0.250**[a]	0.070	**0.495**[b]	0.144	**0.0001**

Table1: Effects of diets on total fatty acids % in muscle

As shown in table 1, compared to the control diet (diet C), addition of extruded linseed (diet L) induced a large increase of the content of CLA, especially of the isomer 9c,11t (P<0.0001). No difference was noted in the major SFA (C16:0 and C18: 0) between treatments. However, the impact of the lipid supplement on fatty acids content of muscle was more marked with diet O, leading to a large incorporation of 18:3n-3 (P<0.0001) and to a large increase in the P/S ratio (P<0.0001) up to the recommended value for the health of consumers. Moreover, diet O decreased the ratio of PUFA n-6 /PUFA n-3 (P< 0.0001) but, with diet L compared to diet C, no significant difference was noted in C18:3n-3 such as noted in the literature (Choi et al., 2000) but quantity and nature of extruded linseed are different between experiments.

About colour characteristics measurements, a slight increase of luminosity L* during the 13 days of meat storage was observed (figure not showed) but with no significant differences between treatments. Figure 1 showed a good stability of a* values until 3 days. From 7 to 13 days, the decrease of redness was more important in O group than in C and groups. The decrease of a* was slightly higher in L group compared to C one. At the end of storage, redness was approximatly two fold higher in C and L groups compared to O group with only a significant difference between C and O group. This indicated a greater myoglobin oxidation rate in meat of animals from O group while feeding animals with extruded linseed (L group) gave meat with myoglobin oxidation identical toC group. It was not showed a significant variation of b* during meat storage and no differences were measured between animals groups (not showed). Some authors (Vatansever et al., 2000; Wood et al., 2003) showed that linseed supplementation slightly decreased colour saturation $(a^2 + b^2)^{1/2}$ of bovine meat comparatively to controls.

Variation of meat discoloration (figure 2) during a refrigerated storage, showed that decrease of R_{630}-R_{580} was more important in O group than in two other animal groups. C and L groups showed similar decrease of R_{630}-R_{580} with values slightly higher (NS) in C group. After 9 days of air storage, mean values of R_{630}-R_{580} in O group were below the limit value of 12.5 which corresponds to 50% acceptability for the consumers (Renerre, 2000). This limit was reached after 10 days in L group and only after 13 days in C group. These results showed the highest reduction of retail shelf life of meat from animals receiving PUFA by oil infusion in the duodenum.

Figure 3 showed an important increase of lipid oxidation measured by accumulation of TBA-RS as soon as 3 days storage. This increase was particularly noted in O group. After 7 days, TBARS values were also above 2 mg MDA/Kg meat in C and L groups and largely exceeded this cut off value, at which rancidity may be detected by consumers (Younathan and Watts, 1959). At the end of storage, TBA-RS values in O group were

approximately two fold higher than values measured in the two other groups and were very high (> 8mg MDA/kg meat) After 7, 9 and 10 days storage, *t*-test showed significant differences between O and L groups. Moreover, lipid oxidation was more important in C than in L group but differences were not significant. This paradoxal effect could be attributed to antioxidants present in linseed grain which could offer better protection against lipid oxidation in membranes. In steers, for Vatansever et al. (1998), linseed (+/- fish oil) showed only small increase in TBA-RS when compared with control. In charolais cattle, it was observed lower TBA-RS values in meat from animals fed pasture, even if PUFA content was higher in meat of these animals (Gatellier et al., in press), compared to mixed-diet.

Conclusions

Compared to controls, including extruded linseed in the finishing diet of steers significantly increase the content of CLA, but not the C18:3n-3 content; but extruded linseed don't change significantly colour stability and lipid oxidation in meat after a refrigerated storage. Bypassing rumen hydrogenation by direct infusion of linseed oil in the duodenum had a significant effect on PUFAn-6/PUFAn-3 and P/S ratios; moreover, infusion of linseed oil increase myoglobin and lipid oxidation. Lipid oxidation induced by linseed oil infusion could be decreased by the use of antioxidants. Further investigations on the relation of PUFA increase and nutritional antioxidant status on beef qualities would be of interest as it was done on other animal species such as turkey (Mercier et al., 1998).

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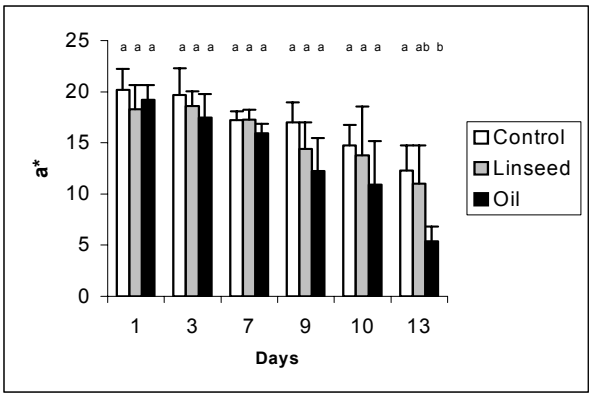

Figure 1: variation of redness a*

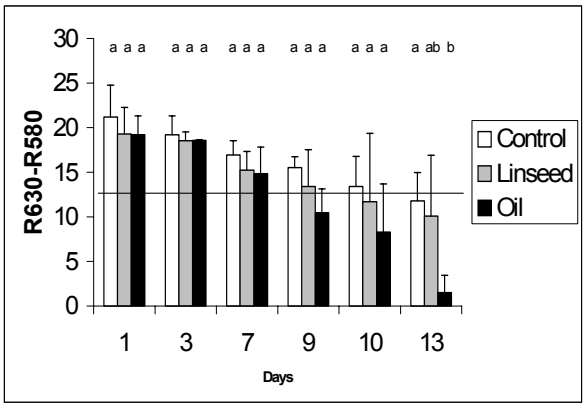

Figure 2: variation of R_{630}-R_{580}

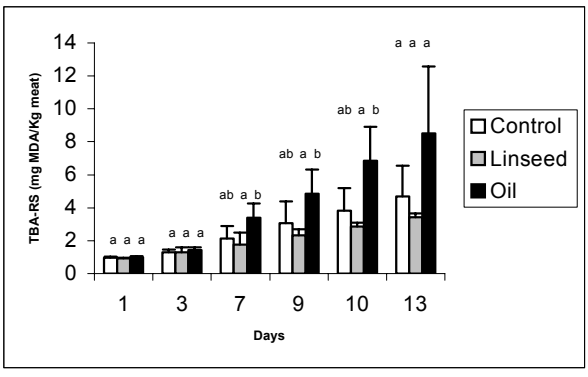

Figure 3: variation of TBARS

EFFECT OF BREED ON MEAT QUALITY AND FATTY ACID COMPOSITION OF KOREAN NATIVE BLACK PIGS, LANDRACE AND YORKSHIRE

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Background

The eating quality and fatty acid composition are known to influence nutritional, technological and sensory qualities of both fresh and processed meat products as important factors that affect the consumer's choice.. Although it is generally accepted that an increased level of the intramuscular fat (IMF) has a positive influence on the sensory qualities of pork (Fernandez et al., 1999), the variation in fatty acid composition also affected firmness of fat, shelf life, and flavor. As fatty acid composition was changed for diet, genetics, sex or fatness, the ratio of the certain fatty acids such as C18:0 and C18:2 provided the best prediction of firmness with the highest correlation with firmness measured subjectively or objectively(Wood et al., 1978). Campo et al.(2003) found that C18:3 produces more intense odors in a model system. In this point, manipulating fatty acids in pork will ultimately influence not only the economics of meat processing, but also the acceptance by the consumer. There were clear effect of breed type on the concentrations of total fatty acids in muscle has been reported. Wood et al. (2004) reported that the *longissimus* and *psoas* muscles of Duroc and Berkshire purebred pigs contained higher concentrations of both neutral lipids and phospholipids than those of Large Whites and Tamworth. Meat of Korean Native Black Pigs (KNBP), on the other hand, has been characterized as redder and higher palatability than that from other commercial breeds especially for Korean consumers. This finding, however, for lack of information, was substantiated by conducting this study.

Objectives

The objective of this experiment was to determine the effect of breed on meat quality of loin muscle and fatty acid compositions.

Materials and methods

Animals : A total of thirty male pigs (10 KNBP, 10 Landrace, and 10 Yorkshire) were sampled from the National Livestock Research Institute's (NLRI) breeding program. KNBP weighed an average of 72 kg; Landrace, 118 kg; and Yorkshire, 118 kg; with ages of 192 days, 201days, and 194 days; respectively. All pigs were transported to the National Livestock Research Institute and conventionally slaughtered for two consecutive days with an electronic stunner (230 volts for 2.5 sec). The carcasses were placed in a 1℃ chiller until the following day and submitted to standard processing procedures and divided into retail cuts. Loin muscle and subcutaneous fat layer samples were separated from each carcass and analyzed for meat quality and fatty acid composition.

Analytical methods: Chemical compositions were analyzed by using methods of Association of Official Analytical Chemists (AOAC) (1996). Water-holding capacity(WHC) was measured by using the method of Ryoichi et al.(1993). WB-shear force(WBS) was measured on cooked steaks(25-mm thick) according to the method described by Wheeler et al.(2000). Color values on freshly cut surface of the WBS block were measured by a chroma meter (Minolta Co. CR 301) for lightness (L), redness (a) and yellowness (b) of CIE after a 30-min blooming at 1℃. Cooking loss was calculated as a percent for the weight changes during cooking for WBS measurement. Total lipids were extracted by using chloroform-methanol (2:1, v/v) according to the procedure of Folch et al.(1957). An aliquot of the lipid fraction was methylated as described by Morrison and Smith(1964). Fatty acid methylesters were analyzed by a gas chromatograph (Varian 3400) fitted with a fused silica capillary column, Omegawax (205, 30 m × 0.32 mm I.D., 0.25 film thickness). The injection port was at 250℃ and the detector was maintained at 260℃. Nitrogen was used as the carrier gas. Results were expressed as percentages, based on the total peak area.

Statistical analysis: Data were analyzed by using the SAS program (1996) and means were separated by the Student-Newman-Keuls' test. To determine the breed effect on samples, data were analyzed as one-factor randomized block experiments with treatments. The level of significance was $p<0.05$.

Results and discussion

Objective meat quality KNBP was significantly higher in fat contents and lower in moisture content than those of the other two breeds (Table 1). Protein and ash contents, however, were not significantly different among three breeds. The loin muscle of KNBP had significantly higher *a* values(redness) than those of the other two breeds. The *L* values(lightness) of KNBP were lower than those of Yorkshire, and the *b* values(yellowness) were higher than those of Landrace($p<0.05$). Consumer perference and choice was believed to depend on the interaction of the objective qualities of the product with expectation of consumers and the consumer prefers pork with a high intensity of pink (Brewer et al., 1998). The loin muscle of the Hampshire breed, according to the studies of Lindahl et al.(2001), has been reportd to have redder and yellower color than the loin muscle of the Swedish Landrace and the Swedish Yorkshire breeds. These differences in color were related to the lower pH and higher glycogen level of Hampshire, resulting in higher internal reflectance and higher pigment content. In this study, postmortem pH_{24hr} values were not significantly different for three breeds (data were not shown). Variations in pigment content between pork breeds have been reported (von Lengerken et al., 1985), but no data that compare the pigment content of the pork from KNBP, Landrace, and Yorkshire were found. Among these three breeds, data on WHC and cooking loss were not significantly different($p>0.05$). However, the Warner Bratzler shear force values of KNBP and Landrace were significantly lower than those of Yorkshire ($p<0.05$)

Fatty acid compositions There were significantly higher levels of C18:1n-9, C16:0, C18:0, 18:2n-6, C16:1n-7, and C14:0 in decreasing order for the intramuscular fat and the subcutaneous fat of three breeds. KNBP had significantly higher levels of C18:2n-6, C18:3n-3 in the intramuscular fat, and C18:2n-6, C18:3n-6, C20:4n-6 in the subcutaneous fat than those of the other breeds. The higher concentrations of C18:2n-6 and C18:3n-3 in Duroc were associated with high fat deposition in muscle adipose cells relative to the other breeds such as Berkshire, Large white, and Tamworth(Wood et al., 2004). Significantly higher levels of C16:1n-7, C20:1n-9, C20:4n-6, and C20:5n-3 in the intramuscular fat and C16:1n-7 and C20:5n-3 in the subcutaneous fat were observed in Landrace and Yorkshire than in KNBP($p<0.05$). However, total SFAs and total MUFAs in the intramuscular fat and subcutaneous fat did not significantly differ among the three breeds (Table 2).

On the other hand, total PUFAs were significantly higher in the intramuscular fat as well as in the subcutaneous fat of KNBP than those of the other two breeds (Fig. 1). Higher PUFA level in the backfat of the three breeds possibly led to a softer fat(Warnants et al., 1999). The ratio of C18:0 to C18:2 was found to provide the best firmness(Whittington, 1986). There were no significant differences in the concentrations of C18:0 among the three breeds($p>0.05$). Total contents of PUFA n-3 were not significantly different among three breeds, but the total contents of PUFA n-6 of KNBP were significantly higher than those of the other two breeds. The ratio of PUFA to SFA (P:S) of KNBP was 0.47, and it is significantly higher than that of Landrace(0.32) and Yorkshire(0.33)($p<0.05$) (Fig. 2). The recommended ratio of polyunsaturated fatty acids(PUFA) to saturated fatty acids(P:S) should be increased to above 0.4(Wood et al., 2003). Since health practitioners have correlated meat with an imbalanced fatty acid intake among the consumers of today, there must be ways to improve the P:S ratio during meat production. In addition, researchers have also reported that the ratio of n-6:n-3 PUFA is a risk factor in cancers and coronary heart disease, especially in the formation of blood clots leading to heart attacks (Enser, 2001). The recommendation, therefore, is for a ratio of n-6: n-3 PUFA less than 4, but that in pork is usually higher than this value. In this study, pork from three breeds had significantly higher ratio of n-6 to n-3 due to their relatively low concentration of n-3 PUFA. Therefore, pork can be manipulated towards a more favorable n-6:n-3 ratio as with the.P:S ratio. Saturated and monounsaturated fatty acids are synthesized in vivo and less readily influenced by diet than the polyunsaturated fatty acids, C18:2n-6 and C18:3n-3, which cannot be synthesized, but can be manipulated by dietary change (Enser et al., 2000). Pork of KNBP, which grew slow and deposited more fat in muscle when compared with other breeds, contained significantly higher PUFA n-6 than that of Landrace and Yorkshire under the same feeding program and environmental rearing condition. Therefore, the feeding program needs

to be established to increase the levels of the PUFA n-3 such as C18:3n-3 for a more balanced and potentially beneficial composition of fatty acid for different breeds.

Conclusions

Breed influenced growth rate and affected the fatty acid composition of intramuscular fat and subcutaneous fat. High levels of polyunsaturated fatty acids, especially for C18:2n-6 and C18:3n-3, were found in KNBP. High levels of monounsaturated fatty acids, especially for C16:1n-7, C20:1n-9, C20:4n-6, and C20:5n-3, were observed in Landrace and Yorkshire. Meat is a major source of fat in the human diet and there is interest in modifying the composition of meat by dietary means to improve the nutritional value. Fat and fatty acids are important because of their effects on human health; thus, it is important to select production options which maximize both meat quality and healthiness in meat production. To improve the nutritive value of pork, manipulation of the fatty acid composition by using dietary means is necessary in the light of today's consumer's health. Thus, it is essential that researchers try to increase the proportion of unsaturated fatty acids in the meat. This can be done by establishing a feeding program that can increase levels of the PUFA n-3. Grass feeding or high levels of C18:3n-3 supplementation, in particular, can be done to put in place a more balanced and potentially beneficial fatty acid composition of different breeds. Our findings in this study showed a significantly higher level of polyunsaturated fatty acids in KNBP compared with that of Landrace and Yorkshire. Thus, further studies on fatty acid metabolism for different breeds must be investigated.

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Table 1. Quality characteristics of Korean Native Black Pigs(KNBP), Landrace and Yorkshire.

Breed	Proximate Composition (%)				CIE			CL[2] (%)	WBS[3]	WHC[4]
	Protein	Fat	Moisture	ash	L	a	b			
KNBP[1]	22.71 (0.29)	4.38 (0.72)^{a}	73.16 (0.51)^{b}	1.03 (0.02)	46.04 (0.91)^{b}	10.96 (0.65)^{a}	4.73 (0.46)^{a}	26.22 (0.83)	6.56 (0.46)^{b}	55.54 (0.95)
L	23.29 (0.20)	1.56 (0.29)^{b}	74.82 (0.17)^{a}	1.05 (0.01)	47.89 (0.85)^{ab}	6.95 (0.37)^{b}	2.83 (0.34)^{b}	26.01 (1.03)	8.92 (0.41)^{a}	54.76 (0.80)
Y	23.40 (0.22)	1.88 (0.19)^{a}	75.33 (0.22)^{a}	1.05 (0.01)	50.00 (1.51)^{a}	7.26 (0.50)^{b}	3.85 (0.47)^{ab}	25.52 (1.57)	7.15 (0.39)^{b}	53.96 (0.71)

[1]KNBP-Korean Native Black Pigs, L-Landrace, Y-Yorkshire; [2]CL - cooking loss; [3]WBS Warner Bratlzer shear force; [4]WHC - water holding capacity; $^{a-b}$ Means with a same superscript within a row are not significantly different ($p<0.05$)

Table 2. Comparison of fatty acid profiles for Korean Native Black Pigs(KNBP), Landrace and Yorkshire

	Intramuscular fat			Subcutaneous fat		
	KNBP	Landrace	Yorkshire	KNBP	Landrace	Yorkshire
C14:0	1.11(0.04)	1.27(0.01)	1.22(0.07)	1.10(0.05)	1.18(0.04)	1.20(0.09)
C16:0	24.78(0.26)	25.46(0.18)	24.89(1.26)	21.48(2.15)	24.80(0.42)	25.12(0.73)
C18:0	15.43(0.29)	15.95(0.43)	15.76(0.81)	14.97(0.69)	15.94(0.25)	15.64(0.81)
C16:1 *n*-7	2.00(0.09)^{b}	2.39(0.14)^{a}	2.71(0.26)^{a}	1.78(0.06)^{b}	2.04(0.06)^{a}	2.24(0.21)^{a}
C18:1 *n*-7	0.06(0.01)	0.09(0.00)	0.07(0.03)	0.04(0.01)	0.04(0.01)	0.04(0.01)
C18:1 *n*-9	40.11(0.81)	42.03(1.25)	43.15(1.40)	40.41(1.12)	40.52(0.24)	39.58(0.60)
C18:2 *n*-6	14.78(0.60)^{a}	11.19(1.06)^{b}	10.41(0.88)^{b}	17.29(0.64)^{a}	12.95(0.61)^{b}	13.59(0.68)^{b}
C18:3 *n*-3	0.67(0.08)^{a}	0.24(0.02)^{b}	0.24(0.02)^{b}	0.83(0.08)	1.00(0.04)	1.02(0.09)
C18:3 *n*-6	0.51(0.12)	0.23(0.01)	0.20(0.02)	0.94(0.03)^{a}	0.51(0.03)^{b}	0.53(0.02)^{b}
C20:1 *n*-9	0.03(0.00)^{b}	0.34(0.13)^{a}	0.36(0.08)^{a}	0.73(0.05)	0.65(0.03)	0.66(0.04)
C20:4 *n*-6	0.21(0.02)^{b}	0.49(0.05)^{a}	0.67(0.14)^{a}	0.14(0.01)^{a}	0.09(0.00)^{b}	0.10(0.01)^{b}
C20:5 *n*-3	0.06(0.00)^{b}	0.11(0.02)^{a}	0.11(0.01)^{a}	0.03(0.01)^{b}	0.07(0.01)^{a}	0.08(0.01)^{a}
C22:4 *n*-6	0.25(0.01)	0.21(0.03)	0.20(0.02)	0.26(0.02)	0.21(0.03)	0.20(0.03)
n-3	0.72(0.08)^{a}	0.35(0.03)^{b}	0.35(0.02)^{b}	0.87(0.08)^{a}	1.07(0.04)^{b}	1.10(0.10)^{b}
n-6	15.76(0.65)^{a}	12.12(1.11)^{b}	11.48(0.90)^{b}	8.63(0.67)	13.76(0.65)	14.41(0.70)

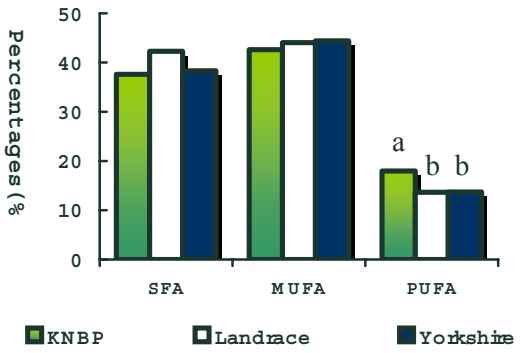

Fig. 1. Comparison of fatty acid contents by breed

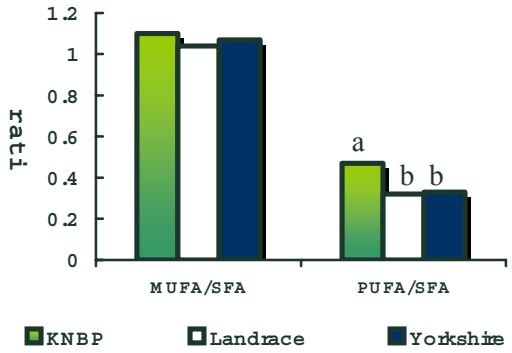

Fig. 2. Comparison of the ratio of M: S and P: S by breed

*SFA=sum of the saturated fatty acids, MUFA=sum of the monounsaturated fatty acids, PUFA=sum of the polyunsaturated fatty acids, n-3=sum of the n-3 fatty acids and n-6=sum of the the n-6 fatty acids

$^{a-b}$ Means with a same superscript within a row are not significantly different ($p<0.05$)

EFFECT OF HIGH PRESSURE TREATMENT ON THE ALLERGENICITY OF BOVINE SERUM ALBUMIN EVALUATED BY HISTAMINE RELEASE ASSAY USING SERA FROM ALLERGIC PATIENTS AND HUMAN BASOPHILIC KU812F CELLS

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Background

In our previous study (Han *et al*., 2000), we indicated bovine serum albumin (BSA) played important role in the allergenicity of beef. Various food-processing techniques have been applied to foods in order to eliminate their allergenic proteins or to reduce levels. The effects of heat treatment on food allergenic proteins have been widely studied by many groups. Heat treatment reduced the sensitization of beef, even if the treatment was less effective on pure BSA under domestic conditions (Werefel *et al*., 1997; Fiocchi *et al*., 1998). On the contrary, in some cases, heat treatment showed negative results. Restani *et al*. (1998) reported that the heat treatment was not able to decrease the BSA capability to bind to immunoglobulin E (IgE).
High pressure treatment has recently been considered a useful food processing techniques and the efficiency of this treatment on meat has been reported by several research groups including that of Suzuki (Suzuki *et al*., 1998). However, to date studies on the effects of high pressure treatment on food allergenicity have not been done, except our previous report (Han *et al*., 2002)

Objectives

Basophils, as well as mast cells, play an important role in the induction of allergic inflammatory responses via release of inflammatory mediators. The purpose of this study is to evaluate the effect of high pressure treatment on the elimination of BSA allergenicity on the basis of histamine release from human basophilic KU812F cells sensitized with sera from allergic patients.

Materials and methods

Sera: Sera from food allergic patients were obtained from Yoshida Hospital (Niigata) for this study.
Heat or pepsin treatment: Heat or pepsin treatment of BSAwas done in captube at 100ºC for 10min and at 37ºC for 60 min, respectively.
High pressure treatment: High pressure treatment of BSA was carried by the procedure of Homma et al. (1994). The sample sealed in a polyethylene bag was pressurized under 100, 200, 300, 400, 500, and 600 MPa at 5~7ºC for 10min using NBIP (Nikkiso Isostatic Processor).
Cells and cell culture: Human basophilic KU812F cells were obtained from the Cell Resource Center for Biomedical Research, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan) and were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS). The cells were cultured at 37ºC in humidified atmosphere with 5% CO_2.
Histamine release: Briefly, in order to bind IgE to high affinity IgE receptor FcεRI on the surface of KU812F cells, the cells (1.0×10^6 cells/ml) were incubated with the sera (diluted 1:100) exhibiting IgE binding activity to BSA in RPMI-1640 medium at 37ºC for 60 min. To evaluate the IgE binding activity of sera, enzyme linked immunosorbent assay (ELISA) was performed according to the method of Han *et al*. (2000). Then the cells were washed twice and resuspended in Tyrode's buffer. Histamine release was measured after stimulation of serum-sensitized cells with BSA with or without pepsin, heat or high pressure treatment at 37ºC for 60 min. After centrifugation, the amount of histamine in the supernatant was measured by means of a fluorometric assay according to the method of Shore *et al*. (1959) with slight modification. Histamine release was expressed as the percentage of the total amount of histamine; spontaneous release, occurring in the absence of any stimulus, was subtracted from all values.

Results and discussion

KU812F cells are known as human basophilic cells, which produce histamine and express high affinity IgE receptor FcεRI. The cells have a potential to release histamine upon stimulation with antigen and its specific IgE. Prior to evaluate the effect of high pressure treatment on the allergenicity of BSA, we established histamine release assay system using KU812F cells in combination with sera from allergic patients. IgE binding activity to BSA of sera used in this study was evaluated by ELISA (Table I). After sensitization of KU812F cells with sera, histamine release from the cells following addition of BSA was investigated. IgE molecules on the cell surface were confirmed by flow cytometric analysis (data not shown). Intact BSA induced histamine release from the cells sensitized with A5 serum exhibiting high IgE binding activity to BSA in a dose dependent manner (Fig, 1).

It seems that cross-linking of IgE specific to BSA on the cell surface followed by aggregation of FcεRI results in histamine release. On the contrary, no significant histamine release by BSA was observed in the cells sensitized with A4 serum exhibiting low IgE binding activity to BSA. Although we cannot eliminate a possibility that IgG specific to BSA may participate in cell activation through IgG receptor FcγRI, our data suggests that this histamine release assay system could be available to detect histamine release by BSA via its specific antibody. Using this histamine release assay system, we evaluated the effects of BSA treated with pepsin, heat and high pressure on the histamine release.

Our previous study revealed that high pressure treatments of beef extract did not show any significant changes in binding with sera from beef allergic patients (Han *et al*., 2002). In addition, SDS-PAGE and immunoblot analysis revealed that BSA treated with high pressure (600 MPa) or heat (100□) is still able to bind serum IgE (unpublished data). Nevertheless, BSA pressurized at ranging from 300 to 600 MPa reduced histamine release from the cells sensitized with A5 serum with significance (Fig.2). The reducing effect of high-pressure treatment gradually increased with the increase of high pressure applied to BSA. In order to show that the result as mentioned above is not a specific case, the histamine release from the KU812F cells sensitized with 6 individual patient sera was examined here. Simultaneously, the effects of heat and pepsin treatments on the histamine release were evaluated. Almost the same results were observed in this study using A5, A17, A45, A77, A125 and P2 serum (Fig. 3). Intact BSA induced about 32 to 42% histamine release, while the BSA pressurized at 600 MPa or heated at 100□ reduced the histamine release to about 6 to 14% and 5 to 19%, respectively. When the BSA treated with pepsin (1000:1 w/w), the histamine release was higher than that of intact BSA. Even though the fragmentation of BSA was confirmed on the SDS-PAGE (data not shown), the structure around the epitope recognized by anti-BSA IgE from sera seems to be retained and facilitate histamine release more easily. It is necessary to pay an attention that there are some cases where the allergenicity is more intensified by the proteolytic enzyme treatment than that of the untreated.

To our knowledge, this is the first study to report that high pressure treatment can decrease allergenicity of food components on the basis of mediator release from basophils or mast cells. Thus, food-processing by high pressure, as well as heat, raises the possibility that the technique might eliminate allergenicity of food components and contribute to attenuation of allergic responses via food allergen specific IgE. Even if high-pressure treatment fails to decrease BSA capability to bind serum IgE, pressurized BSA may undergo some changes to repress the mediator release from mast cells and basophils. Now, we are studying in detail the effect of high pressure treatment on the structure of BSA molecules responsible for this reducing effect on histamine release.

Conclusions

BSA (bovine serum albumin) is the major allergen in beef-allergic patients. SDS-PAGE and immunoblot analysis revealed that high pressure (600 MPa) treatment failed to decrease BSA capability to bind serum IgE. Nevertheless, the pressurized BSA ranging from 300 to 600 MPa lowered the percentage of histamine release from human basophilic KU812F cells sensitized with sera exhibiting high immunoglobulin E (IgE) binding activity to BSA, Under undesirable condition, enzymatic digestion of BSA by pepsin might intensify histamine release more than intact BSA. Besides industrial heat processing but domestic cooking heated nonuniformly, high-pressure treatment could also be effective food-processing technique to reduce the allergenicity of beef.

Acknowledgment

This work was supported by Grant-in-Aid for Scientific Research (A-15208025) from Japan Society for the Promotion of Science (JSPS).

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Table 1. IgE binding activity to BSA of sera from food allergic patients evaluated by ELISA tests.

Allergic Patient's serum	ELISA test (O.D. 405 nm)	IgE binding activity to BSA
A4	0.213	low
A5	1.257	high
A17	1.040	high
A45	1.660	high
A77	1.050	high
A125	1.147	high
P2	1.632	high

ELISA was performed according to the method of Han *et al*. (2000) previously described. Values are expressed as means of two independent experiments.

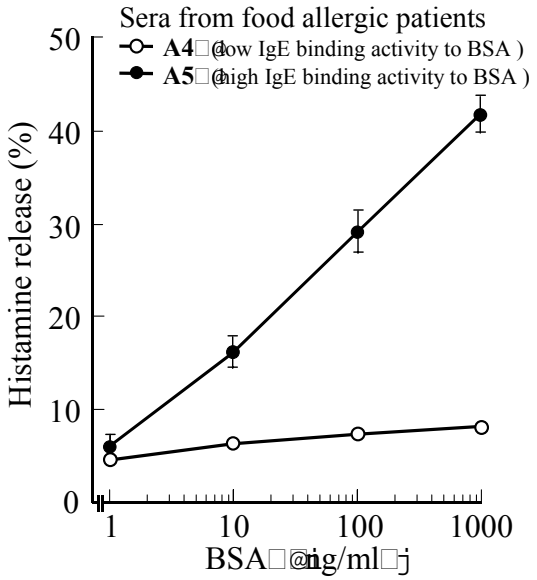

Fig. 1. Effect of BSA on histamine release from KU812F cells sensitized with sera from food allergic patients. Histamine was measured by means of a fluorometric assay. Data are expressed as means ± SE (n = 4).

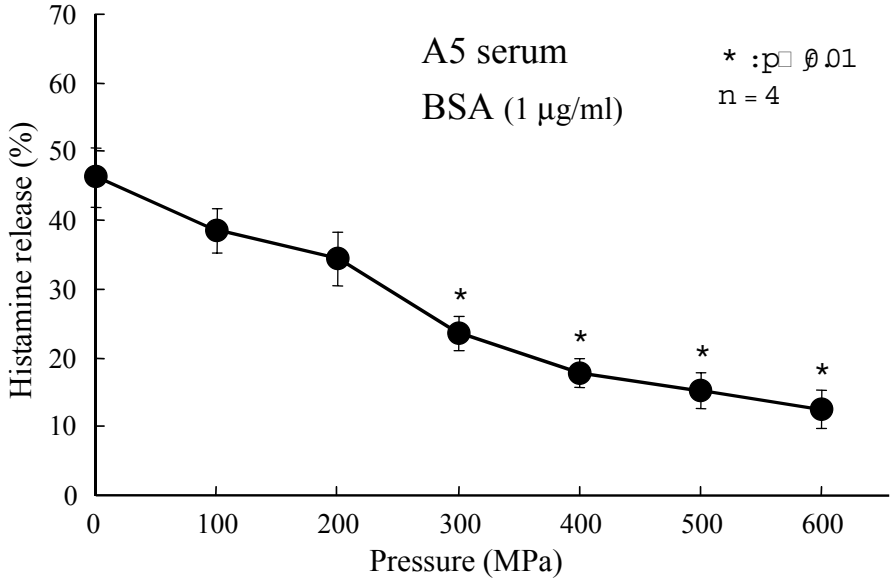

Fig. 2. Effect of BSA treated with high-pressure on histamine release from the KU812F cells sensitized with sera from allergic patients. Cells were sensitized with sera exhibiting high IgE binding activity to BSA (A5), and stimulated by adding 1 μg/ml of BSA pressurized at ranging from 100 to 600 MPa. Histamine released from the cells was measured by means of a fluorometric assay. Data are expressed as means ± SE (n = 4).

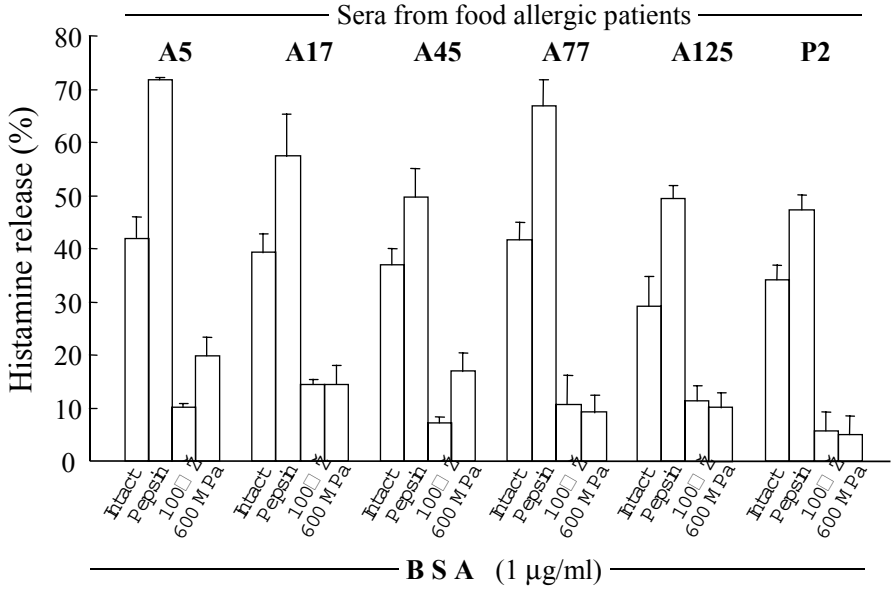

Fig. 3. Effect of digested (pepsin), heated (100 ℃) and pressurized (600 MPa) BSA on histamine release from the KU812F cells sensitized with sera from allergic patients. Cells were sensitized with sera exhibiting high IgE binding activity to BSA (A5, A17, A45, A77, A125, P2), and stimulated by adding 1 μg/ml of processed or intact BSA. Histamine released from the cells was measured by means of a fluorometric assay. Data are expressed as means ± SE (n = 4).

PHYSICO-CHEMICAL CHARACTERISTICS OF RAW AND CANNED OSTRICH MEAT

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Background

Ostrich meat is a relatively new product in the market, and breeding has gained popularity in recent years in many countries. The consumer wants to be aware of their food nutrient composition, since some components may pose a risk factor in coronary heart diseases. Ostrich meat has a relative high pH (5.9) (Sales, 1996) and low intramuscular fat content (1.6%), this fat content was lower compared with beef (4.5%) and turkey (3.8%) (Paleari *et al*., 1998). This interesting characteristic is of particular interest due to the fatty composition of fat. The ostrich meat is low in mono-unsaturated fatty acid but rich in polyunsaturated fatty acids (PUFA) (Paleari *et al*., 1998), confirming the nutritional characteristics of this meat. However, polyunsaturated oils, including the omega 3 fats, are extremely susceptible to damage from heat, light, and oxygen. When exposed to these elements for too long, the fatty acids in the oil become oxidized.

Objectives

The aim of this work was to: (1) prepare a canned product containing ostrich meat; (2) determine food fatty acid composition in raw meat as well as the canned product; and (3) determine the effects of sterilization temperatures on stability of fatty acids in the canned product.

Materials and methods

Vacuum packaged ostrich meat samples from African Black (*Struthio camelus var. domesticus*) corresponding to *M. iliofibularis* and *M. iliotibialis lateralis* were used. The muscles were obtained from a commercial abattoir. Both muscles were cut into about 2.5 cm^3 pieces, mixed together with salt, and precooked in a kettle for 15 min at 60 °C and then for 5 min at 100 °C., when redness disappeared. After preparation, precooked meat was placed in metal containers. In order to measure the temperature TYPE T (copper-constantan) thermocouple was fastened in the geometric center of the container. A Model 692-000 and Design 5-thermocouple-channel data logger (Barnant) were used to collect and record data. The product was sterilizated at 250°F, Z = 18 until reaching a Fo = 9. Sterilization was repeated three times. Nine samples of raw and processed ostrich meat were analyzed in triplicate to measure the following parameters: pH, moisture, ash, protein and fat (AOAC, 2002). Lipid oxidation was determined by measuring thiobarbaturic acid values (TBA) (Pfalzgraf *et al*., 1995) and conjugated dienes (Sirinivasan *et al*., 1996). After extraction of lipids according to Bligh y Dyer (1959), an aliquot of the lipid fraction was transmethylated as described by Park and Goins (1994) using boron-trifluoride in methanol and the produced fatty acid methyl esters were determined on a Hewlett Packard 6890 gas chromatograph equipped with an automatic sample injector and flame ionization detector. Fatty acids were identified by comparing retention times with those of fatty acid methyl ester standards. Results were statistically analyzed by ANOVA test using SPSS for windows version 10.0.6 (1999).

Results and discussion

Means for pH, proximate chemical analysis, TBA, conjugated dienes, and fatty acid composition are presented in Table 1. The pH and proximate composition showed no significant difference ($P< 0.05$) between raw and canned ostrich meat. Values of moisture, fat, protein, and ash are in agreement with those reported by Paleari *et al*. (1998), and Sales (1996), in raw meat. Lipid oxidation indicators (TBA and conjugated dienes) showed that the canning procedure had a significant effect on their values; an increase ($P< 0.05$) in these parameters was observed due to high temperature process. Many factors affect lipid peroxidation, heat

disrupts muscle cell structure, inactivates enzymes and releases oxygen from oxymyoglobin; the release of oxygen from oxymyoglobin produces H_2O_2 and this reaction is increased at 60 °C (Harel and Kanner, 1985). The effect of sterilization on the fatty acids is shown in table 1. Significant differences ($p< 0.05$) were found for saturated and unsaturated fatty acids between raw and canned process. The profile analyzed of fatty acids in this study was similar to that observed by Sales et al (1996) for cooked ostrich meat. Total saturated fatty acids composition was similar in raw and canned meat in this study (32.13 and 32.28%, respectively). According to Armstrong and Bergan (1992) saturated fatty acids are less susceptible to oxidation than unsaturated fatty acids. Total unsaturated fatty acids in canned ostrich meat were also similar than in raw meat. The tendency of temperature influencing some fatty acids was also observed by some researchers like Anderson et al. (1971) and Smith et al. (1989) on beef meat cooked in conventional heating, observed that excessive heating produced cellular tissue break down and permitted lixiviation of hydro soluble nutrients and fatty acids auto oxidation. Unsaturated fatty acids constituted 67.72% of the total fatty acids in the raw and 67.55% canned meat, respectively. Omega-3 fatty acids, especially eicosapentaenoic acid (EPA, 20:5n-3), docosahexanoic acid (DHA, 22:6n-3) and linolenic (18:3n-3) fatty acids had low values in canned meat, mainly linolenic acid. These particular fatty acids (EPA, DHA and linolenic) are of great interest concerning human cardiovascular health.

Conclusions

It can be concluded from this study that canning procedure caused changes in fatty acid composition, principally linolenic (18:3n-3) fatty acid, while the other parameters studied were relatively constant. However, there is evidence that the variation in percentage fatty acids might differ by the diet and the process used.

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Table 1. Average values for pH, proximate analysis, TBA, conjugated dienes and fatty acid composition parameters of raw and canned ostrich meat (Mean ± SD).

Parameters	Raw (n=9)	Canned (n=9)
pH	6.76 ± 0.1	6.72 ± 0.1
Moisture (g/100 g)	75.9 ± 0.3	75.1 ± 1.7
Protein (g/100 g)	21.6 ± 0.7	21.6 ± 1.7
Fat (g/100 g)	1.5 ± 0.1	1.3 ± 0.2
Ash (g/100 g)	1.7 ± 0.2	1.5 ± 0.4
TBA (mg MA/kg)	0.3^{b} ± 0.03	0.7^{a} ± 0.01
Conjugated dienes (µmol/mg)	7.0^{b} ± 2.1	19.9^{a} ± 3.7
Fatty acids (% of total fatty acids)		
C14:0	0.72^{a} ± 0.04	0.66^{b} ± 0.08
C16:0	23.4^{a} ± 0.6	22.7^{b} ± 1.7
C16:1n-7	6.2^{a} ± 0.3	6.0^{b} ± 0.6
C18:0	8.1^{b} ± 0.6	8.9^{a} ± 0.8
C18:1n-9	34.1^{b} ± 0.8	34.7^{a} ± 3.0
C18:2n-6	16.8^{b} ± 1.0	17.4^{a} ± 1.0
C18:3n-6	1.4^{a} ± 0.2	1.1^{b} ± 0.1
C18:3n-3	0.19^{a} ± 0.03	0.04^{b} ± 0.03
C20:1n-9	0.21^{a} ± 0.02	0.13^{b} ± 0.06
C20:4n-6	6.6^{a} ± 1.1	6.2^{b} ± 0.6
C20:5n-3	0.56 ± 0.2	0.55 ± 0.1
C22:4n-6	0.4^{a} ± 0.1	0.3^{b} ± 0.1
C22:6n-3	1.3^{a} ± 0.2	1.1^{b} ± 0.2
Total saturated	32.1	32.3
Total unsaturated	67.7	67.6
Saturated/unsaturated	0.47	0.48

Values in rows with different superscripts differ significantly ($p<0.05$)

PIG HOUSING AFFECTS THE FATTY ACID COMPOSITION OF PORK FAT

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Background

There has been a rapid uptake of low-cost deep litter housing systems for intensive pig production in Australia over the past decade. This housing system provides a low capital investment alternative to intensive pig housing systems, where pigs are raised in insulated buildings, in small groups of 8 to 15 pigs per pen on fully or partially slatted concrete floors. In deep litter systems, pigs are raised in large groups of 100 or more on bedding, such as straw, in canvas covered, naturally ventilated structures. Studies investigating the impact of rearing pigs outdoors in paddocks versus indoors in conventional housing have shown differences in pig growth, carcass quality and pork quality (Nilzen *et al*. 2001; Gentry *et al.* 2002; Gentry *et al.* 2004). Lambooij *et al*. (2004) investigated the effects of housing conditions on pork quality characteristics, and concluded that the differences in pork quality can be substantial when the differences in housing conditions are large. Hence we would expect that the inherent differences between conventional and deep litter housing systems may similarly affect carcass quality and eating quality.

The fatty acid composition in various depots such as subcutaneous, intermuscular and intramuscular fat affects the technological aspects of meat quality. Differing concentrations of fatty acids have been shown to influence the firmness of the fat which has implications for the appearance and cutting of fresh and processed pork (Tume and D'Souza, 1999). Fatty acid composition can also influence fat colour, with fat of a higher melting point appearing whiter than fat with a lower melting point (Wood *et al.* 2003). The consumption of polyunsaturated fatty acids has been found to benefit human health, but increased levels of polyunsaturated fat in meat have detrimental effects on product shelf life as these fatty acids tend to oxidise rapidly and therefore rancidity and colour deterioration is accelerated (Wood et al 2003).

A number of factors can affect the fatty acid composition of various fat depots in pigs. Generally as the age of the pig increases, the fat becomes more saturated (Cameron *et al.* 1990). The rate of fat deposition can also affect the concentration of polyunsaturated fatty acids within the tissue, where the concentration generally increases when the rate of fat deposition slows (Bee *et al* 2004; Rehfeldt *et al* 1994). Unlike ruminants the fatty acid composition of the tissue of pigs is largely a reflection of the fatty acid pattern of the diet (Wiseman and Augunbiade, 1998), particularly in regards to the essential fatty acids such as linoleic and linolenic acid which are preferentially deposited within tissue and can only be obtained through the diet. Ambient temperature also influences fatty acid composition with pigs raised in cooler environments tending to have higher levels of unsaturated fatty acids and softer fat compared to pigs raised in warm environments (Tume and D'Souza, 1999).

Objectives

The aim of this study was to quantify the effect of housing systems currently used in Australia on the fatty acid composition of subcutaneous backfat in the growing pig.

Materials and methods

The experimental design is a 2 x 2 factorial with two housing types, conventional versus deep litter, and two slaughter ages, 13 weeks (about 50 kg liveweight) and 24 weeks (about 110 kg liveweight).

One hundred and fifty two Large White x Landrace female pigs were obtained at weaning from a high health status commercial piggery at 3 weeks of age. Pigs were stratified by weaning weight into two housing treatments, conventional or deep litter and within each treatment pigs were allocated to a predetermined slaughter date based on age. Eight pigs from each housing treatment were slaughtered at 13 and 24 weeks of

age. In the conventional housing treatment pigs were housed in 8 groups of 9 pigs per pen. Each group was randomly allocated to 8 pens in a conventional weaner facility until 9 weeks of age and then randomly allocated to 8 pens in a conventional grower/finisher facility until slaughter. In the deep litter housing treatment eighty pigs were housed together in one large group. At 9 weeks of age the piglets were moved from the deep litter weaner facility to a deep litter grower finisher facility.

The conventional weaner facility was within an insulated thermostatically controlled building. Pens had a mesh floor in the drinking and dunging area and a solid concrete lying area which was heated. Each pen was 1.2 x 3.1 m and equipped with 4 nipple drinkers and a multiple space feeder. The conventional grower/finisher facility was within an insulated shed and pigs were housed in concrete pens with a solid concrete lying area and a slatted area for dunging. Pens were 2.4 x 3.1 m and equipped with two nipple drinkers and one single spaced feeder per pen.

The weaner deep litter facility was within an open ended canvas covered hoop structure 9 x 14.4 m. The shelter had a concrete feeding and drinking platform with the remainder of the shelter bedded with wheat straw. Extra straw bales were placed with the shelter to provide added protection from the weather. The deep litter housing system was quipped with two multiple spaced feeders and 6 bowl drinkers. The grower finisher deep litter facility was an open ended canvas covered hoop structure that had been divided down the length into two pens. The experimental pigs were housed in one pen (4.5 x 22 m) and at one end of the shelter was a feeding and drinking platform equipped with a multiple spaced feeder and four bowl drinkers.

The pigs were phase fed commercial, cereal based diets *ad libitum* as per industry practice. They had *ad libitum* access to fresh water. Individual liveweights and feed supplied per pen were recorded weekly.

The pigs were transported to a commercial abattoir (one and a half hours travel time) and slaughtered within one hour of arrival. Within 40 minutes post slaughter fat was collected from the hot carcasses. Subcutaneous backfat samples were taken (15g from the dorsal midline in line with the last rib) and stored in two 5 ml polypropylene containers on dry ice. The samples were transported to the lab in dry ice and stored at -80^{o}c until the fatty acid profiles were determined.

Fatty acid profiles were be determined by extracting the lipid from the tissue samples (Bligh and Dyer, 1959). Fatty acid methyl esters prepared and the fatty acid composition was determined via gas chromatography following the AOAC Official Methods of Analysis (1981).

Data were analysed by using Genstat 2002 (Lawes Agricultual Trust, Rothamsted Experimental research Station: Rothamsted, UK) to conduct a two-way analysis of variance.

Results and discussion

There were no significant differences ($P>0.05$) in liveweight, hot standard carcass weight and subcutaneous backfat thickness at the P2 site between housing treatments within slaughter groups (Table 1.)

The fatty acid profiles of pigs slaughtered at 13 and 24 weeks of age are described in Table 2. The profiles indicate that as pigs age, the level of saturated fatty acids within subcutaneous backfat significantly increases ($P<0.001$), whilst the level of polyunsaturated fatty acids, 18:2 and 18:3 was significantly reduced ($P<0.001$). Subsequently the overall proportion of saturated to unsaturated fatty acids increased with age ($P<0.001$). This is in agreement with numerous studies (Cameron *et al.* 1990; review by Tume and D'Souza, 1999) who reported that an increase in weight of the pig was also accompanied by increased fat firmness through an associated increase in saturated fatty acids. Linoleic (18:2) and linolenic acids (18:3) are essential fatty acids and cannot be synthesised by the pig, therefore these fatty acids must be sourced from the diet (Wiseman and Agunbiade, 1998). The percentage of monounsaturates 16:1 and 18:1 did not change over time.

Pigs housed on deep litter had significantly higher percentages of 14:0 (myristic) ($P=0.04$), 16:1 (palmitoleic) ($P=0.01$) and a lower percentage of 18:0 (stearic) ($P=0.05$) in subcutaneous backfat compared to pigs housed in conventional systems. Myristic and palmitoleic acids have been positively associated with firmer fat (Piedrafita et al. 2001). Palmitoleic acid has also been found to be positively correlated to pork

flavour (Cameron *et al*. 1990; Cameron *et al.* 2000; Kimata *et al*. 2001) while stearic acid has been negatively associated with pork flavour attributes (Kimata *et al*. 2001). Hence the higher percentage in myristic and palmitoleic acids in reported in this experiment, may indicate that pork from pigs raised in deep litter housing systems may have firmer fat compared to pork from pigs raised in conventional housing systems. This may also improve the cutability of both fresh and processed pork, and enhance shelf life via a reduced rate of oxidative rancidity. The higher palmitoleic and lower stearic acid percentage in pigs raised in deep litter housing systems may also result in improved flavour, as palmitoleic acid is positively correlated with flavour while stearic acid is negatively correltated with flavour, compared to pigs raised in conventional housing systems. In contrast, Bee *et al*. (2004) reported that pigs raised outdoors only had a lower percentage of stearic acid (18:0) compared to pigs raise indoors. Although there were differences in the levels of individual fatty acids reported in this experiment, there was no significant difference in the total proportion of saturated and unsaturated fatty acids ($P>0.05$) (Table 3).

Conclusions

The results from this experiment indicate that pigs housed on deep litter had significantly higher levels of myristic (associated with improved fat firmness) and palmitoleic acid (associated with improved fat firmness and flavour), and a significantly lower level of stearic acid (associated with reduced flavour) in subcutaneous backfat compared to pigs housed in conventional systems. Therefore raising pigs in deep litter housing systems may have a positive effect on both carcass quality and eating quality compared to pork from pigs raised in conventional housing systems.

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Table 1. Liveweight, carcass weight and P2 backfat of pigs housed in conventional or deep litter housing systems and slaughtered at 13 and 24 weeks of age.

Age	13 weeks				24 weeks			
Housing type	Conventional	Deep litter	lsd	P-value	Conventional	Deep litter	lsd	P-value
Live weight (kg)	47.2	44.8	7.12	0.495	120.1	109.0	16.29	0.165
Hcwt (kg)	30.1	28.2	5.31	0.461	81.8	80.2	11.66	0.752
P2 (mm)	8.63	8.75	1.50	0.861	17.5	16.87	4.32	0.761

Table 2. Selected fatty acids indicated as a percentage of total fatty acids within the subcutaneous backfat of pigs slaughtered at 13 and 24 weeks of age

	Age			
	13 weeks	24 weeks	lsd	P-value
14:0 (%)	1.34	1.58	0.087	<0.001
16:0 (%)	21.50	24.80	0.692	<0.001
16:1 (%)	2.52	2.70	0.325	0.272
18:0 (%)	10.85	13.00	0.979	<0.001
18:1 (%)	39.73	39.49	1.51	0.753
18:2 (%)	17.53	13.20	1.065	<0.001
18:3 (%)	1.84	1.35	0.128	<0.001
Total Saturated (%)	35.51	40.82	1.53	<0.001
Total Monounsaturated (%)	43.72	43.53	1.63	0.816
Total Polyunsaturated (%)	20.78	15.65	1.23	<0.001
Saturated:unsaturated	0.552	0.692	0.041	<0.001

Table 3. Selected fatty acids indicated as a percentage of total fatty acids in the backfat and belly fat of pigs raised in conventional or deep litter housing systems

	Housing			
	Conventional	Deep Litter	lsd	P-value
14:0 (%)	1.543	1.615	0.069	0.042
16:0 (%)	23.89	23.91	0.501	0.951
16:1 (%)	2.809	3.12	0.239	0.012
18:0 (%)	11.91	11.28	0.633	0.052
18:1 (%)	40.02	39.63	1.090	0.472
18:2 (%)	14.19	14.68	0.837	0.246
18:3 (%)	1.49	1.58	0.100	0.064
Saturated (%)	38.98	38.40	1.041	0.270
Monounsaturated (%)	44.22	44.11	1.200	0.863
Polyunsaturated (%)	16.8	17.48	0.982	0.169
Saturated:unsaturated	0.64	0.63	0.028	0.396

BULL PRODUCTION FACTORS AFFECTING PROXIMATE AND MINERAL COMPOSITION OF COOKED *LONGISSIMUS* STEAKS

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Background

Beef is one of the most preferred foods by Venezuelans. Its chemical composition has been well characterized in foreign countries where research (Byers *et al*., 1988) has indicated that intrinsic (gender, species, etc.) and extrinsic factors (plane of nutrition, growth regulation, castration, etc.) are largely responsible for the variation found in beef nutrient composition. Nevertheless, information regarding the proximate and mineral composition of beef produced under tropical conditions is scarce. It is well known that recommended dietary allowances (RDA) are referred to nutrients supplied by the food once it has been processed or cooked. According to Ramos Galvan (1995) this aspect has not been taken into account when designing food composition tables, which express the content of a specific nutrient per 100 g of net weight without indicating the edible portion and/or the refuse of the food. In order to diminish the risk of transmissible diseases by consumption of contaminated meats, thoroughly cooking has been recommended. Over-cooking might produce protein losses, especially from those zones more exposed to heat (Maynard *et al*. 1981). On the other hand, significant losses on mineral content might occur due to leaking or dilution, depending on cooking procedures (Ramos Galván, 1985). Beef cattle producers of Venezuela are constantly searching for new management alternatives to increase cattle productivity and to avoid the traditional need for beef imports (Morón *et al.*, 1999). Bull production offers the advantages of a faster and more efficient growth with a higher yield of lean beef as compared to steers (Huerta and Ríos, 1993). Strategic supplementation has been recommended to complement nutritional deficits of grasses, and to reach elevated production indices and yield from grazing herds (Rowe, 1999). Likewise, the use of anabolic implants allows for accelerating growth rate and improving the production efficiency of grass fed beef (Araujo *et al.* 1991). Efforts to improve beef cattle productivity through better genetics, nutrition and growth regulation must now take into consideration marketplace trends for leaner and more nutritious foods.

Objectives

The purpose of this study was to evaluate the effects of anabolic implant regimes and a supplementation strategy of grass-fed bulls on proximate and mineral composition of cooked beef *longissimus* steaks

Materials and methods

Animals

Seventy-seven bulls representing seven breed-types (Brahman, Angus, Romo Sinuano, Senepol, Simmental, commercial Zebu crossbred and ¾ Bos taurus) were raised in a ranch (Hato Santa Luisa) located at the Western Llanos of Venezuela, under the same pre- and post-weaning conditions including a common antiparasitic treatment at 90 days of age, vaccination program and supplementation with a mineral mixture. The fattening trial was conducted when the dry season started. The zone corresponds to a tropical dry forest with an annual temperature that varies from 22 to 29°C. This savannah area presents a hydric deficit during the rainy season (May-October). The precipitation averages 1,400 mm/year, and most (60%) of it occurs during June to August.

Implant regimes and strategic supplementation

Implant regimes were as follows: 1 = Ralgro™ (72 mg) administered to bulls at 0 d on fattening with reimplantation at d 90 (**RAL-RAL**); 2 = Combined strategy, consisting of Revalor™ administered to animals at 0 d on fattening followed by reimplantation with 72 mg of Ralgro™ on d 90 (**RAL-REV**). Bullocks were

randomly allotted to one of the two following treatments: a) Mineral supplementation *ad libitum* that served as a control diet; and b) Strategic supplementation *ad libitum*, consisting of an adjustment ration of 10% of feather flour, 77.9% of rice flour, 5% of molasses, 7% of minerals and 0.1% de ionophore (Salocin™), during 58 d; followed by a second ration of 49.9% of cotton seed, 28.0% of rice flour, 7.0% of minerals, 10% of feather flour, 5.0% molasses, and 0.1% ionosphere (Salocin™), which was offered during the following 114 d.

Sample collection

Animals were slaughtered ca. 500 kg liveweight. At 48 h *post-mortem* carcasses were reduced to wholesale cuts. One 2.5 cm thick steak (*longissimus dorsii*) was excised from each carcass and individually vacuum-packaged in multi-laminar plastic bags (Cryo-vac™) using a Koch-Ultravac™ packaging machine. Each sample was identified by animal number and kept frozen at -22ºC. To be prepared for chemical analyses, steaks were cooked on an electric broiler (Oster™) to reach an internal endpoint temperature of 70ºC, and trimmed to zero fat cover and other surrounding muscles. Cooked lean samples were ground for homogenization with a Black & Decker food manual processor and packaged by duplicates in hermetically sealed plastic (Zip-lock) bags and immediately stored at -20ºC until chemical analyses.

Chemical analyses

Except for total lipids (by the Folch *et al.* 1957 method), proximate analysis was performed according to the A.O.A.C. (1990). Except for phosphorus (by the A.O.A.C., 1990 method), mineral analyses were conducted by atomic absorption and/or atomic emission with ashing procedure (A.O.A.C, 1990), following the analytical methods described by Perkin-Elmer (1994).

Statistical Analyses

A completely randomized design with an unbalanced number of animals was used. Proximate and mineral composition data were subjected to an analysis of variance (ANOVA) by using the procedure PROC GLM of the Statistical Analysis System (SAS, 1996) to test differences due to supplementation and implant regime. The least squares means (LSMEANS) were separated by Tukey-Kramer´s test (SAS, 1996).

Results and discussion

Effect of implant regime and strategic supplementation on the proximate and mineral content:

ANOVA revealed a significant effect ($P<0.01$) of implant regime on the total intramuscular lipid content (Table 1). Beef derived from animals implanted with RAL-REV showed 0.4g more total lipids than those implanted with RAL-RAL. Lee *et al*. (1990), found that trembolone acetate plus 17β estradiol implanted in the stocking phase did not cause any decrease in the content of muscle lipids. Table 2 shows the effect of RAL-RAL on the content of Cu. The *longissimus* muscle of bulls implanted with RAL-RAL presented 0.01 mg less Cu than that of bulls implanted with the RAL-REV implant combination. Sodium was the only mineral affected by supplementation ($P<0.05$) (Table 3). Cooked samples from the strategic supplemented group had 2.1 mg of sodium than those samples obtained from the control group-

Effects of supplementation x implant regime on proximate and mineral composition

ANOVA revealed that beef derived from supplemented animals which had been implanted with RAL-REV had a higher protein content than those subjected to the other treatments. However, samples from animals fed with the control diet and implanted with RAL-REV showed lesser protein content than counterparts implanted with RAL-RAL. This would explain the synergistic effect of the strategic protein mineral ionophore supplementation and androgenic implant on the activation of protein anabolism and documents that the effectiveness of growth regulation regimes is regulated by the nutrition program provided.

Conclusions

The enhancement of protein accretion depends not only of the use of anabolic growth regulators, but also, it depends of nutrition of the animal, indicating the importance of an integrated nutrition-growth regulation plan in beef production.

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Table 1
Least Square means ± standard error for the nutrient content of 100g of cooked longissimus sample, according to implant regime

Component [a]	Implant regime		P value
	RAL-RAL (n = 43)	RAL-REV (n = 33)	
Protein	35.52 ± 0.16	35.88 ± 0.20	NS
Moisture	59.92 ± 0.52	59.37 ± 0.64	NS
Dry matter	40.06 ± 0.52	40.63 ± 0.63	NS
Ash	1.36 ± 0.02	1.36 ± 0.02	NS
Total lipids	3.63 ± 0.07	4.03 ± 0.07	0.0003

[a:] g/100g cooked muscle
NS: Non significant (P> 0.05)

Table 2
Least Square means ± standard error for mineral content of cooked *longissimus* sample, according to implant regime

Component[a]	Implant regime		P value
	RAL-RAL (n = 43)	RAL-REV (n = 33)	
Ca	10.29 ± 0.26	10.27 ± 0.31	NS
Mg	29.61 ± 0.30	29.76 ± 0.37	NS
Na	70.48 ± 0.57	70.11 ± 0.70	NS
K	413.45 ± 2.58	406.97 ± 3.13	NS
P	230.98 ± 1.82	233.03 ± 2.20	NS
Fe	3.05 ± 0.09	3.01 ± 0.11	NS
Cu	0.07 ± 0.003	0.08 ± 0.003	0.0003
Zn	5.80 ± 0.10	5.86 ± 0.13	NS
Mn	0.01 ± 0.0008	0.01 ± 0.001	NS

[a]mg/100g cooked muscle
NS: Non significant (P> 0.05)

Table 3
Least Square means ± standard error for the mineral content of cooked *longissimus* sample, according to supplementation

Component [a]	Treatment		P value
	Strategic Supplement (n = 27)	Control (n = 49)	
Ca	10.02 ± 0.32	10.54 ± 0.24	NS
Mg	29.85 ± 0.89	29.52 ± 0.28	NS
Na	71.34 ± 0,5	69.24 ± 07	0.02
K	409.20 ± 3.30	411.26 ± 2.37	NS
P	230.00 ± 2.32	234.08 ± 1.67	NS
Fe	3.15 ± 0.12	2.91 ± 0.08	NS
Cu	0.08 ± 0.004	0.07 ± 0.003	NS
Zn	5.92 ± 0.13	5.73 ± 0.10	NS
Mn	0.01 ± 0.001	0.01 ± 0.0008	NS

[a]mg/100g cooked muscle
NS: Non significant (P> 0.05)

Table 4
Least Square means ± standard error for the nutrient content of 100g of cooked *longissimus* sample, according to supplementation x implant regime

Component	Treatment				P Value
	Strategic Supplementation		Control		
	RAL-RAL (n = 17)	RAL-REV (n = 10)	RAL-RAL (n = 26)	RAL-REV (n = 23)	
Protein, g	35.36 ± 0.25[a/c]	36.21 ± 0.33[b/c]	35.67 ± 0.20[b/c]	35.53 ± 0.21[b/d]	0.05
Moisture, g	59.89 ± 0.81	58.64 ± 0.81	60.00 ± 0.66	60.10 ± 0.70	NS
Dry matter, g	40.12 ± 0.81	41.37 ± 1.1	40.03 ± 0.66	39.90 ± 0.70	NS
Ash, g	1.37 ± 0.02	1.37 ± 0.03	1.35 ± 0.02	1.34 ± 0.02	NS
Total lipids, g	3.61 ± 0.10	3.99 ± 0.13	3.66 ± 0.08	4.07 ± 0.09	NS

[a,b/c,d:] different letters in the same row (within the same treatment) indicate significant differences (P<0.05).
NS: Non significant (P> 0.05)

FATTY ACID COMPOSITION OF INTRAMUSCULAR LIPIDS IN VARIOUS BEEF BREEDS AND GENOTYPES

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Background

Fatty acid composition of dietary fats is of great importance in human nutrition and health. Numerous studies (Sadi et al., 1996; Turek et al., 1996; Zhang et al., 1999) have demonstrated that dietary fatty acids with different degrees of saturation exert various effects on human health. Additionally, the state of saturation of the fatty acids influences the meat flavour (Purchas et al., 1979; Wood and Enser, 1997) and the consistency of adipose tissues (Bozzolo et al., 1990; Martin et al., 1999). Increased unsaturation results in greater flavour changes in ruminants, including beef, than in pork (Melton, 1990). Breed- age- and sex-related differences in the fatty acid composition of beef cattle have been widely demonstrated (Eichhorn et al., 1986; Zembayashi et al., 1995; Huerta-Leidenz et al., 1996; Perry et al., 1998; Malau-Aduli et al., 2000; Laborde et al., 2001).

Objectives

The aim of our study was to evaluate the effects of breed and sex on growth performance, carcass characteristics and fatty acid composition attributes of the longissimus muscle in Limousin, Simmental, Red Angus, Belgian Blue x Simmental, Belgian Blue x Limousin, Simmental x Limousin growing finishing bulls and heifers.

Materials and methods

Fifty-seven (25 bulls and 32 heifers) growing finishing beef cattle were fattened. The animals were housed in free-stall barn with an open corral, fed *ad libitum* a diet consisting of maize silage, alfalfa hay (NEm=6.8; NEg=3.2 MJ/kg) concentrate mixture (NEm=4.0; NEg=2.5 MJ/kg) and corn (NEm=6.2; NEg=9.5 MJ/Kg). The proportion of the concentrate was increased from about 30 to 40 % of the DM during the fattening period. Bulls and heifers were slaughtered at age of 554 to 520 days, and weight of 617 to 543 kg, respectively. The left side of each carcass was sampled approximately 24 hours *post mortem*. Muscle samples were excised from the longissimus dorsi muscle at the 12th rib. Each sample, consisting of approximately 10 g of tissues, was stored at –20°C in a small plastic bag until the fatty acids were analyzed.

Total lipid was extracted by the method of Folch, Leeas and Sloane-Stanley (1957). The fatty acid methyl esters were separated and analysed by gas liquid chromatography according to Husvéth, Karsai and Gaal (1982), using an automated gas liquid chromatograph (Carlo Erba HRGC 5300) equipped with a dual flame ionisation detector and a packed glass column. Fatty acids were identified by comparing their retention times to those of known standard mixtures of fatty acid methyl esters quantified by a Shimadzu C-RGA integrator. The results were expressed as a weight percentage distribution of total fatty acid methyl esters. Data were initially recorded and listed as the percentages of individual fatty acids in the samples. The total saturated fatty acids (SFA) were calculated as the sum of C14:0, C16:0 and C18:0. Monounsaturated fatty acids (MUFA) comprised C16:1n-7, C18:1n-9, C18:1n-7, and C20:1n-9, whereas total polyunsaturated fatty acids (PUFA) was calculated as the sum of C18:2n-6, C18:3n-3, C18:3n-6, C20:4n-6, C20:5n-3, C22:4n-6, C22:5n-3, and C22:6n-3 fatty acids. Statistical analysis of the data was performed using SPSS 9.0 for Windows (Statistical Package for the Social Sciences, 1996).

In the first step the effects of sex and breed were examined with General Linear Model (GLM) procedure applying the following equation:

$$yij = \mu + Ai + Mj + AixMj + eij$$

(yij= being the observed value of the ith age and jth muscle type, μ = mean value common to all observations, Ai = fixed effects of sex, Mj = fixed effects of breed, AixMj = interaction between sex and breed, eij= the error term). Treatment means were compared by least significant differences (LSD).

Significant differences between the treatments are reported at $P < 0.05$. Statistical models and factors fitted for least square analysis are shown in Table 1.

Results and discussion

Intramuscular lipid content and fatty acid composition of the longissimus muscle in growing finishing bulls and heifers are shown in Table 2. Breed and sex had significant effect on MUFA and PUFA content. With respect to SFA, sex and breed effect was significant ($P<0.05$) only in case of stearic acid (C18:0). Belgian Blue x Simmental bulls had the highest, while heifers of this genotype had the lowest, C18:0 percentages. This difference suggests that sex had a high impact on stearic acid content of the muscles. Dietary intake of SFA has been attributed to elevate serum cholesterol level and increased risk of cardiovascular disease in humans (Hegsted et al., 1965). In the MFA group only the oleic acid (C18:1n-9) content of the longissimus muscle differed significantly ($P<0.001$) in all models. Limousin heifers had the highest oleic acid level in their muscles, while Belgian Blue x Limousin crossed bulls had the lowest percentage. Most of the significant differences were found in polyunsaturated fatty acids such as C18:2n-6; C18:3n-3 and C18:3n-6. From a nutritional and dietetic point of view, enhanced PUFA content of beef is advantageous as it increases flavor and decreases cardio-vascular risks, which translate to a more healthful product. Belgian Blue x Limousin bulls had the highest percentage of PUFA the lowest values being detected in Limousin bulls. The opposite results were found in SFA percentages. The longissimus muscles of Belgian Blue x Limousin crossed bulls had the lowest values of SFA the highest proportions being detected in Limousin bulls. SFA and MUFA proportions were higher in heifers, whereas PUFA content of the longissimus muscle was superior in bulls.

Conclusions

Both sex and breed had significant ($P<0.05$) effect on the intramuscular TL content and on fatty acid composition with the exception of saturated fatty acids (SFA). Belgian Blue x Limousin crossed animals had the lowest proportion of SFA and monounsaturated fatty acids (MUFA), and consequently, the highest level of polyunsaturated fatty acids (PUFA). The highest SFA and the lowest PUFA contents were found in Limousin breed.

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Table 1. Significance of factors and models

Fatty acids	Sex	Breed	Sex × Breed
Intramuscular Total Lipid	***	**	**
C14:0	**	NS	NS
C16:0	***	*	**
C16:1 *n-7*	**	NS	NS
C18:0	***	*	***
C18:1 *n-9*	***	***	***
C18:1 *n-7*	NS	NS	NS
C18:2 *n-6*	***	***	***
C18:3 *n-6*	NS	***	***
C18:3 *n-3*	***	***	***
C20:1 *n-9*	NS	NS	NS
C20:4 *n-6*	**	**	**
C20:5 *n-3*	NS	NS	NS
C22:4 *n-6*	NS	NS	NS
C22:5 *n-3*	*	*	NS
C22:6 *n-3*	NS	NS	NS
Saturated FA[1]	NS	NS	NS
Monounsaturated FA	***	***	***
Polyunsaturated FA	***	***	***

NS: not statistically significant; *: P<0.05; **: P<0.01; ***: P<0.001; FA[1]: Fatty Acids

Table 2. Intramuscular total lipid (ITL) content (g/kg wet wt) and fatty acid composition (wt%) in heifers and bulls of different genotypes

Fatty acid	Red Angus		Sim x Li		Sim	Limousin		BB. x Sim		BB. x Li	Total
	bulls	heifers	bulls	heifers	Bulls	bulls	heifers	bulls	heifers	bulls	
ITL	1.9±0.9[a]	5.9±2.3[a]	1.7±0[a]	3.7±1.7	1.8±1.3[a]	1.6±0.0	4.3±1.0	1.3±0.8	3.4±1.3	0.8±0.7	2.7±1.2
C14:0	1.7±0.5[a]	2.0±0.3	1.5±0[a]	2.0±0.6	1.6±0.3[a]	2.1±0.2	2.1±0.4	1.7±0.3[a]	2.1±0.5	1.6±0.4[a]	1.9±0.5
C16:0	18.8±2.7[a]	21.3±0.8	18.7±0[a]	21.1±2.1	19.1±1.3[a]	20.7±1.6	21.8±1.5	18.3±1.4[a]	21.1±1.7	15.7±4.1	20.2±2.3
C16:1n7	2.0±0,6[a]	2.9±0.5	2.4±0	2.9±0.6	2.1±0.5[a]	2.2±0.2[a]	2.9±0.8	1.9±0.4	2.2±1.6	1.5±0.1	2.4±0.9
C18:0	12.3±2.0[a]	10.1±0.9	10.0±0	9.3±0.6	12.4±1.6[a]	11.9±0.1[a]	10.0±1.2	12.7±3.3[a]	9.3±1.5	11.3±0.9[a]	10.8±1.9
C18:1n9	25.7±3.2[b]	30.8±2.3	27.9±0[a]	31.3±3.1	26.3±2.4[a]	27.0±0.4[a]	31.6±3.6	25.3±2.2[b]	29.8±1.3	18.0±4.8	28.4±4.1
C18:1n7	0.8±0.1	0.8±0.1[b]	1.2±0	0.9±0.2	1.0±0.1	0.8±0.1	0.9±0.2	1.0±0.2[b]	0.8±0.3	1.0±0.2	0.9±0.2
C18:2n6	6.4±1.7[a]	2.7±0.5[a]	5.8±0[a]	3.8±1.7	8.8±1.8	5.8±1.8[a]	4.3±1.8	7.0±2.7[a]	3.7±1.2	9.8±5.6	5.3±2.7
C18:3n6	25.3±5.0[a]	24.6±2.8	26.0±0[a]	22.8±1.9	18.9±1.7	23.2±1.4[a]	19.6±1.4[a]	23.9±4.1[a]	24.1±2.6[a]	31.5±0.9	23.0±3.9
C18:3n3	0.3±0.1	0.2±0	0.2±0	0.2±0.1	0.6±0.1[a]	0.2±0.1	0.2±0.1	0.3±0	0.2±0	0.5±0.3[a]	0.3±0.2
C20:1n9	0.5±0.2	0.3±0.1	0.7±0[a]	0.3±0.1	0.3±0	0.5±0.2	0.3±0.2	0.4±0.1	0.4±0.3	0.4±0.0	0.4±0.2
C20:4n6	1.3±0.4[b]	0.7±0.2[a]	1.7±0	1.1±0.6	2.1±0.7[a]	1.1±0.7	1.5±0.8[ab]	1.3±0.5[b]	1.0±0.3	1.0±0.1	1.3±0.6
C20:5n3	0.2±0	0.4±0.3[a]	n.d.	0.2±0	0.2±0.2	n.d.	0.2±0	0.1±0	0.1±0	0.3±0	0.2±0.2
C22:4n6	0.3±0.2	0.1±0[a]	n.d.	0.4±0.3	0.2±0.1	0.4±0.2	0.4±0.4	0.8±0.6[a]	0.4±0.4	0.2±0	0.4±0.3
C22:5n3	0.2±0.1	0.2±0.1	n.d.	0.2±0.1	0.4±0.2	0.3±0	0.2±0.3	0.3±0.2	0.2±0.1	0.8±0[a]	0.3±0.2
SFA	32.8±4.4	33.4±1.4	30.2±0[b]	32.4±2.7	33.2±1.9	34.6±1.7	33.9±2.0	32.7±3.0	32.5±1.8	28.6±5.5[a]	32.9±2.7
MUFA	29.0±3.3[a]	34.8±1.9	32.2±0[b]	35.4±3.4	29.6±2.6[a]	30.4±0.5[a]	35.6±3.5	28.6±2.1[a]	33.3±2.2	20.8±4.7	32.1±4.4
PUFA	33.7±6.8	28.6±2.7	33.7±0	28.2±3.8	31.1±3	29.9±0.6	25.8±3[a]	33.4±5.0	29.4±2.5	43.4±6.1[a]	30.3±5.1

Sim: Simmental; Lim: Limousin; BB.: Belgian Blue;
Within a raw and sex, means lacking common letter (a,b) differ significantly ($P<0.05$). Values are means±S.D.
SFA: saturated fatty acids
MUFA: monounsaturated fatty acids
PUFA: polyunsaturated fatty acids

THE CHEMICAL COMPOSITION OF THE MEAT TYPE DRAKES MUSCLES FROM BREEDING STRAINS

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Background

There are 5 strains of meat type ducks that have been produced in the Department of Poultry Breeding in Dworzyska and, that were recognised by Polish Ministry of Agriculture and Country Development as breeding strains. Two of them A44 and A55 have been the sire, and three of them P66, P77 and K11 have been the maternal strains. They, except K11 have been intended to broiler production – Astra K, K1 and K2. The A55 male and P66 female ducks have been the parental combination for broiler production.
The parental combinations of those strains for production of two strain crosses have been characterized by high: laying, fertilisation, and hatching as well as good vitality. However, the two strain crosses have been characterized by: very good musculature, small fatness and big feed efficiency per 1kg gain (Mazanowski 2002).
The ducks from A55 strain have been characterized by more favourable parameters such as: body weight, percentage of skin with subcutaneous fat and abdominal fat content, as well as water holding capacity than from A44 (Mazanowski and Ksiazkiewicz 2004) .
The characteristic of both P66 and P77 ducks has been alike. However, the body weight of K11 has been significantly lower than P66 and P77 (Mazanowski 2002). They are characterized by resistance to hard environment conditions, big ability to crossing and laying.
Such parameters as : body weight at 7 weeks of rearing, slaughter yield, content of breast and leg muscles, skin with fat, bones and fat in carcass, geometrical body measurements, reproductive trait, fall down of ducks during rearing were determined for all above mentioned strains (Mazanowski 2002). Additionally for sire strains chemical composition (protein, fat, moisture and ash), water holding capacity, pH 15 and pH 24 were determined (Mazanowski and Ksiazkiewicz 2004).
There are no adequate data on the chemical composition of maternal strains and composition of fatty acid, amino acid and cholesterol content in muscles of the all above mentioned breeding strains.

Objectives

The objective of the work was to evaluate and compare the chemical composition of the breast (BM) and leg (LM) ducks' muscles from A55 and P66 breeding strains.

Materials and methods

The material consisted of the breast and the leg muscles isolated from 10 male ducks 7 week old selected from population of 60 heads of each strain. The weights of birds being close to arithmetic mean of strains for male (A55-2869g and P66-2617g). Ducks were fed by standard mixture containing 19.6% crude protein and 11.96 MJ metabolizable energy (ME) to 3 weeks of rearing and 18.2% crude protein and 11.73% MJ ME from 4 to 7 weeks.

Each kind of muscle was separately ground and homogenized before analysis, 24 hours after slaughter. The analyses were made in 6 repetitions.
The analyses were carried out using the following methods:
Protein - multiplying nitrogen content with Kjeltec System 1026 by 6.25.
Fat – with Soxtec System HT2 using the petroleum benzine as the extraction solvent.
Moisture – by drying at 105^0 C.

Cholesterol – using enzymatic Human test in the extract prepared by Folch et al. (1957) procedure. The saponification was carried out by the Rhee et al. (1982) method.
Amino acid composition – by the procedure described by Skrabka-Blotnicka et al. (1997).
Fatty acids - with Agilant Techn. 6890N gas chromatograph. The methyl esters of fatty acids were separated on the CP-Sill 88 (Chrompack 100 x 0,25 mm) column, at the temperature from 165 to 200^{0}C. The temperature rose by 2^0 C /min. The helium was used as carrier gas.
The Duncan's multiple range test was used for establishing the differences between means.

Results and discussion

Comparing the chemical composition of breast and leg muscles in both investigated strains, only significant differences in cholesterol content were found. The A55 breast muscles comprised less cholesterol than P66, inversely A55 leg muscles comprised more cholesterol than P66 (table1).
The higher protein content and lower moisture and fat content in both kinds of A55 muscles was found than reported by Mazanowski and Ksiazkiewicz (2004).
The breast muscles from A55 birds comprised more : ILE, LEU, LYS, TRP, and less THR comparing to P66 (table2). However, the leg muscles from A55 comprised more PHE+ TYR, THR and less TRP than from P66.
The limited amino acid index was lower than 84% only for TRP (68-78%). The amino acids limiting the biological values of proteins established by Woloszyn (2002) for Mullard ducks were MET+CYS (22-45%) and TRP that was on the same level as in the muscles of investigated ducks.Taking into consideration the biological values of protein the A55 breast muscles appeared the most favourable, but the A55 leg muscles the least favourable.
The fatty acids from C4 to C22 were detected in all the investigated muscles.
The contents of C4 – C12 acids were lower than 0.1% of total fatty acids content. Among saturated fatty acids C16:0 was dominant (22.3% BM P66 , 19.9% LM P66 and LM A55, 21.9% BM A55), next C18:0 (from 9.1% LM P66 to 10.5% BM A55). The C18:1 fatty acids dominated among the monounsaturated (MUFA) fatty acids. It was in the lower percentage in lipids of A55 (BM 27.41% and LM 34.90%) comparing to P66 (29.41% and 36.32 % respectively). The presence of such fatty acids as C18 :2, C18:3, C20:4, C20:5, and C22:6 that belong to the essential polyunsaturated fatty acids (PUFA) has been very important. Lipids of A55 breast muscles comprised more C18:3 (1.30%), C20:5 (1.12%) and less C20:4 (7.11%) as compared to P66 (1.03%, 0.91% and 7.49% respectively).
The lipids of leg muscles from A55 comprised more C20:4 (6.63%), C20:5 (0.45%) and less C18:2 (13.4%) than P66 (6.10%, 0.38% and 14.06% respectively). The contribution of fatty acids C18:2 (14.2%) and C22:6 (3.4%) did not significantly differ in lipids of breast and leg C18:3 (1.13%), C22:6 (2.6-2.8%) muscles of both strains.
The ratios of n-6/n-3 fatty acids were very favourable (from 3.3 to 4.4) for all investigated muscles, from the human health point of view. They were close to recommended which amounted to 4 (Leskanich and Noble 1997).
Though, it was established the significant differences between contents of fatty acids in lipids influenced by the strains, they were not so large so that they were recognized as significant from the practical point of view. The contribution of saturated fatty acids and PUFA were higher and MUFA were lower in lipids of the breast than of the leg muscles regardless of strains (table3).

Conclusions

It is hard to say which investigated strain is more favourable, because the differences – though statistically significant – were small and some components were in more favourable quantities in the A55 muscles and the others in the P66 muscles.
It is evident that muscles from both examined strains have been characterized by the high nutritional value.

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Table 1. The chemical composition of ducks' muscles from A55 and P66 strains.

Component	Breast muscles					Leg muscles				
	A55		P66			A55		P66		
	x	SD	x	SD	D	x	SD	x	SD	D
Protein%	21.4	0.28	21.8	0.52	ns	20.8	0.40	20.8.	0.40	ns
Lipids%	1.3	0.09	1.3	0.16	ns	1.7	0.11	1.8	0.13	ns
Moisture%	75.9	0.53	76.1	0.54	ns	76.3	0.79	76.4	0.59	ns
Cholesterol mg/100g	71.2	0.80	82.2	2.16	*	66.8	0.31	65.2	0.79	*

Where : x – average value of 6 tests; SD – standard deviation; D- difference; ns – not significant;
*- significant difference, P< 0.05

Table 2. Essential amino acids' content in muscles of ducks'[% proteins]

Amino acid	Breast muscles					Leg muscles				
	A55		P66			A55		P66		
	x	SD	x	SD	D	x	SD	x	SD	D
PHE+TYR	8.08	0.12	7.83	0.32	ns	8.42	0.21	7.93	0.18	*
ILE	6.14	0.96	5.91	0.19	*	5.54	0.26	5.77	0.21	ns
LEU	8.45	0.19	8.13	0.11	*	8.40	0.23	8.66	0.22	ns
LYS	9.57	0.25	8.90	0.14	*	9.61	0.20	9.62	0.26	ns
MET+CYS	3.11	0.17	3.23	0.15	ns	3.42	0.18	3.30	0.18	ns
THR	4.13	0.15	5.22	0.06	*	5.66	0.26	5.29	0.09	*
TRP	0.78	0.03	0.70	0.04	*	0.68	0.02	0.74	0.03	*
VAL	7.01	0.11	6.93	0.11	ns	6.96	0.12	6.54	0.17	*

Description – as in table 1

Table 3. The contribution of different fatty acids in lipids from ducks' muscles [% lipids]

Fatty acid	Breast muscles		Leg muscles	
	A55	P66	A55	P66
Saturated	34.2	34.5	30.9	30.4
Mono unsaturated MUFA	30.0	32.0	38.8	39.2
Polyunsaturated PUFA	28.9	28.7	25.6	25.0
Trans isomers	4.4	3.6	1.0	1.2
Σ n-6	21.3	21.6	20.0	20.2
Σ n-3	6.6	6.0	2.1	2.1
n-6/n-3	3.3	3.6	4.4	4.3

SALIVA INCORPORATION DURING MEAT BOLI FORMATION IN RELATION TO CHEWING EFFICIENCY

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Background

Meat undergoes important structural changes during chewing (breakdown of fibers as well as saliva incorporation). During this transformation, sensory quality (texture and flavor) of meat is perceived, and determines the acceptability of the meat (Harris, 1972; Mathevon *et al*., 1995; Mathonière *et al*., 2000).

When swallowing, the properties of the boli make an important contribution to the overall acceptability of meat. It is therefore relevant to characterize these properties as well as the changes that occur in meat structure during chewing in relation to the initial properties of meat in order to get consumer-oriented clues in the understanding of meat acceptability. Cooked meat texture prior to chewing has been widely investigated and many mechanical measurements have been validated for its evaluation (Lepetit and Culioli, 1994; Culioli, 1995). Mechanical properties usually correlate with texture assessments performed during sensory analysis sessions (Bouton *et al.*, 1975; Boccard *et al.*, 1981). In contrast, properties of meat boli as affected by saliva incorporation are only sparsely documented. In a previous study (Mioche *et al.*, 2002), saliva incorporation was evaluated by the variation of the weight of the samples before and after chewing. This method does not allow quantification of the meat juice lost under bite force. Therefore, methods to determine total saliva incorporation need to be developed in order to get a better estimation of net saliva incorporation along with the juice lost from the meat.

Objectives

This study aims to investigate 1) the relationship between saliva incorporation and juice release during chewing as well as 2) the relationship between saliva incorporation and the structure of the mat boli when swallowed.

Materials and methods

Subjects

Fifteen healthy elderly subjects with very different chewing efficiencies participated in the study; eight dentate (4 male, 4 female, 66.6 ± 3.1 years) and seven complete denture wearers (3 male, 4 female, 68.7 ± 5.9 years). The subjects were selected after dental examination. All dentate subjects had at least eight pairs of natural post-canines teeth. Denture-wearing subjects had been edentulous for at least five years. They wore full dentures with a correct interocclusal relationship and a satisfying prosthetic stability. Subjects felt comfortable with their dentures, which had been integrate for at least 6 months. All subjects declared to eat meat on a regular basis and all of them were able to chew the samples in the present study. All subjects were thoroughly informed and gave their consent. The protocol was approved by the Regional Ethical Committee.

Sample

Two different textures of beef meat were obtained from the muscles *Semimembranosus* of the same animal by combining different aging times and cooking temperatures ad modem (Mathevon *et al*., 1995). One muscle was aged for 3 days at 4°C and then fully cooked to 80°C (tough meat). The other was aged for 15 days at 4°C and then cooked to 65°C (tender meat). After cooking, meat was vacuum-packed, placed at -20°C (maximal storage 3 month) and then cut into cubes (5.1g ± 0.3). Just before using, the samples were thawed by immersing the packs in a 15°C water-bath for 1h.

Data acquisition:

1) Saliva collection

Subjects' saliva was collected with salivette (Sarstedt, Germany) in stimulated condition. Salivation was stimulated by chewing parafilm (American National Can, Chicago, Il., USA). Afterwards, two salivette were placed at the aperture of parotid ducts and the subjects were asked to chew a third one for 1 min. Salivette

were frozen at –20°C until centrifugation at 1000g for 10 min and analysis. Saliva from three salivettes of each subject was then pooled.

2) Boli collection

Subjects were asked to chew a cold meat sample without swallowing, and to spit out the bolus when they felt that a swallow would normally be triggered. Textures were randomized among subjects and six replicates were analyzed for each texture giving twelve boli per subjects. Every four samples, the subjects were given a tender meat sample to eat in order to reset their natural chewing pattern. After collection, all boli were frozen at –20°C until analysis.

Food bolus analysis

Dry matter measurement

Possible losses of matter due to unexpected swallows were reported (Mishellany, Woda and Peyron, 2003). In order to evaluate the losses in meat, total dry matter of each bolus was controlled and compared to total dry matter of initial samples, calculated from cooked meat dry matter content and initial sample weight. Lower amounts of dry matter in boli than in cooked meat should reflect meat loss. For dry matter determination, samples were weighed before and after being placed in a 110°C oven for 24 hours.

Saliva incorporation measurements

Three methods were tested to evaluate saliva incorporation.

1) Weight increase: increase between the weight of meat samples and the weight of boli was determined weighing meat samples before and after chewing, which allowed saliva impregnation. To determine the losses of food matrix, boli weights were recalculated from losses evaluated by dry matter measurement.
2) Volume increase: increase in the volume of the sample was considered saliva incorporation. Volumes were measured immersing samples in water and measuring water volume increase using a graduated pipette with 0.2 ml accuracy. The volume was determined from five meat samples to obtain original meat density and from four frozen boli for each texture.
3) Marking of saliva: one bolus was collected for human secretory Immunoglobulin A (sIgA) analysis, using sIgA as a salivary marker. Boli were squeezed and the mix of meat juice and saliva was collected. The concentration of saliva in a sample was calculated using a human sIgA radial immuno-diffusion (RID) kit (The Binding Site, United Kingdom). Saliva collected with salivette was used as a reference and compared with values measured from the juice mix of saliva and meat in the boli. The quantity of sIgA in the boli was calculated from the sIgA rate in the boli obtained by RID and the quantity of the boli liquid phase obtained by dry matter measurement. From these values of sIgA in the boli and the rate of sIgA in saliva, the volume of saliva incorporated in boli was calculated.

Mechanical measurement

Samples used in this section were previously used for volume measurements. The mechanical properties of the food boli were measured by applying a shear test using a double-bladed cell with a displacement rate of 60 mm/min (Culioli, 1995). After thawing at room temperature, two boli per texture and per subject were gently placed into a U-shape mould (70 x 10 x 10 mm). Samples then had a section of 10 x 10 mm with a length depending on the bolus size. After removal from the mould, they were sheared. Three to five measurements were performed on the same bolus, 5 mm apart from each other, without any interference from one measure to another one. The maximum shear force was calculated from the force-distance curves and expressed as stress relative to the initial bolus section area. The replicates were performed to get information about structure homogeneity.

Statistical Analysis

Statistical analyses were carried out with SAS Analyst (SAS software, version 8.01, 1999 – SAS Institute Inc., NC, USA). To analyze correlations between variables, Pearson correlation coefficients were calculated. The SAS Mixed Model procedure was used to study the effect of dental status on saliva incorporation and on boli texture analyzed by shear tests.

Results and discussion

Total dry matter was determined in order to identify possible losses due to unexpected partial swallows. The texture of meat affected significantly the material losses, which varied from 5.6% to 12% of the initial sample weight for tender meat and tough meat, respectively. The losses were not significantly different between the two groups of subjects. This method allowed to highlight losses which were not identified in a

previous study (Mioche *et al.*, 2002). In similar experiments performed with brittle foods, the losses could reach an average of 60 % of the initial sample weight (Mishellany, Woda and Peyron, 2003). Losses may be due to food thinning with saliva during mastication. These losses appeared to be out of subject control as the losses occurred for all subjects whatever the food product. Losses appeared to be related to the cohesiveness of the food, and therefore, their determination might be relevant for the acceptability of meat.

The variables describing saliva impregnation correlated with each dental status and each meat texture, except in dentate subjects chewing tough meat, where volume increase values did not correlate with the other variables. These correlations are illustrated in figure 1 showing correlations between values obtained by weight increase measurements and by the levels of sIgA on boli from tough meat. With tender meat, the Pearson coefficients of correlation were 0.796 ($p<0.05$) and 0.933 ($p<0.01$) for dentate subjects and denture wearers respectively. Variations in meat sample weight during chewing did not allow reliable separation of lost meat juice and saliva incorporation. Therefore, saliva intake during boli formation was underestimated. Volume measurements were equally underestimated, since this method did not allow quantifying the loss of meat juice, and the accuracy of the measurement was insufficient in terms of the volumes measured (from 5 to 12 ml). Using sIgA as a salivary marker permitted to determine juice losses but this method took longer and was more expensive to perform than the other two. However, the values obtained from the three methods were not significantly different.

Saliva incorporation does not vary along with food boli properties. No correlation was observed between shear stress and saliva incorporation values. Denture wearers produced boli that was less disorganized than that of the dentate subjects for each meat texture (figure 2) with the same amount of saliva. With tough meat, 5.167 ml (± 1.615ml) and 6.254 ml (± 2.832ml) of saliva was incorporated by dentate subjects and denture wearers, respectively. In this case, denture wearers characterized by reduced mastication efficiency, had their salivary flow stimulated by their denture (Veyrune and Mioche, 2000). Therefore, these values were not significantly different. However, meat texture induces large variations in the amount of saliva incorporated, tougher and dryer meat requiring more saliva than tender and juicy meat to trigger a swallow.

Conclusions

Evaluation of unexpected food matrix losses by controlling dry matter reduces the underestimation of saliva incorporation. Among the three methods tested to evaluate saliva impregnation in boli, the one using a salivary marker gave the highest estimation of saliva incorporation, but the values obtained were not significantly different from those obtained by measuring increases in weight and volume. Furthermore, it was observed that changes in chewing efficiency had a direct effect on the dynamics of meat bolus formation. With respect to the amount of saliva, the denture-wearers swallowed boli that was less disorganized to that of the dentate subjects. Therefore, saliva incorporation was not linked to the level of meat disorganization. This may suggest that a certain level of loose moisture is required to trigger a swallow.

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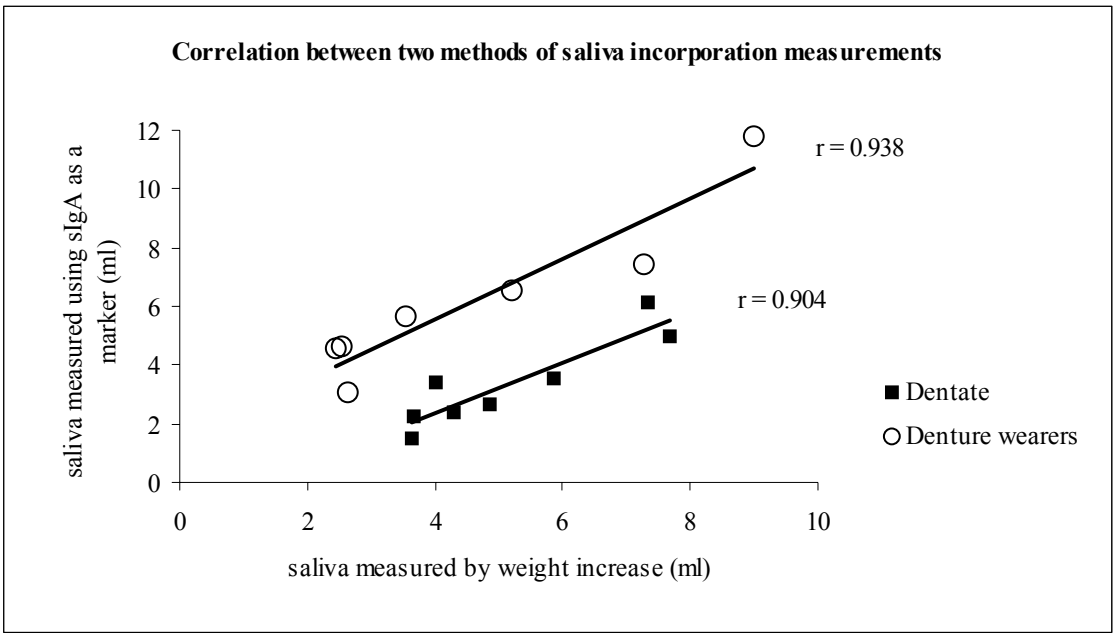

Figure 1. Correlation between the values of saliva incorporation obtained using human sIgA as a salivary marker and obtained by variations in meat sample weight corrected by food matrix losses, for each dental status, only values for tough meat are showed.

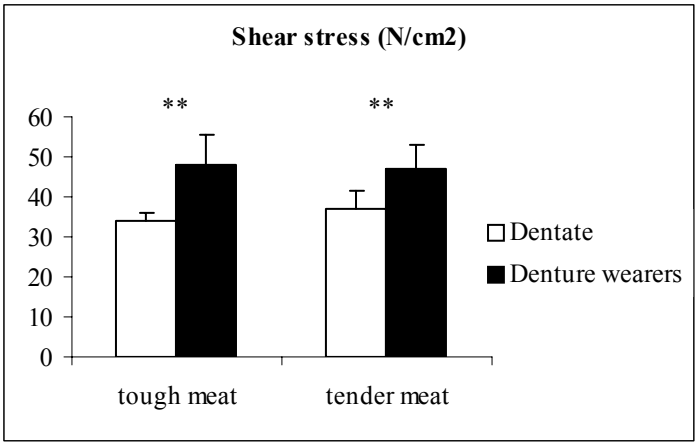

Figure 2. Variations in the shear resistance of meat boli with dental status

CONJUGATED LINOLEIC ACID AND THE RATIO OF ω6:ω3 FATTY ACIDS ON THE LIPID METABOLISM OF BROILER CHICKENS

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Background

From the point of view of food safety it has been a growing concern of the poultry industry to improve not only the yield but also the composition of the poultry products, maximizing the deposition of protein and reducing the fat content. Quality and quantity of fat present in the food of animal origin has been an important criteria of nutritional evaluation. Conjugated linoleic acid (CLA) is a functional metabolite that has the potential of reducing carcass fat deposition (Akahoshi et al., 2003). This effect is related to the modification of gene expression of lipogenic enzymes (Bauman, 2001) showing that the hepatic lipogenisis and fat deposition may be reduced by the supplementation of CLA in the feed. CLA may reduce adipose tissue mass by minimizing accumulation of triglycerides in adipocytes. t10c12-CLA inibits activity of lipoprotein lipase in vivo (Park & Pariza, 2001). Also, there are reports of the effects of CLA in the increase of carnitine palmitoil transferase activity, a key enzyme on the β-oxidation (Akahoshi et al., 2003). Supplementation with CLA also reduced significantly the level of liver fat in broilers (Badinga et al., 2003) and total triglycerides and cholesterol in the plasma of rabbits (Corino et al., 2002). The use of CLA in association with oils rich in ω3 fatty acids or in diets that have a balanced ratio of ω6:ω3 has optimized the CLA effect (Aydin et al., 2001) showing that the CLA effect depend upon the amount of fatty acids ω6 and ω3 in the diet. Therefore, since CLA has the potential of alter the genetic expression of the lipogenic enzymes, it is believed that the use of CLA in association with different sources of fat in the diet may improve the productive efficiency as well as the carcass yield.

Objectives

The aim of these studies were to evaluate the dietary supplementation of CLA and the ratio of ω6:ω3 on the lipid metabolism of broiler chickens.

Materials and methods

Two studies were conducted simultaneously using 100 male or female Ross broiler chickens with 21 days of age at the start of the experiment. Birds were selected from a population of 300 animals so that, male or female birds used in the studies were within of 10% of the mean body weight of the population. The experimental design was a completely randomized in a factorial arrangement 2 x 5 (two oil source, i.e. soybean or canola oil and five levels of CLA supplementation, i.e. 0.0, 0.25, 0.50, 0.75 and 1.00). Oils used were supplied by Bunge Alimentos and CLA (Lucta – CLA 60) by BASF. The control diet had 4% of soybean or canola oil. CLA levels were obtained by isometrically replacing soybean or canola oil in the control diet. The ratio of ω6:ω3 fatty acids in the soybean and canola oil was 12:1 and 3.5:1 respectively. From 1 to 21 days of age chicks were raised in a corn-soy starter diet with 21% protein and 2,950 Kcal of metabolizable energy per Kg of diet. From 22 to 45 days of age the experimental diets were corn-soy diets formulated to at least reach nutrient levels recommended by the NRC (1994). The final body weight was determined individually at 45 days of age, when birds were killed by cutting the jugular vein. Blood samples were collected from all 10 birds. Abdominal fat pads and livers were collect from the five birds with body weight closest to the mean body weight of the treatment. Total serum cholesterol was measured using spectrometry (LABTEST – cholesterol liquiform). ANOVA and the F test (5% level) were used to compared results of the effect of oil sources and regression analysis for CLA levels.

Results and discussion

The effect of oil source on final body weight of males and females chickens is shown in Table 1. For both sexes, supplementation with canola oil resulted in heavier birds ($P < 0.05$) when compared to soybean oil.

The effect of CLA supplementation on final body weight of female birds was significant and better explained by a cubic response. It decreased until the level of 0.20% of CLA and increased to a maximum between 0.75 and 0.80% of CLA (Figure 1). Research with growing swine have also shown that CLA supplementation improved feed efficiency, growth and carcass composition (Thiel-Cooper et al., 2001). On the other hand, Badinga et al. (2003) showed a reduction on the body weight of broilers receiving CLA supplementation. Abdominal fat pad content of females have shown a significant interaction between oil sources versus levels of CLA. A linear reduction in abdominal fat pad content was observed on females receiving canola oil and CLA (Figure 2). Probably, this effect of the CLA was due to a higher body fat content in the heavier females fed canola oil. The effect of CLA supplementation on reduction in body fat of heavier animals was also reported by Corino et al. (2003). Table 2 shows the effect of oil source on liver weight ($P < 0.05$). Canola oil fed male and female birds had a reduction in liver weight ($P < 0.05$) when compared to soybean oil fed birds. An interaction effect between canola oil and CLA was also verified on the liver weight of females ($P < 0.05$). The results follows a cubic response where liver weights were reduced to a minimum at 0.25% CLA supplementation and increased to a maximum at 0.80% of CLA (Table 2). There was effect of the oil source on the serum cholesterol levels of females ($P < 0.05$). Total serum cholesterol was lower (115,1 mg/dl) in females fed canola oil when compared to that of females fed soybean oil (131.8 mg/dl). In both sexes, the supplementation with CLA in association with two sources of oil influenced ($P < 0.05$) the lipid metabolism of birds (Figures 3, 4 and 5). Badinga et al. (2003) reported a significant reduction in liver lipids due to CLA supplementation of birds diets. Fatty acids production in birds occur mainly in the liver, therefore the lighter livers of male and female birds fed canola oil found in this study may be due to a reduction in the endogenous production of lipids as also shown by the reduced serum cholesterol levels in the females. The significant interaction effect between oil sources and levels of CLA on liver weight and serum cholesterol levels suggested that the CLA effect may be related to the ratio of omega 6 to omega 3 fatty acids in the diet. Brown et al., (2001) reported that culture of pre-adipocytes supplemented with CLA and sunflower oil (rich in omega 6 fatty acids) resulted in higher content of triglycerides when compared to the cultured treated with only CLA, showing that the anti-adipogenic effect of CLA on the pre-adipocytes can be reversed. Therefore, it is reasonable to think that in studying the CLA effect on the lipid metabolism is important to take in consideration the fatty acid composition of the diet as well as the ratio of omega 6 to omega 3 fatty acids. Zanini et al. (2003) reported a reduction in total fat, cholesterol and saturated fatty acids in the carcass of roosters fed canola oil. The ratio of omega 6 to omega 3 in theirs study was 6,6:1 that is within the range of 4:1 to 10:1 recommended to humans (British Nutrition Foundation, 1991). The results of this study suggest that effects of oil source and CLA levels on abdominal fat pad, liver weight and serum cholesterol of females may be related to a reduction in the hepatic lipogenesis.

Conclusions

The results of this study have shown that:

1. Canola oil fed birds were heavier than soybean oil fed birds.
2. The CLA response on abdominal fat deposition depend upon body weight, sex and the source of fat added to the diet.
3. The effect of CLA in reducing total serum cholesterol was dependent of oil source.
4. Liver weight in both sexes and serum cholesterol in the females were reduced in the canola oil diet.

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Table 1 - Final body weight of male and female birds fed diets supplemented with soybean or canola oil and CLA levels.

	FINAL BODY WEIGHT (g)		
	MALES		
CLA (%)	SOYBEAN OIL	CANOLA OIL	$\bar{x}$ CLA
0,0	2248,12	2637,50	2442,81
0,25	2200,00	2640,62	2429,31
0,50	2284,37	2635,62	2460,00
0,75	2404,37	2677,50	2540,94
1	2435,00	2586,23	2510,62
$\bar{x}$ OIL SOURCE	2314,37 [b]	2635,50 [a]	
	FEMALES		
CLA (%)	SOYBEAN OIL	CANOLA OIL	$\bar{x}$ CLA[1]
0,0	2055,00	2263,12	2159,06
0,25	1997,50	2163,75	2080,62
0,50	2148,75	2246,25	2197,50
0,75	2146,87	2341,25	2244,06
1	2116,87	2276,25	2196,56
$\bar{x}$ OIL SOURCE	2093,00 [b]	2258,12 [a]	

[a,b] Averages values within the same line with no common superscript differ significantly by the test F (P<0.05)
[1]Cubic effect (P<0.05)

Table 2 - Liver weight of male and female birds fed diets supplemented with soybean our canola oil and CLA levels.

	LIVER WEIGHT (%)		
	MALES		
CLA (%)	SOYBEAN OIL	CANOLA OIL	$\bar{x}$ CLA
0,0	1,87	1,66	1,76
0,25	1,89	1,57	1,73
0,50	2,00	1,71	1,85
0,75	1,91	1,81	1,86
1	1,93	1,68	1,81
$\bar{x}$ OIL SOURCE	1,92 [a]	1,68 [b]	
	FEMALES		
CLA (%)	SOYBEAN OIL	CANOLA OIL[1]	$\bar{x}$ CLA
0,0	1,79[a]	1,86[a]	1,82
0,25	1,93[a]	1,61[b]	1,77
0,50	1,90[a]	1,73[b]	1,82
0,75	1,95[a]	1,82[a]	1,89
1	1,90[a]	1,74[b]	1,82
$\bar{x}$ OIL SOURCE	1,89 [a]	1,75 [b]	

[a,b] Averages values within the same line with no common superscript differ significantly by the test F (P<0.05)
[1]fCubic effect (P<0.05, R^2 0.97)

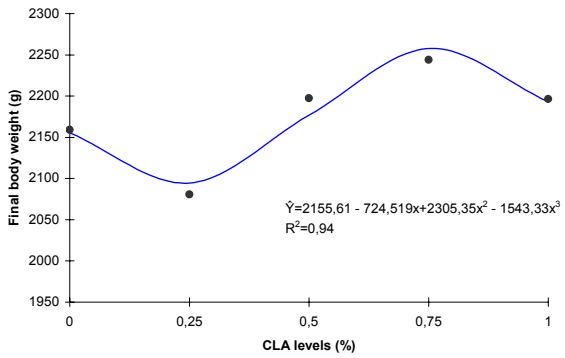

Figure 1. Final body weight of female birds supplemented with CLA

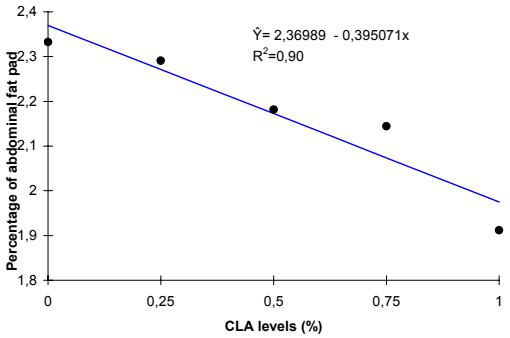

Figure 2. Percentage of abdominal fat pad of female birds fed canola oil diets and supplemented with CLA.

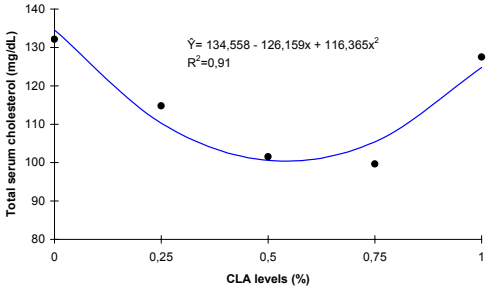

Figure 3. Total serum cholesterol (mg/dl) of female birds fed canola oil and supplemented with CLA.

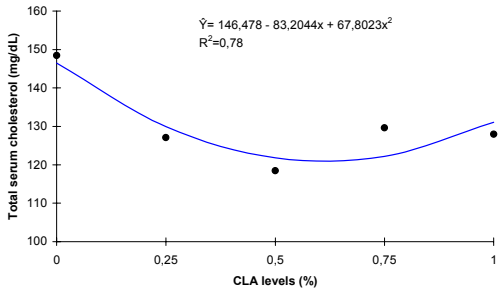

Figure 4. Total serum cholesterol (mg/dl) of male birds fed canola oil and supplemented with CLA.

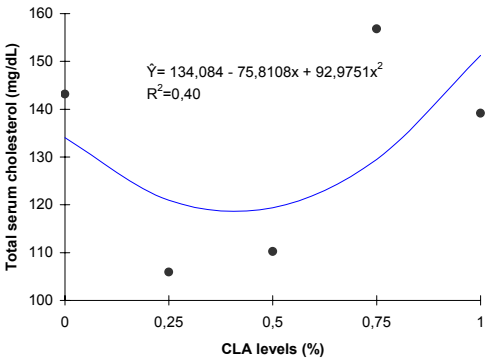

Figure 5. Total serum cholesterol(mg/dl) of male birds fed soybean oil and supplemented with CLA.

CONJUGATED LINOLEIC ACID AND THE RATIO OF ω6:ω3 FATTY ACIDS ON THE OXIDATIVE STABILITY OF CHICKEN MEAT

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Background

Studies to change the composition and the quality of lipids present in the carcass of meat producing animals have increased lately. Products high in lipids, as meats, have call the attention of the meat scientists due to the high probability to suffer auto-oxidative reactions. The intake of rancid products may cause a great deal of health problems to the population due to its toxic effect on the cells (Ferrari, 1999). Adding conjugated linoleic acid (CLA) to the animal feed it is a reasonable way to improve meat lipid quality, therefore, its nutritional value. Dietary supplementation with CLA may change the composition of lipids produced by the liver (Belury & Kempa-Steczko, 1997) as well as reduce the total lipid concentration in rats (West et al., 1998). These alterations on the lipid profile by CLA supplementation may also result in changing on the oxidative stability of tissues. Joo et al (2002) supplemented finishing swine diets with CLA and observed that thiobarbituric acid-reactive substances (TBARS) values in the meat were higher for animals fed the control diet. Similar results were reported by Corino et al. (2002) in the meat of rabbits fed diets with 0,5% CLA. Previously, Du et al. (2000) reported that the oxidative stability of broiler meat is improved by using CLA supplemented diets. No information were found in the literature about the oxidative stability of broiler meat fed diets supplemented with different oil sources and enriched with CLA. It is believed that this association may improve carcass lipid composition as well as carcass quality.

Objectives

The purpose of this study was to evaluate the dietary supplementation of broiler diets with CLA and oil sources to produce different ratios of ω6:ω3 fatty acids on the oxidative stability of broiler meat submitted to refrigeration or freezing storage temperatures.

Materials and methods

One study was conducted using 100 male Ross broiler chickens with 21 days of age at the start of the experiment. Birds were selected from a population of 300 animals so that males birds used in the study were within of 10% of the mean body weight of the population. The experimental design was a completely randomized in a factorial arrangement 2 x 5 (two oil sources, i.e. soybean or canola oil and five levels of CLA supplementation, i.e. 0.0, 0.25, 0.50, 0.75 and 1.00%). Oils used were supplied by Bünge Alimentos and CLA (Lucta CLA 60) by BASF. The control diets had 4% of soybean or canola oil. CLA supplementation levels were obtained by isometrically replacing soybean or canola oil in the control diets. From 1 to 21 days of age chicks were raised in a corn-soy diet with 21% protein and 2.950 Kcal of metabolizable energy per Kg of diet. From 22 to 45 days of age the experimental diets were corn-soy diets formulated to at least reach nutrient levels recommended by the NRC (1994). At 45 days of age birds were killed by a cut in the jugular vein. Samples of breast and thigh meat were collected and part kept under refrigeration at 5˚C and the other part kept under freezing conditions at -20˚C. Oxidative stability was measured on meats kept under refrigeration after the third day of storage and on the frozen meat on days 25, 50, 75 e 100 of storage. Oxidative stability was measured using the procedure described by Tarladgis et al (1960) for the TBARS values. The F test at 5% of significance was used to compare results between sources of oils when interactions were not detected. When there was an interaction ($P<0.05$), it was used the SNK test to compare results between sources of oils. Regression analysis was used to report the effects of CLA levels.

Results and discussion

Under storage conditions fats and the fat content foods may undergo oxidation. The rate of oxidation depends upon the type of food and the storage conditions. The malonaldehyde is formed primarily by the oxidation of insaturated fatty acids, being the reaction more intense as the level of insaturation of the fat increases (Janero, 1990). Under freezing conditions our results (table 1) show that oxidation of the both breast and thigh meat occurred slowly and followed a linear pattern as storage time increased. Gava (1984) reported that in frozen food the enzymatic reactions occur at a slow but continuing rate. Lipids oxidation in highly insaturated fats particularly in red meat is a matter of concern due to the high levels of iron and phospholipids. On the thigh meat, during the first 50 days of storage was observed a significant interaction (oil sources x levels of CLA) on TBARS values. These results were better explained by a quadratic function (table 1 and figure 1, 2 and 3, $P < 0.05$). However on days 75 and 100 of storage this effect was not observed ($P > 0.05$). A significant interaction (oil sources and CLA levels) was also observed on TBARS values of breast meat (table 1). At day 25 of storage, independently of the oil source, was observed a significant reduction on TBARS values on thigh meat that plateau between 0.50 and 0.75% of CLA supplementation (Figure 1 and 3). At 25 days of storage, supplementation with 0.50% in association with soybean oil resulted in lower TBARS values for both breast and thigh meat when compared to that of canola oil supplemented birds. On breast meat of birds fed the soybean oil there was a linear reduction ($P<0.05$) of TBARS values with increasing CLA levels at 25 days of storage. However, as storage time increased the interactive effect of oil source and levels of CLA on TBARS values of breast meat was better explained by a quadratic function. On days 50 and 100 of storage, a reduction on oxidation of the breast meat was observed with soybean oil and supplementation of CLA, stabilizing at the level of 0.50% and increasing again with higher levels. At 50 days of storage, levels of 0.75% of CLA in association with canola oil resulted in lower oxidation on both breast and thigh meat when compared to soybean oil. At this storage time, even without CLA supplementation the oxidation of breast meat was lower in birds fed canola oil. At 75 days of storage, again 0.75% of CLA in association with canola oil resulted in lower breast meat oxidation. Also, there was a linear reduction on TBARS values with growing levels of CLA on breast meat of canola fed birds. These overall results showed a synergic effect between CLA and oil source improving oxidative stability on breast and thigh meat during storage. Shantha et al.(1995) reported that CLA is a stable fat and its deposition on carcass fat may reduce the oxidative potential. Previously Shantha et al. (1994) have suggested that the stability of the levels of CLA on the meat during storage were probably related to its higher stability when compared with fats higher in polyunsaturated fatty acids. On the other hand, Du et al. (2000) suggested that the effect of CLA on meat stability quality is related to its effect on the increase in saturated fat content. Fresh food can be stored under refrigeration for a limited period of time, which reduce the rate of microbiological and enzymatic deterioration. The results of oxidative stability of breast and thigh meat stored under refrigeration are shown in table 2. Significant interaction (oil sources and levels of CLA) on refrigerated meat was observed (Figure 4 and 5). On the breast meat a linear reduction on TBARS values was observed with increased levels of CLA in association with canola oil (figure 4). Variations on TBARS values of breast meat of birds fed soybean oil was best explained by a quadratic function with reductions up to 0.50 % levels. However, the 0.25% level of CLA in association with soybean oil produced the lowest TBARS value on breast meat when compared with canola oil. On the thigh meat of broilers fed canola oil, variations on TBARS values followed a cubic function (figure 5). Therefore, under refrigeration supplementation of 1% of CLA in association with canola oil produced the lowest oxidation levels on the thigh meat when compared to that of birds fed soybean oil.

Conclusions

Considering the conditions under which this experiment was carried out, it can be concluded that:

1. The improve in the oxidation stability by CLA and dietary fat allows the use of diets with highly unsaturated fatty acids with no impairment of chicken meat quality

2. Oxidation stability of broiler breast and thigh meat was improved by CLA and oil supplementation.

3. On the frozen thigh meat CLA supplementation in association with canola or soybean oils resulted in higher oxidative stability during the first 50 days of storage. For breast meat the maintenance of meat quality was even longer up to 100 days.

4. Under refrigeration, breast and thigh meat quality was improved by the synergic effect of CLA and dietary oil. CLA in association with canola oil resulted in lower TBARS values for both breast and thigh meat, when compared with soybean oil.

5. The effect of levels of CLA on TBARS values depend upon the oil source used in the diet, the storage condition as well as the duration of storage.

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Table 1 - TBARS values (mg of MDA/kg meat) of frozen breast and thigh meat of broilers fed diets with canola or soybean oil and CLA under different storage time.

	TBARS VALUES			
	25 DAYS OF STORAGE TIME			
	THIGH MEAT		BREAST MEAT	
CLA (%)	SOYBEAN OIL[2]	CANOLA OIL[2]	SOYBEAN OIL[1]	CANOLA OIL
0.0	0.167a	0.179a	0.095a	0.082a
0.25	0.106a	0.094a	0.056a	0.043a
0.5	0.058b	0.129a	0.051b	0.098a
0.75	0.070a	0.082a	0.05a	0.048a
1	0.084b	0.121a	0.039b	0.078a
	50 DAYS OF STORAGE TIME			
	THIGH MEAT		BREAST MEAT	
CLA (%)	SOYBEAN OIL[2]	CANOLA OIL[2]	SOYBEAN OIL[2]	CANOLA OIL
0.0	0.187a	0.187a	0.165a	0.111b
0.25	0.125a	0.112a	0.112a	0.109a
0.5	0.156a	0.148a	0.104a	0.129a
0.75	0.207a	0.142b	0.180a	0.106b
1	0.216a	0.140b	0.165a	0.115b
	75 DAYS OF STORAGE TIME			
	THIGH MEAT		BREAST MEAT	
CLA (%)	SOYBEAN OIL	CANOLA OIL	SOYBEAN OIL	CANOLA OIL[1]
0.0	0.267	0.238	0.196a	0.193a
0.25	0.231	0.227	0.161a	0.185a
0.5	0.224	0.252	0.173a	0.143a
0.75	0.218	0.203	0.176a	0.115b
1	0.230	0.235	0.160a	0.106b
	100 DAYS OF STORAGE TIME			
	THIGH MEAT		BREAST MEAT	
CLA (%)	SOYBEAN OIL	CANOLA OIL	SOYBEAN OIL[2]	CANOLA OIL
0.0	0.325	0.268	0.206	0.232
0.25	0.305	0.269	0.176	0.219
0.5	0.296	0.349	0.170	0.221
0.75	0.368	0.355	0.215	0.215
1	0.358	0.327	0.235	0.198

[a,b] Averages values within the same line with no common superscript differ significantly by the SNK test (P<0.05)
[1] Linear effect (P<0.05)
[2] Quadratic effect (P<0.05)

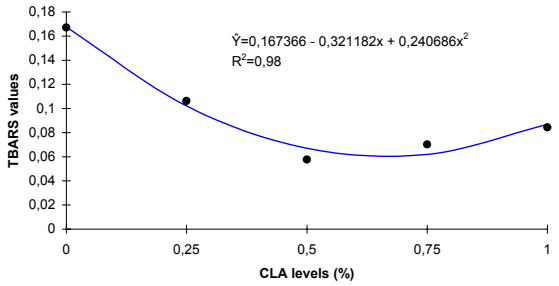

Figure 1. TBARS values (mg of MDA/kg meat) of frozen thigh meat of broilers fed diets with soybean oil and CLA, stored for 25 days.

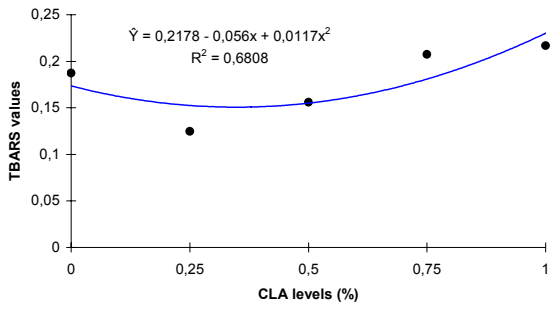

Figure 2. TBARS values (mg of MDA/kg meat) of frozen thigh meat of broilers fed diets with soybean oil and CLA, stored for 50 days.

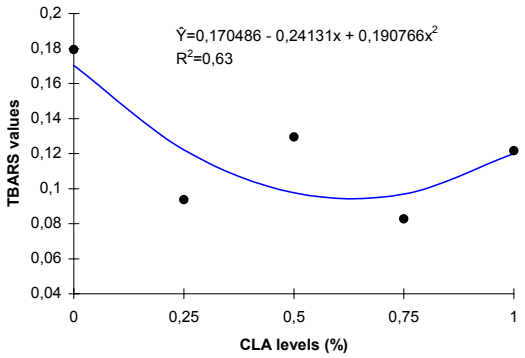

Figure 3. TBARS values (mg of MDA/kg meat) of frozen thigh meat of broilers fed diets with canola oil and CLA, stored for 25 days.

Table 2 – TBARS values (mg of MDA/kg meat) of refrigerated breast and thigh meat of broilers fed diets with canola or soybean oil and CLA.

	TBARS VALUES			
	THIGH MEAT		BREAST MEAT	
CLA (%)	SOYBEAN OIL	CANOLA OIL[3]	SOYBEAN OIL[2]	CANOLA OIL[1]
0.0	0.158b	0.214a	0.149a	0.140a
0.25	0.152a	0.159a	0.066b	0.150a
0.5	0.119a	0.122a	0.121a	0.108a
0.75	0.176a	0.152a	0.091a	0.112a
1	0.156a	0.124b	0.106a	0.095a

[a,b] Averages values within the same line with no common superscript differ significantly by the SNK test (P<0.05)
[1] Linear effect (P<0.05)
[2] Quadratic effect (P<0.05)
[3] Cubic effect (P<0.05)

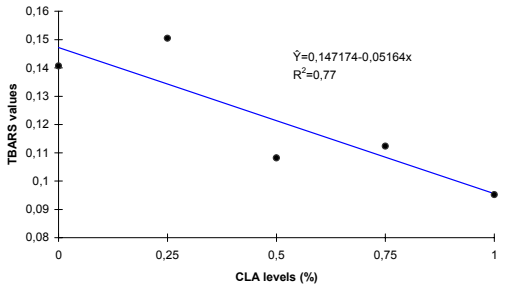

Figure 4. TBARS values (mg of MDA/kg meat) of refrigerated breast meat of broilers fed diets with canola oil and CLA.

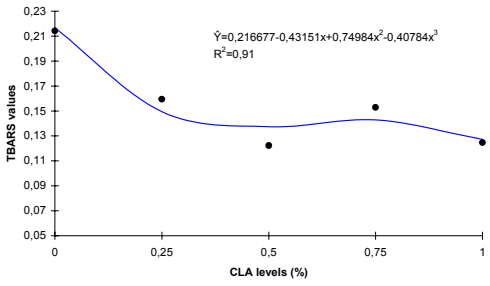

Figure 5. TBARS values (mg of MDA/kg meat) of refrigerated thigh meat of broilers fed diets with canola oil and CLA.

LIPID COMPOSITION OF LAMB MEAT, AFFECTED BY GENOTYPE AND SEX

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Background

In the last two decades the breeding of sheep in Slovenia has grown up. According to Zagožen (1984), humid climate allows production of meat and milk, but not very high quality wool. Sheep breeding has, therefore, been directed to more meaty crossbreeds, which has enabled the consumption of lamb meat to increase. For the consumers, this trend creates refreshment to nutrition as well as gastronomy.

Lamb meat is a category of red meats, which nutritionally is not highly appreciated because of high fat content and fairly high proportion of saturated fatty acids (SFA). In comparison to other red meats, lamb contains the highest proportion of SFA (52.1%) (beef (44.9%); pork (42.5%)), the lowest proportion of monounsaturated fatty acids (MUFA) (40.5%) (pork (47.9%); beef (49.3%)), and an intermediate proportion of polyunsaturated faty acids (PUFA) (5.0%) (beef (4.3%); pork (8.3%)) (Southgate, 1993). Lipid content and composition of lamb meat are affected by different factors: genotype, animal fattening, animal live weight, age and sex. Breeds for meat production contain less fat than those for milk production (Wood *et al*., 1980, Croston *et al.*,1987, Fisher *et al.,*2000) and there are significant differences in FA composition between different milky breeds (Arsenos *et al.,* 2000). Animal age significantly affects intramuscular fat content (IMF), which is lower in meat of younger animals (Failla *et al*, 1996, Čepin & Žgur, 2003) as well as FA composition (higher degree of SFA, and lower degree of MUFA and PUFA as an animal gets older) (Cifuni *et al.,*2001). The data on the influence of animal sex on IMF content are: female animals contain more fat (Hammel and Laforest, 2000; Mc Clinton and Carson, 2000), but in other experiments no difference was found between sexes (Vergara *et al., 1999)*. There are no data concerning the influence of sex on the FA composition of lamb meat. Cholesterol content of lamb meat varies from about 60 mg/100g to 140 mg/100g (Sevi *et al.,* 1997, Arsenos *et al.,* 2000), and is dependent on the genotype, fatness, age and sex of the animal, as well as analytical method applied.

Objectives

The aim of this research was to investigate the effect of genotype and sex of lambs on the intramuscular fat (IMF) content, cholesterol content and fatty acid composition of meat.

Materials and methods

The experiment was conducted on 24 lambs of two genotypes: improved autochthon Jezersko-Solcavska breed (JSR) and crossbreed between Jezersko-Solcavska and Texel breed (JSR×T). 12 animals (6 male and 6 female) represented each genotype. Lambs were raised by the same farmer under the same feeding system, and were slaughtered at 33–52 kg live weight, at the age range from 91 to 159 days. Left *Longissimis dorsi* muscle from the 7th to the last thoracic vertebra was removed from each carcass 24 h *post mortem,* and the subcutaneous fat was trimmed off. The loins were homogenized with a blender, packed into polyethylene bags and frozen at –21 ºC±1°C until analysed.

Intramuscular fat (IMF) content was determined using the method described in AOAC Official Methods 991.36 Fat (Crude) in Meat and Meat Products (A.O.A.C. 991.36., 1997). The fatty acid composition was determined using the modified *in situ* transesterification method after Park and Goins (1994), as well as the capillary Gas-Liquid Chromatography. The cholesterol content was determined by the modified method adapted from Naeemi *et al.* (1995), as well as with HPLC. All analyses were made in duplicate.

The data were statistically analysed by the least squares method using the GLM procedure (SAS Software. Version 8.01, 1999). The statistical model for IMF content, fatty acid composition and cholesterol content of

lamb included the effects of genotype (G_i; i=JSR, JSR×T), sex (S_j; j=male, female) and repetition (R_k; k=1-6): $y_{ijkl} = \mu + G_i + S_j + R_k + e_{ijkl}$.

Results and discussion

Results of studied effects (genotype and sex) on the quality parameters of lamb meat are presented in table 1.

Lamb meat of two genotypes (JSR + JSR×T) and both sexes contained 2.2 g of intramuscular fat (IMF) and 67.5 mg of cholesterol per 100 g. The IMF content was not influenced by genotype, but was significantly lower in male animals (1.90% vs. 2.45%), which is in accordance with statements of Hammel and Laforest (2000) and McClinton and Carson (2000). The cholesterol content was not affected by genotype or sex, and the relatively low concentrations found correspond with literature (Arsenos *et al.,*2000).

Table 1. Influence of genotype and sex on IMF (g/100g) and cholesterol (mg/100g) content and fatty acid composition (expressed as relative proportion) of the lamb meat (mean values)

Parameter		Breed			Sex		
		JSR (N = 48)	JSR×T (N = 48)	Sign.	Male (N = 48)	Female (N = 48)	Sign.
IMF (g/100g)		2.12	2.14	Ns	1.90	2.45	*
Cholesterol (mg/100g)		64.43	70.52	Ns	70.18	64.77	Ns
Fatty acid:							
Lauric	C 12:0	0.02	0.06	Ns	<0.01	0.08	*
Myristic	C 14:0	2.84	3.11	Ns	2.67	3.28	Ns
Myristoleic	C 14:1, n-5 c	1.79	2.83	Ns	2.79	1.83	Ns
Palmitic	C 16:0	29.67	29.43	Ns	30.04	29.06	Ns
Palmitoleic	C 16:1, n-7 c	2.65	2.54	Ns	2.72	2.46	Ns
Margaric	C 17:0	1.01	1.16	Ns	1.5	1.12	Ns
10-heptadecenoic	C 17:1, n-7 c	0.72	0.62	Ns	0.62	0.72	Ns
Stearic	C 18:0	17.71	6.39	Ns	18.29	15.82	Ns
Oleic	C 18:1, n-9 t	3.53	4.01	Ns	4.47	3.06	***
Oleic	C 18:1, n-9 c	24.73	21.65	Ns	17.39	28.98	Ns
Linoleic	C 18:2, n-6 c,c	10.66	11.44	Ns	13.27	8.83	***
γ-linolenic	C 18:3, n-6 c,c,c	0.40	0.51	Ns	0.33	0.58	Ns
α-linolenic	C 18:3, n-3 c,c,c	0.59	0.96	*	0.72	0.84	Ns
Gadolenic	C 20:1, n-9 c	0.25	0.26	Ns	0.23	0.28	Ns
11,14-eicosadienoic	C 20:2, n-6 c	2.75	3.22	Ns	23.76	2.19	***
Arachidonic	C 20:4, n-6	0.18	0.42	*	0.25	0.35	Ns
5,8,11,14,17-eicosapentaenoic (EPA)	C 20:5, n-3	0.35	0.87	Ns	0.86	0.36	Ns
Erusic	C 22:1, n-9 c	0.08	0.09	Ns	0.08	0.10	Ns
4,7,10,13,16,19-docosahexaenoic (DHA)	C 22:6, n-3	0.07	0.45	Ns	0.46	0.07	Ns
SFA		51.25	50.14	Ns	52.05	49.35	Ns
MUFA		33.74	31.99	*	28.31	37.43	Ns
PUFA		15.01	17.86	Ns	19.65	13.23	***
n-3		1.02	2.28	Ns	2.04	1.27	Ns
n-6		11.24	12.36	Ns	13.85	9.76	***
P/S		0.29	0.37	*	0.29	0.27	***
IA		1.07	1.07	Ns	1.08	1.06	Ns
n-6/n-3		6.56	5.12	*	6.10	5.50	Ns
HH		1.06	0.98	Ns	0.86	1.19	Ns

Sign. - Level of significance: *** – $P \leq 0.001$ highly statistically significant; ** – $P \leq 0.01$ statistically significant;
* - $P \leq 0.05$ statistically significant; Ns – $P \geq 0.05$ statistically not significant;
N=Number of observations;
P/S=PUFA/SFA;
AI=atherogenic index = (C12 + 4 C14 + C16 + trans FA) / (PUFA + C18:1 + MUFA) (Ulbricht *et all*., 1991);
HH=(C18:1 n-9 + C18:2 n-6 + C20:4 n-6 + C18:3 n-3 + 20:5 n-3 + C22:5 n-3 + 22:6 n-3) / (C14:0 + C16:0) (Santos-Silva *et all.,* 2002).

Fatty acid (FA) composition was affected by genotype and sex. The meat of JSR breed vs. JSR×T breed contain significantly higher proportion of α-linolenic (C18:3, n-3), arahidonic (C20:4, n-6) FA, higher MUFA and n-6/n-3 ratio and lower P/S ratio.

The meat of female lambs shows significantly higher total IMF content (2.45% vs. 1.90%) and higher proportion of lauric (C 12:0); proportion of oleic (C 18:1, n-9t), linoleic (C 18:2, n-6) and eicosadienoic acid (C 20:2, n-6c), but proportion of PUFA, n-6 FA and P/S index were significantly lower than in meat of male lambs.

The lambs tested in our experiment showed higher P/S indexes (0.3 – 0.4) than previously reported in literature (0.10 – 0.15) (Rowe *et al.,* 1999; Cifuni *et al*., 2001), reaching the minimum value recommended by Food Advisory Committee (Enser *et al.,* 1998).

Conclusions

Lamb meat of two genotypes (JSR + JSR×T) and both sexes contain 2.2 g/100g IMF and 67.5 mg/100g cholesterol. Neither parameter was affected by genotype or sex.

Fatty acid (FA) composition of meat lipids was affected by genotype and sex when expressed by the P/S ratio. JSR breed and female lambs had lower P/S ratio than the JSR×T breed and male lambs, respectively.

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50th International Congress of Meat Science and Technology

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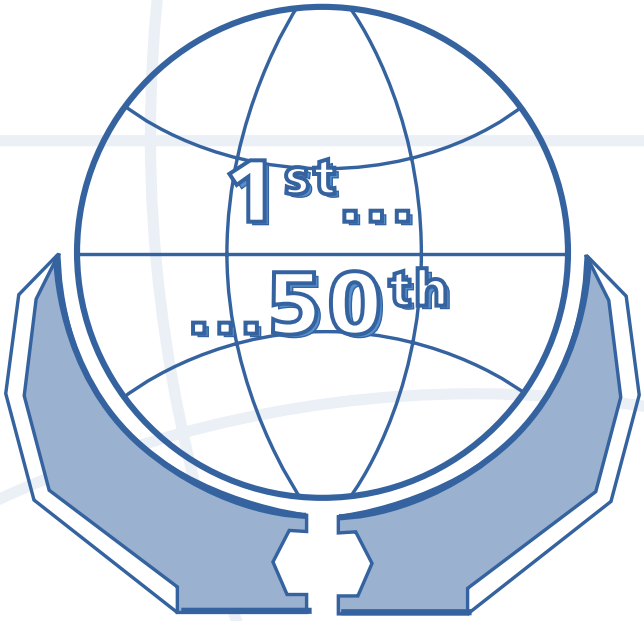

FEEDING AND MEAT QUALITY

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Introduction

Traditionally, the term 'meat quality' covers inherent properties of meat decisive for the suitability of the meat for eating, further processing and storage including retail display. The main attributes of interest are safety, nutritional value, flavour, texture, water-holding capacity, colour, lipid content, lipid composition, oxidative stability and uniformity. However, the dramatic changes at the international market over the past decade also require high standards of quality assurance regarding diversity and of aspects related to environmental, ethical and animal welfare problems in the production of meat. Consequently, quality is now to be considered a complex and multivariate property of meat, which is influenced by multiple interacting factors including the conditions under which the meat is produced. Production conditions include management system, breed, genotype, feeding, pre-slaughter handling and stunning, slaughter method, chilling and storage conditions.

Feeding strategy is the management factor, which is most actively used as a quality control tool in the production of meat and in relation to improvement and/or control of performance, animal welfare, safety, nutritional value, and eating and technological quality. Most feeding strategies tested and implemented in meat production have until recently been based on 'passive' effects, i.e. the uptake and incorporation of specific feeding components or chemical/structural behaviour in the intestinal tract, e.g. contribution to lipid content and composition in relation to nutritional value (Wood, Richardson, Nute, Fisher et al., 2004), technological quality (Allen & Foegeding, 1981; Sheard, Enser, Wood, Nute, Gill & Richardson, 2000) and/or anti-oxidative status (vitamin E) in relation to storage life and technological quality (Faustman & Cassens, 1989; Morrissey, Buckley & Galvin, 2000; Granit, Angel, Akiri, Holzer et al., 2001) and also to minimize pathogen growth in the intestinal tract (Engberg, Hedemann & Jensen, 2002; Hansen, Bach Knudsen, Jensen & Kjærsgaard, 2001a,b) and off-flavour formation by regulation of microbial fermentation in the intestinal tract (Jensen, Cox & Jensen, 1995). However, the use of feeding to optimise livestock performance and lean meat percentage has also produced a considerable biological understanding of the influence of diet composition on muscle deposition.

In view of our present knowledge of the effect of feeding on meat quality, it might be relevant to ask whether additional focus on individual feedstuffs in relation to meat quality is an area where we can expect significant breakthroughs in the years to come? The answer might be ***no***, if the traditional thinking of feed *versus* meat quality continues to dominate. On the other side, if the focus changes towards an understanding of how feeding influences biological mechanisms and the outcome of these in relation to specific meat quality parameters, a picture begins to emerge of a huge potential for future production of diverse and specific meat qualities.

The present paper will pinpoint a few critical areas where a strengthened emphasis on a basic understanding of the influence of specific feeding strategies on biological responses may help to revolutionise future management practices aiming at the production of meat of the required quality. A fundamental understanding of muscle physiological and physical processes, and their interactions in relation to gene expression and environmental stressors, will be fundamental to exploiting future meat science and production through a systems biology[1] line of thought. Considering the already extensive knowledge of feed and meat quality, feeding seems the optimal tool in the further elucidation of physiological and physical events of importance for demanded meat qualities. Feeding can hereby become a key parameter together with the impending presence of genomes of most of the significant livestock in establishing a systems biology line of thought in the future quality assurance of meat. Simultaneously, the understanding obtained in employment of the discrete feeding strategies necessary for data accumulation to a systems biology approach will ensure temporary short-term solutions for specific meat quality problems.

In the following a few examples of feeding-dependent meat quality aspects of high priority from both an economic and/or socioeconomic point of view will be highlighted in relation to their potential as quality assurance tools. Furthermore, their contribution to the knowledge of importance for a coming systems biology line of thought in meat science will be discussed.

Feeding-regulated muscle protein turnover

Basic principles of muscle growth

Muscle growth is the major determinant of the performance of meat-producing animals. The amount of meat produced is related to the number of muscle fibres and the growth of the individual muscle fibre. Muscle fibres are formed during the embryonic and foetal stages, and the number is fixed around birth in most mammals. Thus postnatal growth is related to growth in the cross-sectional area (hypertrophy) and length in the fibres by adding additional sarcomeres. Postnatal growth is determined by the difference between two dynamic processes; i) the rate of protein synthesis and ii) the rate of protein degradation, referred to as the protein turnover. Thus, during postnatal muscle growth the rate of synthesis exceeds the rate of degradation. With increasing age both the rate of synthesis and degradation decrease and become equal in adult animals (for review see Oksbjerg, Gondret & Vestergaard, 2004). The rate of protein turnover is also related to the fibre type frequency, being higher in slow-twitch fibres than in slow-twitch fibres (Garlick, Maltin, Baillie et al., 1989, Goldspink, 1996).

Influence of feeding-regulated muscle protein turnover on specific meat quality parameters

The growth rate of farm animals is related to muscle protein turnover. Thus the more positive the muscle protein balance becomes the better is the growth performance of farm animals, hereby making this parameter economically essential in meat production. Moreover, maintenance of maximal positive muscle protein balance throughout the life of farm animals with minimal feed intake (superior feed conversion rate) is decisive for the environmental load during production. The proteolytic potential in the muscle at the time of slaughter has long been regarded as an important factor in the tenderisation process in meat, which claims

[1] Systems biology is an emerging field that aims at system-level understanding of biological systems. What is meant by a "system level"? In contrast to molecular biology, which has its focal point on molecules, systems biology focuses on systems that are composed of molecular components. Although systems are composed of matters including diversities and functionalities of components, the core of systems lies in dynamics and it cannot be described merely by enumerating components of the system. Moreover, both structure and components of the system play indispensable roles in forming the symbiotic state of the system. Within this context, *i*) understanding of structure of the system, such as gene regulatory and biochemical networks, as well as physical structures, *ii*) understanding of quantitative and qualitative dynamics of the system, and construction of theory/models with powerful prediction capability, *iii*) understanding of control methods of the system, and *iv*) understanding of design methods of the system, are the landmarks to assess to exploit how much we understand the system. For a further definition of systems biology, see Ideker, Galitski & Hood (2001) and Kitano (2002).

high muscle protein turnover in healthy animals at the time of slaughter. Consequently, management of muscle protein turnover may enable control of the three important meat quality attributes - *price*, *tenderness* and *sustainability*.

Relationship between muscle protein degradation in vivo and tenderisation post mortem

Several reports suggest that a relationship between the rate of muscle protein degradation and the rate and extent of tenderness in meat exists (Table 1). Thus, in situations where the rate of protein degradation is decreased, this may lead to increased muscle growth but decreased tenderness, e.g. *i*) treatment with β-adrenergic agonists (for review see Beermann, 1993), *ii*) restrictive feeding (Kristensen, Therkildsen, Riis, Sørensen, et al., 2002), *iii*) bulls versus steers (Morgan, Wheeler, Koohmaraie, Crouse & Sawell, 1993) and *iv*) animals possessing the callipyge gene (Lorenzen, Koohmaraie, Shackelford, Jahoor et al., 2000). In contrast, treatment with porcine growth hormone results in increased muscle growth by stimulating both the rates of synthesis and degradation without change in tenderness, which most probably can be explained by simultaneous increase in lean tissue (Sevé, Ballèvre, Ganier, Noblet et al., 1993; Oksbjerg, Petersen, Sørensen, Henckel et al., 1995). Finally, short term fasting for 5 days leads to increased rate of muscle protein degradation resulting in increased tenderness in lambs (McDonagh, Fernandez & Oddy, 1999). The link between the rate of muscle protein degradation and tenderness development may be coupled to the calpain system, which is known to be the rate-limiting proteolytic system disassembling the myofibrillar proteins to their individual constitutive proteins (Goll, Thompson, Taylor & Ouali, 1992; Koohmaraie, Kent, Shackleford, Veiseth & Wheeler, 2002). Having established a link between the rate of protein degradation and postmortem tenderisation, the challenge becomes to implement this into a feeding strategy.

Table 1. Factors affecting protein degradation parameters and shear force in muscle and meat, respectively, of meat producing mammals.

	Muscle Growth	FDR[a]	Calpastatin activity	Shear Force
β-adrenergic agonist	⇑	⇓	⇑	⇑
pGH[b]	⇑	⇑		⇔
Bull *vs* Steer	⇑	⇓	⇑	⇑
Fasting (short time)	⇔	⇑	⇓	⇓
Fasting (long time)	⇓	⇓	⇑	⇑
Callipyge gene	⇑	⇓	⇑	⇑

[a]Fractional Degradation Rate; [b]porcine Growth Hormone

For decades it has been recognised that compensatory growth may occur after a period of feed restriction in most farm animals, and the phenomenon has continuously been supported to take place in pigs (McMeekan, 1940; Critser, Miller & Lewis, 1995), cattle (Abdalla, Fox & Thonney, 1988; Rossi, Loerch, Keller & Willett, 2001), broilers (Washburn & Bondari, 1978; Acar, Petterson & Barbato, 2001) and sheep (McManus, Reid & Donaldson, 1972; Kabbali, Johnson, Johnson, Goodrich & Allen, 1992). The degree of compensatory growth or the index is calculated as: Index = (A-B)/A, where A is the difference in weight between feed-restricted animals and *ad libitum* fed animals following the restriction period and B is the difference between *ad libitum* fed animals and compensatory animals at the end (at slaughter) of the period (Hornick, Van Eenaeme, Gerard, Dufrasne, & Istasse, 2000). The degree of compensatory index may dependent on;

- Degree of restriction
- Length of the restriction period
- Length of the period of compensatory growth
- Sex
- Genotype.

During compensatory growth both the rate of protein synthesis and degradation are elevated according to findings in rat (Milward, Garlick, Stewart & Nnanyelugo, 1975) and cattle (Jones, Starkey, Calkins & Crouse, 1990). Consequently, implementation of a compensatory growth strategy in production of meat-producing animals could be a means to improve tenderness of meat.

However, the increase in protein turnover during compensatory growth is dynamic. Thus, initially during compensatory growth increased protein synthesis is evident while protein degradation remains low as a consequence of the former restricted feeding regime. Later on also the rate of protein degradation increases gradually and eventually exceeds the rate of protein degradation of control *ad libitum* fed animals. Thus, one of the goals to successfully implement a compensatory feeding approach in the production of meat of high quality is to establish the length of the compensatory period which results in highest muscle protein degradation potential at the time of slaughter. Recently, Therkildsen, Riis, Karlsson et al. (2002) showed that the optimal length of the compensatory period in pigs was between 42 to 50 days with regard to elevated protein degradation rate at slaughter. This was verified by measuring the activity of μ-calpain, m-calpain and their inhibitor calpastatin as indicators of protein degradation and total RNA and elongation factor-2 (eEF-2) as indicators of protein synthesis.

Having established the optimal length of the compensatory period of pigs in relation to elevated protein degradation rate at slaughter we have continued studies on the effect of compensatory growth response on muscle protein turnover and tenderness (Therkildsen, Vestergaard, Busk, Jensen et al., 2004; Kristensen et al., 2002; Kristensen, Therkildsen, Aaslyng et al., 2004). In general these studies showed that both castrated male pigs and female pigs exerted compensatory growth, while tenderness was only improved in meat from female pigs, see Figure 1 (Page 15). The reason for this is unknown at present; however, the compensatory growth response resulted in decreased intra-muscular lipid in castrated male, which may counteract the increase in tenderness by elevated protein degradation. In female pigs no effect of compensatory growth response on intra muscular-lipid was observed (Kristensen et al., 2004).

Use of a compensatory growth approach has also been verified to increase tenderness in meat from cattle (Allingham et al., 1998), and studies including young bull calves are now in progress in our laboratory, where the compensatory growth response may prove to be of even larger significance regarding the tenderness of beef compared to pork. The preliminary data show that both muscle protein degradation and synthesis reach a maximum level in bull calves exhibiting compensatory growth that exceeds the level found in continuously *ad libitum* fed calves. Thus there may be a time during compensatory growth, which would be the optimal time of slaughter with respect to tenderness development *post mortem* (Therkildsen, 2004).

Relationship between feed regulated muscle protein turnover and sustainability
The compensatory growth model used by Oksbjerg, Sørensen & Vestergaard (2002) and Therkildsen et al. (2004) resulted in a more efficient production in terms of feed, as the feed conversion ratio was improved by 5% in pigs exerting compensatory growth over the total period of growth. This will result in a lower environmental load with nitrogen and phosphorus. Similar results have been found in ruminants with

improved feed conversion ratio in periods involving compensatory growth, whereas the overall feed conversion ratio of both the restrictive period and the realimentation period is likely to be the same as in ruminants fed *ad libitum* throughout the experimental period (Abdalla et al., 1988; Carstens, Johnson, Ellenberger & Tatum, 1991; Sainz, De la Torre & Oltjen, 1995; Rossi et al., 2001).

Feed-induced manipulation of muscle energy levels

Basic principles of post mortem muscle metabolism

Glycogen plays the leading role as substrate for energy metabolism of living muscle as well as in the *post mortem* metabolism during which muscle converts to meat. The *post mortem* conversion of muscle to meat is an energy-demanding process, which requires ATP. After exsanguination, the energy needed for the *post mortem* process is mainly obtained from ATP produced by anaerobic metabolism and partly from phosphorylation of ADP by creatine phosphate (Henckel, Karlsson, Jensen, Oksbjerg & Petersen, 2002). The anaerobic metabolism of glycogen results in formation of lactate and a simultaneous decline in pH. Both the degree (Hamm, 1960; Bendall, 1973) and rate (Offer, 1991; Offer & Cousins, 1992) of *post mortem* metabolism are known to influence critical meat quality attributes, which is why control of glycogen and creatine phosphate levels at the time of slaughter may enable control of the vital technological quality parameters, e.g. water-holding capacity and sensory characteristics including colour and juiciness.

Influence of feeding-regulated glycogen levels on specific meat quality parameters

Sugar feeding, e.g. supplementation of high levels of sucrose or other digestible carbohydrate sources, a few days prior to slaughter or during overnight lairage has for decades been known to increase muscle glycogen stores and thus reduce $pH_{24\ h}$ (Briskey, Bray, Hoekstra, Phillips & Grummer, 1959; Fernandes, Smith & Armstrong, 1979). This is a short-term effect (Fernandez, Tornberg, Mågård & Göransson, 1992), where glucose is transported from the gastrointestinal tract via the circulation to the liver and the muscle, and subsequently incorporated into the glycogen stores. Such an increase in glycogen stores does not call for changes in the expression of any of the key enzymes of glycogenesis, as the increase is solely of a regulatory nature.

In contrast, other diet-induced regulations of the glycogen pools in monogastrial animals require changes in metabolic pathways and the glycogen synthesis apparatus. In this context muscle glycogen stores are reduced in rats (Lapachet, Miller & Arnall, 1996) and rabbits (Gierus & Rocha, 1997) using diets with a high fat content and digestible carbohydrate content. Similar results have been obtained after feeding standard diets with increased fat content to pigs from 25 kg live weight to slaughter (Lauridsen, Nielsen, Henckel & Sørensen, 1999). Recently, feeding diets high in fat (approx. 17-18%) and protein (22-24%) in combination with a low content of digestible carbohydrate (< 5%) to pigs during the last three weeks of finishing has been shown to reduce muscle glycogen stores in *M. longissimus dorsi* without influencing the overall growth performance (Rosenvold, Lærke, Jensen, Karlsson et al., 2001a; Rosenvold, Petersen, Lærke, Jensen et al., 2001b; Rosenvold, Lærke, Jensen, Karlsson et al., 2002). The combination of low digestible carbohydrate and high fat content in the diet causes a muscle glycogen-reducing effect (Figure 2, Page 16). The reduction in muscle glycogen content improved the WHC of *M. longissimus dorsi*, *M. biceps femoris* and *M. semimembranosus* (Rosenvold et al., 2001a; 2002). This reduction in muscle glycogen level did not result in higher $pH_{24\ h}$, but a higher $pH_{45\ min}$, suggesting a diet-induced delayed *post mortem* conversion of glycogen to lactate.

Bee (2001) has shown that only a minor reduction in digestible carbohydrate content (~20%) with a simultaneous iso-energetic supplement in fat has no influence on *post mortem* glycogen stores in porcine *M. longissimus dorsi* and the red part of *M. semitendinosus*. Hence, feed-induced reduction in muscle glycogen content, which affects pH_{45} and WHC in the porcine muscle, as observed by

Rosenvold et al. (2001a; 2001b; 2002b) seems to claim a critical ratio between fat and digestible carbohydrate. In support of this theory, Leheska, Wulf, Clapper, Thaler, and Maddock (2002) found no effect on *post mortem* glycolytic potential or pork quality attributes upon feeding a low digestible carbohydrate/high protein diet during the last two weeks prior to slaughter.

In ruminants several studies too indicate that muscle glycogen levels are at least to a certain extent responsive to finishing composition (for review see Immonen, 2000), despite the general belief that muscle glycogen stores are hardly affected by diet composition, as the ruminal fermentation is expected to dominate the availability of gluconeogenic precursors for hepatic glucose production. In an experiment with intensive (concentrate) or extensive (pasture) feeding with or without finishing feeding with concentrate of Friesian bulls, Vestergaard, Oksbjerg & Henckel (2000) found a decreased concentration of glycogen in *M. longissimus* and *M. semitendinosus* from bulls fed with pasture (E) compared with bulls fed on concentrate (I). However, when the E-bulls were finished on a concentrate ration for 10 weeks and had a compensatory feed intake compared with the I-bulls, the glycogen concentration increased and exceeded the level seen in the I-bulls.

Although muscle glycogen stores at the time of slaughter have long been recognised to be decisive for meat quality (Briskey, 1964), the physiological nature of the regulation of muscle glycogen stores is poorly understood (Graham & Adamo, 1999; Roach, 2002). This applies especially in *post mortem* muscle as existing knowledge is mainly based on findings in *in vivo* muscle. However, the recent identification of a key enzyme, glycogenin, and renewed interest in the existence of the two forms of glycogen, proglycogen and macroglycogen, may remedy this fact.

Proglycogen and macroglycogen are distinguished on the basis of size and protein content. The existence of two pools of glycogen was first studied extensively forty years ago (Stetten & Stetten, 1960). However, Lomako, Lomako, Whelan, Dombro, Neary & Norenberg (1993) were the first to describe the two forms in detail. The pool with a high ratio of carbohydrate to protein (maximum M_w ~10^4 kDa) is termed macroglycogen. The pool with relatively high protein content to carbohydrate (M_w<400 kDa) is termed proglycogen. It is known that the macroglycogen pool increases with high muscle glycogen concentrations (Jansson, 1981; Adamo & Graham, 1998; Hansen, Derave, Jensen & Richter, 2000; Graham, Adamo, Shearer, Marchand & Saltin, 2001). Moreover, dietary energy favours the *post* exercise synthesis of proglycogen over that of macroglycogen (Adamo, Tarnopolsky & Graham, 1998; Derave, Gao & Richter, 2000) and the metabolism of the two pools appears to depend on the type of exercise (Asp, Daugaard, Rohde, Adamo & Graham, 1999; Graham et al., 2001).

Proglycogen is degraded in favour of macroglycogen during the first 45-60 min *post mortem* in porcine muscle (Charpentier, 1966; Rosenvold, Essén-Gustavsson & Andersen, 2003). Moreover, total glycogen and the concentration of proglycogen have been found to be higher in pigs showing a rapid pH decline *post mortem* and subsequent development of inferior meat quality (pale, soft and exudative meat) (Wismer-Pedersen & Briskey, 1961). Reduced muscle glycogen stores as a result of a 3-week strategic finishing period are reflected in a reduction in the macroglycogen pool (Rosenvold et al., 2003). Interestingly, the subsequent reduction in the rate of *post mortem* glycolysis observed in muscle from these animals was due to reduced metabolism of the proglycogen pool. Recently, it was found that the increased glycogen level in pigs carrying the RN^- gene is reflected in larger macroglycogen stores (B. Essén-Gustavsson, unpublished observations), which might explain why early *post mortem* glycolysis is not enhanced in pigs carrying the RN^- gene compared with that of non-carriers. These preliminary data clearly show that a complete understanding of how feeding influences glycogen pools might be critical in setting up quality assurance schemes for the production of high quality meat.

Significance of vitamin E supplementation on muscle glycogen level

Vitamin E stripped diets have been reported to reduce liver glycogen stores in rats (Schroeder, 1974) and muscle glycogen stores in pigs (Lauridsen et al., 1999). Recently, we found that supra-nutritional vitamin E supplementation on the day prior to slaughter in both a strategic finishing diet known to reduce muscle glycogen stores and control diets gave rise to significantly higher muscle glycogen levels and tended to decrease WHC of meat from supplemented pigs (Rosenvold et al., 2002). Schroeder (1974) suggested that metabolic changes caused by vitamin E depletion were controlled by cyclic AMP, which is central in the co-ordinated control of glycogen synthesis and breakdown (Stryer, 1988). The hypothesis was rationalised on the fact that the result of vitamin E deficiency (e.g. increased catabolic activity) can be mimicked by the administration of glucagon, which stimulates glycogenolysis, lipolysis and the activity of certain catabolic enzymes through an increase in the cellular concentrations of cyclic AMP (Jost & Rickenberg, 1971 in Schroeder, 1974). It has also been noted that adenylate cyclase, the enzyme responsible for the synthesis of cyclic AMP, is localised mainly in the cellular membranes where vitamin E could play a major role (Srivastava, Robin, & Thakur, 1992). Moreover, vitamin E possibly inhibits protein kinase C (Mahoney & Azzi, 1988), which catalyses the phosphorylation and inactivation of glycogen synthase - the rate-limiting enzyme for glycogen synthesis (Ahmad, Lee, DePaoli-Roach, & Roach, 1984). However, the mechanism behind this effect of vitamin E on protein kinase C is still debated (Azzi et al., 2001). Thus, a vitamin E-induced inhibition of protein kinase C would increase the activity of glycogen synthase and stimulate glycogen synthesis, as found in the studies by Schroeder (1974), Lauridsen et al. (1999) and Rosenvold et al. (2002). Even though none of these studies can identify the mechanism for the significance of vitamin E supplementation on muscle glycogen levels, they do indicate that vitamin E has a direct effect on the mechanisms controlling glycogen metabolism. Considering the positive effect of vitamin E supplementation of farm animals on other meat quality parameters, e.g. colour and oxidative stability (Jensen, Lauridsen, & Bertelsen, 1998) and the negative effect on WHC in the study of Rosenvold et al. (2002), one could speculate whether an optimal level of vitamin E supplementation exists, where improved colour and oxidative stability are obtained before the possible elevation of muscle glycogen stores and increased drip formation overrides these positive effects. However, this calls for a better understanding of the influence of vitamin E supplementation on muscle glycogen stores.

Creatine

In contrast to many years of general belief, dietary supplementation with creatine monohydrate in humans, has been found to increase intramuscular creatine load by 20% (Balsom, Söderlund, Sjödin & Ekblom, 1995; Greenhaff, 1996). Moreover, it has been suggested that creatine supplementation increases the muscle energy stores as phosphocreatine (Balsom, Söderlund & Ekblom, 1994; Casey Constantin-Teodosiu, Howell, Hultman & Greenhaff, 1996). Supplementation with creatine monohydrate is known to increase weight gain (Maddock, Bidner, Carr, McKeith, Berg & Savell, 2000; Berg & Allee, 2001; Stahl, Allee & Berg, 2001; Young, Bertram & Oksbjerg, 2004a), probably by increased water retention in lean tissue (Balsom et al., 1995; Juhn, 1999). Moreover, muscle protein may also increase as the synthesis of myosin heavy-chain probably increases upon creatine supplementation in chicken skeletal muscle cells (Ingwall, Morales & Stockdale, 1972). A feed-induced increase in energy stores, as a consequence of elevated muscle phosphocreatine level should according to the generally accepted theory delay the glycogen metabolism in *post mortem* muscle and thus slow down the pH decline in the muscle, which minimize potential protein denaturation during the conversion of muscle to meat. A reduction in protein denaturation will consequently increase water-holding properties of the meat (Offer, 1991; Bertram, Dønstrup, Karlsson et al., 2001). Creatine monohydrate supplementation for five days has been shown to reduce the rate of the early *post mortem* pH decline and decrease cooking loss (Maddock et al., 2000; Berg & Allee, 2001; Stahl et al., 2001). Moreover, the incidence of PSE was significantly reduced in supplemented pigs (Maddock et al., 2000). However, extending the supplementation

period to 10 or 15 days had no (O'Quinn et al., 2000) or negative (Stahl et al., 2001) effect on technological pork quality, and high concentrations of creatine had even adverse effects on chicken meat quality (Young, Karlsson & Henckel, 2004b). The PSE-reducing effect of creatine monohydrate supplementation observed by Maddock et al. (2000) could be explained by the fact that half the pigs were carriers of the Halothane gene, and that the effect of creatine monohydrate supplementation was most pronounced in meat from pigs carrying the gene. However, in a consecutive study Maddock et al. (2002) found no significant effects of creatine monohydrate supplementation on technological pork quality. Effects of dietary creatine on meat quality traits are thus very diverse, and recent results by Young et al. (2004a) also demonstrate how differently two breeds of pigs are affected by creatine supplementation. Young et al. (2004a) showed that pure Duroc pigs had an increased pH both early *post mortem* and after 24 h, as well as reduced drip loss after dietary creatine treatment. Similar dietary creatine addition, however, had no systematic affect on *post mortem* pH in pure Landrace pigs, and drip loss was not significantly affected, although two independent drip loss methods indicated increased drip loss upon creatine supplementation (Figure 3, Page 17). These opposing results indicate that genetics play a major role for meat quality attributes related to dietary creatine treatment of pigs, and could be used in the elucidation of underlying mechanisms.

Genomics versus feeding - Nutrigenomics

The genetic influence on meat quality comprises differences among breeds as well as differences among animals within the same breed. With the exception of a few monogenic effects (major genes) in the different farm animals, e.g. the calipyge gene in sheep, the myostatin gene in cattle, the halothane, RN^-, 'intramuscular fat' and 'androstenone' genes in pigs, the heritability of most attributes relating to the quality of meat is low to moderate (0.15–0.30) (Sellier & Monin, 1994; Sosnicki, Wilson, Sheiss, & Vries, 1998). These differences in quality attributes are caused by a large number of genes with small effects, polygenic effects. The best approach to genetically improve meat quality is to identify relevant DNA-markers directly in populations under selection (de Vries, Faucitano, Sosnicki & Plastow, 2000). This calls for continuous meat quality measurements on the nucleus population of breeding organisations. However, this is a very long-term approach, which is extremely expensive, and as full assessment of meat quality can only be done after slaughter, the data have to be collected on culled animals and cannot be obtained on potential breeding animals. Another way to utilize the genetic potential of farm animals, considering the emerging development of molecular biological techniques, is the introduction of a molecular approach, e.g. DNA therapies, for altering biological processes of importance for meat quality (Grant & Gerrard, 1998). However, such an approach will hardly become ethically acceptable for conventional exploitation.

Alternatively, a fundamental understanding of the biological processes, which are directly linked to meat quality attributes, will rationalise a marker-assisted DNA approach, as this allows sampling on live breeding animals. Considering that most traits of interest for meat quality have a multifactorial background, i.e. meat quality attributes are due to interaction between the genetic potential and the environment, with feeding being the most decisive factor in meat production renders that a nutrigenomic approach seems most optimal in the exploitation of biological processes of direct importance for vital meat quality attributes. A nutrigenomic approach in relation to meat quality will in this way kill two birds with one stone, as it will support both a marker-assisted DNA approach and include a basic understanding of the most pronounced interaction of importance for meat quality, namely the interaction between the genome and the feed.

Nutrigenomics – an emerging strategy in modern meat science?

As illustrated in the above paragraphs, feeding has a regulatory effect on biological processes in muscle, which is directly reflected in the quality of the meat from the fed animals. This is in accordance with the fact that specific diet components *inter alia* are known to regulate gene expression within the cells. The knowledge of the complex interaction between the individual nutrients and their interaction on the genome of farm animals, which represent 30-40,000 genes within each species, is practically an unexplored area. The major reason for this is that the technical resources for such an understanding have only become accessible within the past few years. The ongoing mapping of the genomes of the principal farm animals together with the progress within information technology and molecular biological techniques will undoubtedly accelerate this process in the future.

The key element that distinguishes nutrigenomic from nutrition research is that the observable response to diet, or phenotype, is analysed or compared in different genotypes (or individuals). Classical nutrition research essentially treats all test individuals as genetically identical, and hereby misses a considerable part of the potential differentiation, which is essential in the future production of meat. Likewise, molecular biology and biochemical approaches show the same limitations as classical nutrition. Consequently, to include the environments, here represented by feeding, in relation to the expression or activity of variant forms (within groups or individuals defined by Single Nucleotide Polymorphisms (SNPs), haplotypes, and other polymorphism) of normal genes will allow a new essential understanding, which will have significant implications on future strategies to control meat qualities.

Conclusions

Considering a pro-active quality control system in the future production of high quality meat, the traditional way of using feeding as a quality control tool has outlived its usefulness. However, as emphasized by above results regarding fed induced control of muscle protein turnover and muscle energy levels and subsequent influences on important meat quality attributes of importance for the industry and/or consumers, feeding seems the optimal tool to explore biological processes of importance for meat quality development. Via a nutrigenomic approach feeding could be the base to initiate a systems biology line of thought in meat science, which subsequently could change from the present qualitative strategy to a quantitative strategy enabling development of optimal decision support systems for future meat quality control.

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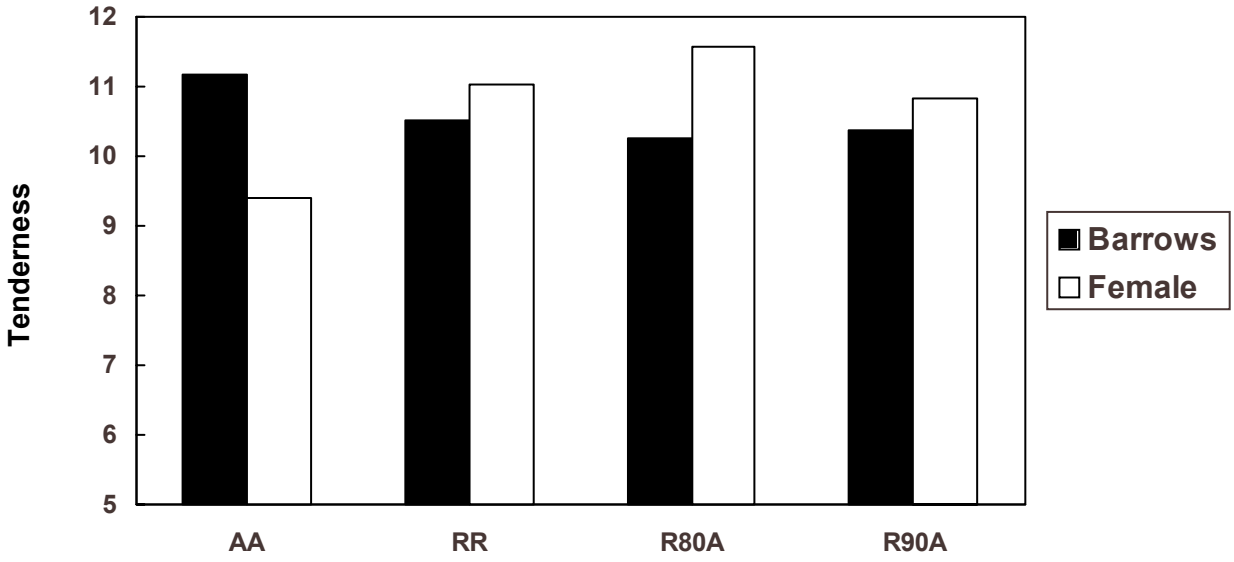

Figure 1. Tenderness (scored at a scale from 1-15, with 15 being extremely tender and 1 being extremely tough) of muscle longissimus dorsi from female and castrate pigs fed different from weaning (day 28) to slaughter (day 140). AA: Pigs fed *ad libitum* throughout the experiment; RR: Pigs fed restrictively (60% of *ad libitum*) throughout the experiment; R80A: Pigs fed restrictively (60% of *ad libitum*) from weaning to day 80 followed by *ad libitum* feeding to slaughter; R90A: Pigs fed restrictively (60% of *ad libitum*) from weaning to day 90 followed by *ad libitum* feeding to slaughter.

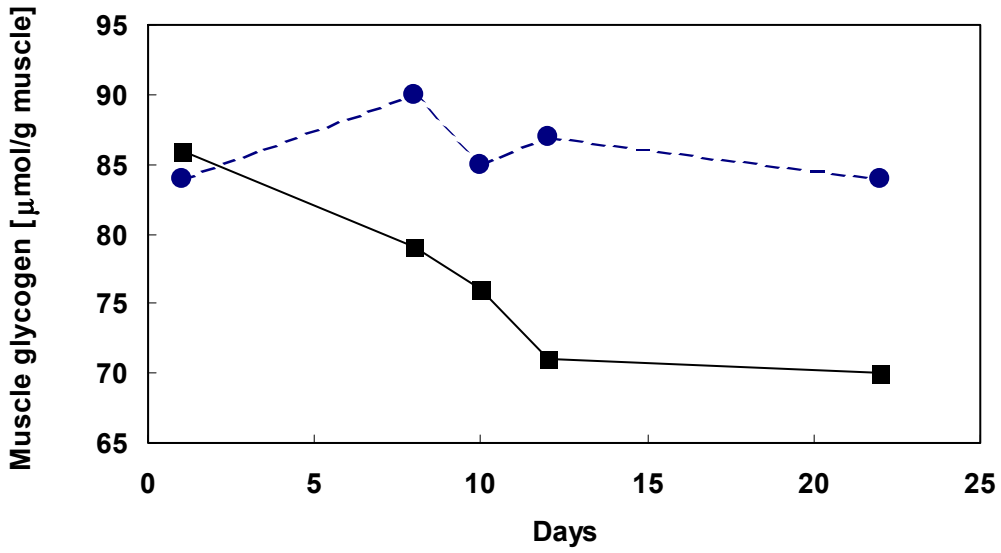

Figure 2. Muscle glycogen levels in muscle longissimus dorsi from slaughter pigs fed control diet (■—■) and after change to experimental diet (●- -●). Control diet: 19.5% soybean meal; 58% barley; 21% wheat; 1% animal and vegetable fat; 0.2% vitamin mineral mixture. Experimental diet: 50% rape seed cake; 10% soybean meal; 33% raw potato starch; 1% sugar beet molasses; 6% animal and vegetable fat; 0.2% vitamin mineral mixture.

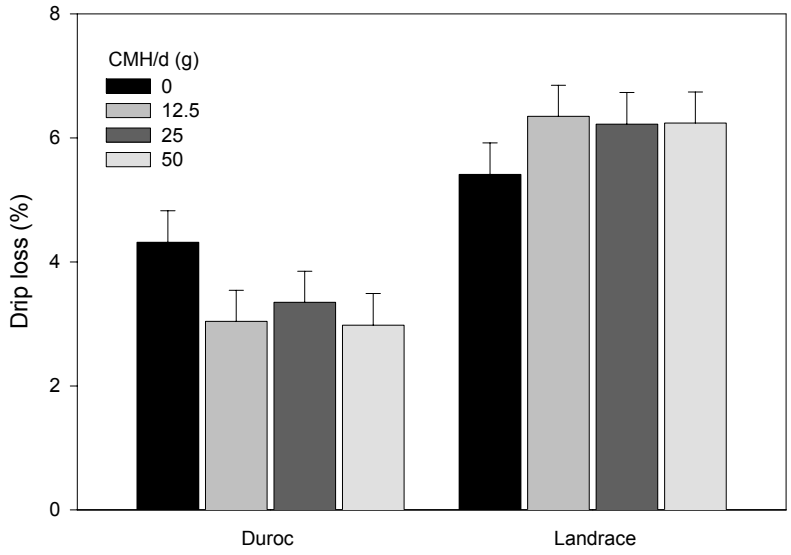

Figure 3. Water-holding capacity determined as drip loss (%) from muscle longissimus dorsi of creatine monohydrate (CMH) supplemented Duroc and Landrace pigs. Values are given as LSmeans.

THE FUTURE OF BSE FROM THE GLOBAL PERSPECTIVE

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Abstract

Although the BSE risk of most countries in Europe has been assessed and they have implemented both measures to control BSE and extensive surveillance systems, complete and valid data is still not consistently available. Globally, data is largely unavailable. Assessments based on incomplete or invalid information could lead to false conclusions. The BSE risk of countries throughout the world must continue to be assessed, and improvements in collection of surveillance data must be made, both in countries already reporting BSE cases and worldwide, in order to evaluate the global BSE picture and assure that cattle and products can be safely traded.

*Keywords**:** BSE, surveillance, risk assessment, GBR*

BSE in the world: what we know today

After bovine spongiform encephalopathy (BSE) was first diagnosed in cattle in the United Kingdom (UK) in 1986 (Wells et al., 1987), it became clear that the disease had been spread throughout Europe through trade in live animals, meat and bone meal (MBM) contaminated with the BSE agent, and feeds containing this MBM. Now, it is evident that the disease agent has continued to spread throughout the world through global trade in these products (Figure 1).

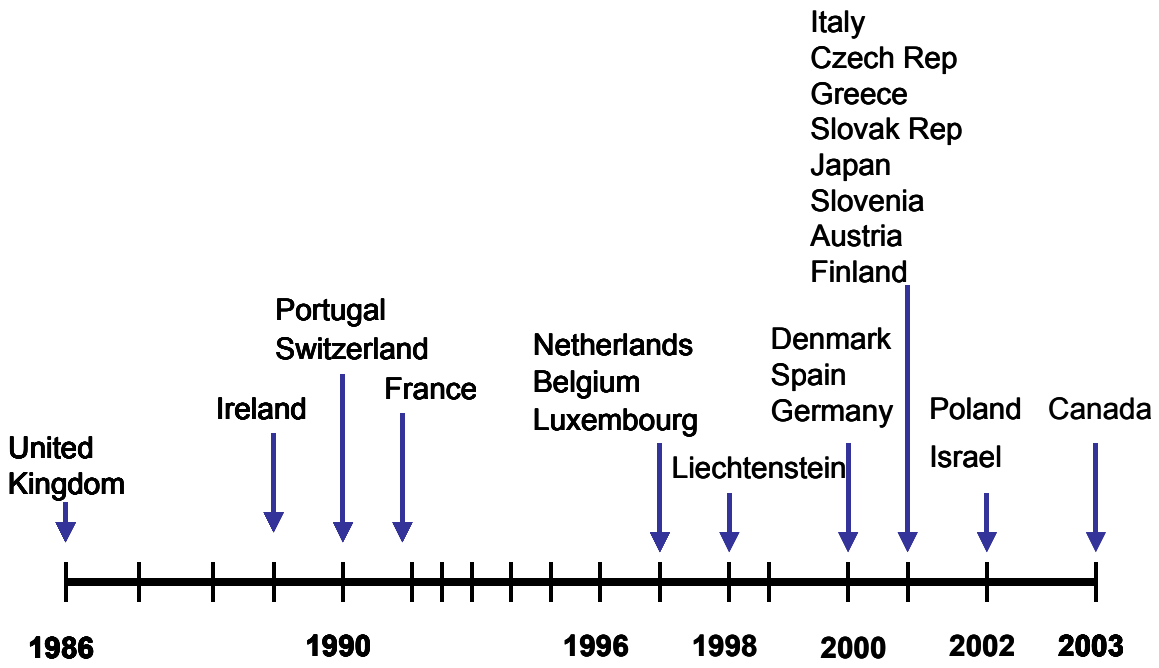

Figure 1: Year of first reporting of indigenous BSE cases

Extensive epidemiological studies have traced the cause of BSE to animal feed containing inadequately treated ruminant MBM (Wilesmith, Wells, Cranwell & Ryan, 1988), which was then recycled through the rendering/animal feed chain and amplified over time. It is possible that this amplification cycle began in the UK as early as the 1970s (Wilesmith, Ryan & Atkinson, 1991). The appearance of indigenous BSE cases throughout the world (e.g. Japan in 2001, Israel in 2002, North America in 2003) (Office International des Epizooties, 2004c) shows that, once the BSE agent is introduced, this cycle can be propagated outside Europe.

However, it was only after implementation of active surveillance programs targeted to BSE risk populations that many countries (in Europe and elsewhere) finally detected BSE, and year after year more countries detect their first BSE cases. Most of these countries had previously considered themselves to be “BSE-free” and many had exported BSE risk products. Trade in these products still continues among some countries that are considered “BSE-free” but possibly could have undetected cases. Therefore it is essential to be able to determine if an exporting country could have BSE before the first case is detected.

Risk Assessment & the GBR

This determination of the BSE status of a country can only be made on the basis of the outcome of a BSE risk assessment, which evaluates imports, surveillance data, and internal factors. This recommendation is codified in the BSE chapter of the Terrestrial Animal Health Code of the World Organisation for Animal Health (Office International des Epizooties, 2004a).

On the basis of the recommendation of the OIE, the Scientific Steering Committee (SSC) of the European Commission initiated the "Geographical BSE Risk Assessment" (GBR) for several countries. The GBR is defined as a qualitative indicator of the likelihood of the presence of one or more cattle being infected with BSE, pre-clinically or clinically, within the native cattle population of a country at any given point in time. In countries already reporting BSE, the GBR gives an indication of the level of infection (Scientific Steering Committee, 2000a, Scientific Steering Committee, 2000b, Scientific Steering Committee, 2002a, Scientific Steering Committee, 2002b, Scientific Steering Committee, 2002c).

The GBR qualitatively assesses risk factors that contribute either to the potential for introduction of BSE into the country or to the ability to cope with an introduction by asking the following questions:

- Had the agent been introduced to the country by import of potentially infected cattle or by feed with MBM, and if so to what extent ("external challenge")?

  In the assessment it is assumed that the BSE agent can only be introduced into a country by imports of cattle and MBM.

- What would happen if the agent were introduced into the animal production system, i.e. would it be amplified or eliminated ("stability of the system")?

  When risky imports are found to have occurred, the system's ability to minimize the exposure of cattle is evaluated in terms of the use made of MBM, the use made of SRM, the rendering conditions, and the feeding systems.

Currently 63 countries have been assessed (Table 1). These countries have been categorized into the following four levels defined by the SSC:

GBR I: highly unlikely that any BSE infected cattle are present.

GBR II: the presence of any BSE infected cattle is still unlikely, but it cannot be excluded.

GBR III: the presence of BSE infected cattle is likely or, if cases already were discovered, the number of BSE cases identified during the last 12 months is below 100 per million adult cattle.

GBR IV: more than 100 BSE cases per million adult cattle were discovered in the last 12 months.

The GBR has proven to be a useful tool in assessing countries in terms of their potential BSE risk. Before the detection of the first cases in many "BSE free" countries, the GBR showed that a risk could be present by categorizing them as GBR III. For example, Germany, Italy, Spain, the Czech Republic, the Slovak Republic and Poland, none of which had reported indigenous cases of BSE at the time of the original assessment, were included in GBR III and subsequently reported BSE in their national herds. Israel detected their first BSE case before the assessment was finalized, but the draft report already indicated GBR III. Thus, the results of the GBR (or any objective, comprehensive risk assessment) can be practically and reliably used to estimate the likelihood of a BSE problem in countries and categorize countries according to their risk.

However, as with all risk assessments, valid data is required to give a valid outcome. Until 2002, the GBR only considered imports of live cattle and MBM from countries with confirmed BSE cases as an external challenge. The subsequent detection of BSE cases in some GBR II countries (e.g. Austria, Finland, Slovenia and Canada) indicated that the approach underestimated the risk. In 2002 the method was changed to consider imports from all GBR III countries as an external challenge, even in the absence of reported cases.

Table 1: GBR level of countries assessed through June 2004

GBR I	Argentina*, Australia, Botswana, Brazil*, Chile*, El Salvador, Iceland, Namibia, New Caledonia*, New Zealand*, Nicaragua, Norway, Panama, Paraguay*, Singapore*, Swaziland, Uruguay*, Vanuatu*
GBR II	Colombia, Costa Rica, India, Kenya, Mauritius, Nigeria, Pakistan, Sweden, USA
GBR III	Albania, Andorra, Austria, Belarus, Belgium, Bulgaria, Croatia, Denmark, Canada, Cyprus, Czech Republic, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Ireland, Israel, Italy, Latvia, Lithuania, Luxembourg, Malta, Poland, The Netherlands, Romania, San Marino, Slovak Republic, Slovenia, Spain, Switzerland, Turkey
GBR IV	United Kingdom, Portugal

* Newly assessed in 2002/2003

Therefore, all GBR I and II countries have had to be re-assessed under these criteria. Re-assessments of some countries are complete and some are still ongoing. However, these re-assessments were initiated before the BSE cases in North America were reported and so, again, some re-assessments are necessary to account for this increased risk. This emphasizes the fact that, as the BSE situation changes worldwide, national risk assessments should be updated continuously using the most current data in order to remain valid and useful. As well, the fact that assessments should be carried out for all countries is clearly shown.

Risk assessments can also be used to ask additional questions, such as what are the BSE risk populations within the country, in order to guide development of a surveillance system. Although initial assessments of BSE risk can be performed using challenge data and internal stability data, further assessment requires additional information, such as surveillance data, from within the country, making risk assessment an iterative process.

Surveillance

Surveillance programs for BSE should be determined by, and commensurate with, the outcome of a national BSE risk assessment, as recommended by the Terrestrial Animal Health Code of the OIE (Office International des Epizooties, 2004b). The two major objectives for BSE surveillance are to determine whether BSE is present in the country, and, if present, to monitor the extent and evolution of the prevalence over time, thus monitoring the effectiveness of measures in place. It is important to remember that the number of BSE cases reported by a country can only be evaluated within the context of the quality of the national surveillance system. However, although OIE provides general guidelines, it does not currently provide specific guidelines for an appropriate level of surveillance for the different BSE risk categories. This makes the comparison and evaluation of national surveillance systems even more difficult.

Passive surveillance

Until 1999, BSE surveillance was limited to the notification of clinically suspected cases (passive surveillance). Mandatory reporting is certainly a basic requirement for a surveillance system, and in many countries it was assumed that this level of surveillance would allow early detection of a BSE outbreak.

However, passive surveillance relies solely on the identification and reporting of clinical suspects by farmers, veterinarians and others involved in handling animals to the veterinary authorities. For this system to function effectively, several factors must be considered (Doherr, Heim, Fatzer, Cohen, Vandevelde & Zurbriggen, 2001):

- Disease awareness at every level and in every sector is essential. Cases are often overlooked because the more dramatic clinical signs are normally only observed at the end-stage of the disease. Farmers, veterinarians, slaughterhouse personnel and others handling cattle play a crucial role and should be trained to recognize even the subtle, early signs of BSE. This requires an extended, long-term education program.

- The willingness to report suspected BSE cases is also essential. There must be minimal negative consequences to the identification of a positive case at the farm level, i.e. measures must be "reasonable". The motivation is low for a farmer to notify a suspect case if their whole "life-work" could be destroyed, therefore all consequences should be understood and accepted by the farmers and a reasonable compensation paid. Moreover, the stigma associated with BSE – especially with the first cases of BSE detected – should not be underestimated. These issues can be improved through proactive communication of information.

- Adequate laboratory competence must be available to ensure appropriate handling and examination of brain or other tissues collected through the surveillance system.

Because these factors vary greatly, both among countries and within countries over time, the results of passive BSE surveillance systems are subjective and evaluation and comparison of reported case numbers should be made carefully.

For example, the legal framework for passive surveillance within all EU member countries states that cattle of all age with clinical signs consistent with BSE should be examined for BSE. However, even within this stringent EU framework, variability exists, and some EU countries with a known BSE problem do not test the minimum number of animals set forth in the OIE code (Office International des Epizooties, 2004b). In the OIE code section, the minimum number of animals exhibiting one or more clinical signs of BSE (clinical suspects) that should be tested (based on the total cattle population over 30 months of age) is indicated, and suggestions are that the number is even too low. However, analysis of the number of tests performed in the EU between 2001 and 2003 showed that the number of suspect cattle examined varied between 2 and 408 per million adult cattle. (European Commission, 2002, European Commission, 2003, European Commission, 2004). Therefore, despite the OIE code recommendations, much less the stringent EU regulations, in some member states passive surveillance seems to be nonexistent and, with a few exceptions, it may be actually declining (Table 2).

Given this variability in the factors involved and the difficulty in effectively implementing a passive system, it has become obvious that passive surveillance alone is not sufficient to establish the real BSE status of a country, therefore, other solutions were sought.

Active surveillance

Other groups of cattle that are at increased risk of having BSE have been identified and can be actively targeted within a national surveillance system to improve the chances of identifying positive animals in the overall population and improve the validity of the data. These risk groups include cattle with signs of disease non specific to BSE (e.g. weight loss, loss of production) and cattle that died or were killed for unknown reasons. In different countries these animals may be defined as sick slaughter, emergency slaughter, fallen stock, or downer cows. It is known from the pathogenesis of the disease that cattle younger than 30 months rarely test positive, so the age of the population tested also is important.

Table 2: Number of clinically suspected cases of BSE per million adult cattle in the European Union, 2001-2003

Country	Clinical suspects per million cattle		
	2001	**2002**	**2003**
Austria	2	4	2
Italy	3	29	19
Finland	8	15	13
Greece	10	0	3
Germany	32	55	136
Sweden	36	37	23
France	43	18	39
Netherlands	54	23	15
Denmark	81	42	42
Spain	136	20	21
Luxembourg	140	140	40
Ireland	142	142	92
Belgium	161	186	111
UK	228	174	91
Portugal	408	188	128

For example, in January 1999 Switzerland initiated an active, targeted surveillance scheme to enhance the detection of BSE cases in the adult cattle population (Doherr, Oesch, Moser, Vandevelde & Heim, 1999). In addition to the mandatory reporting of all suspect clinical cases, all cattle that are in the above groups and that have at least four permanent incisors are tested. Additionally, a random sample of apparently healthy cattle is examined during regular slaughter to minimise diversion of questionable animals to slaughter (i.e. to improve compliance with passive surveillance and reporting). The development of rapid BSE tests (Scientific Steering Committee, 1999, Scientific Steering Committee, 2002d) facilitated testing of the increased number of samples collected through this system.

n the spring of 2000, a similar surveillance approach was introduced in Western France (Calavas et al., 2001). In January 2001, the EU introduced a standard system for the active screening of cattle for BSE (Heim & Kihm, 2003), in which all animals in the entire risk population (i.e. all cattle that have died or been killed on farm or during transport, fallen stock, and cattle sent to emergency/sick slaughter) over 24 months of age are tested. Additionally, all cattle subject to regular slaughter over 30 months of age are tested. Through this program, many more positive cases were detected and the number of reported cases in the EU increased in 2001 and 2002. In the 15 original countries of the EU (EU 15) in 2003 1,364 cattle were positive of more than 10 million tested. This suggests an overall downward trend in the number of cases. Interestingly, Spain and Portugal were the only countries in the EU 15 with increased cases in 2003 (figure 2). The basis for this reported increase is unclear.

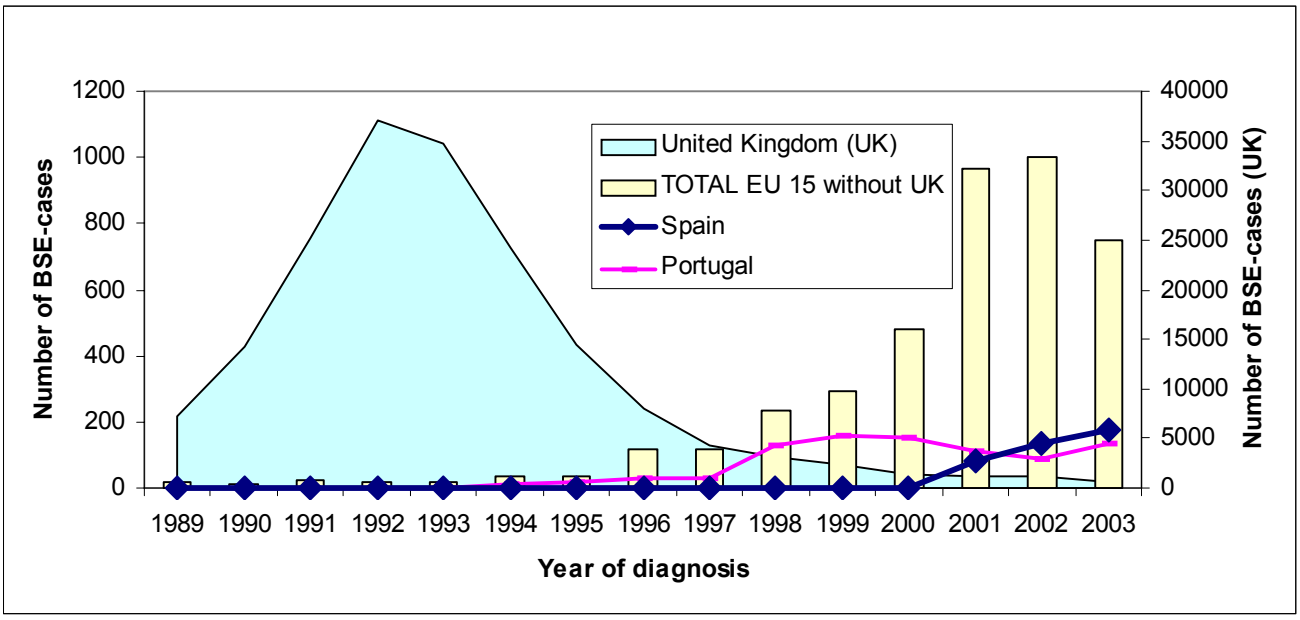

Figure 2: Number of BSE cases in the 15 original member states of the European Union from 1989 to 2003

Despite the fact that all EU member countries have the same legal requirements for surveillance (except UK and Sweden which have special regulations), other variability and inconsistencies can also be identified.

One evaluation of the data from 2001 and 2002 concluded that active BSE surveillance was operating reasonably in most member states although anomalies persisted (Bird, 2003). However, it is clear that some of the countries with a very low number of cases also tested fewer animals (Figure 3 and 4) and that the number of cattle tested varies substantially among member states. For example, the risk population tested has ranged between 0.62% and 5.86%, and the population of cattle at regular slaughter has ranged between 7.5% and 37.9% (excluding UK and Sweden) of the total adult live cattle population. Although some variations in the number of tests performed could be explained by different production and detection systems, the deviation is that significant that it can only be explained with an imperfect implementation of surveillance. This emphasizes that legal requirements alone are not sufficient and enforcement of implementation is crucial.

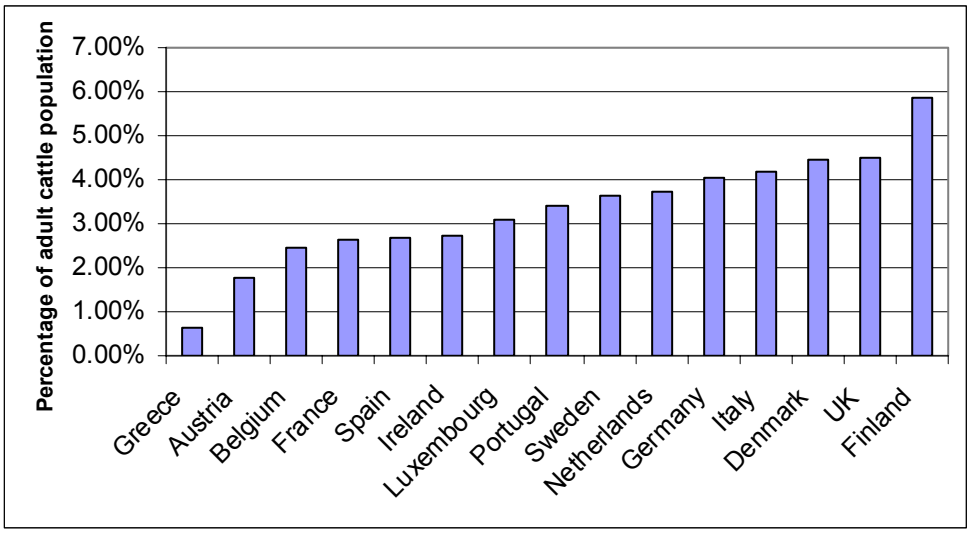

Figure 3. Cattle tested from the risk population, shown as a percentage of the adult live cattle population

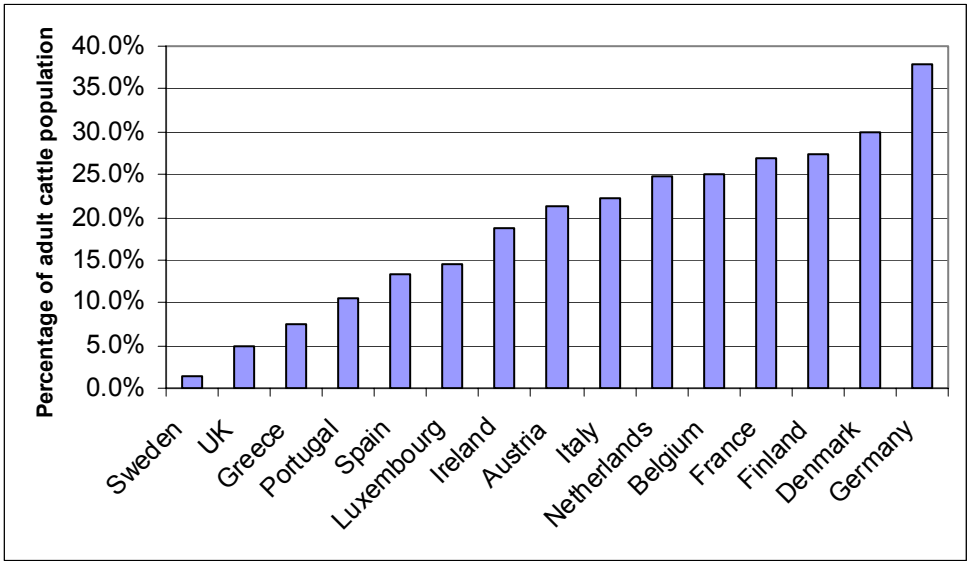

Figure 4. Cattle tested from the regular slaughter population, shown as a percentage of the adult live cattle population

The variability in the cattle population tested also affects the interpretation of the results. If risk populations, as described above, are tested, the efficiency of testing (i.e. the number of tests performed to find one positive) increases. If testing focuses on regular slaughter cattle, especially cattle younger than 30 months (or even younger than 24 months), a country may report a very high number of tests performed and low number of cases, but these numbers only give part of the picture. Most reports of tests performed and cases reported are not age adjusted, making country-to-country comparisons difficult. In addition, testing large numbers of low risk animals greatly increases the cost of a surveillance program, which may be especially important for countries with limited resources (Table 3).

Table 3: Number of cattle tested and BSE positive cases in the risk and the regular slaughter populations in the European Union

	Regular slaughter population		**Risk population**	
Year	2002	2003	2002	2003
number of cattle tested	7’511’862	8’716’481	1’030’484	1’295’770
number of BSE positives	237	265	938	783
rate of positives: 1 of	31’696	32’892	1099	1655
Costs to find one positive BSE case (assuming €70/ sample)	**€ 2,2 million**	**€ 2,3 million**	**€ 76901**	**€ 115’841**

Taken together, the different aspects of the variability in implementation make it extremely difficult to judge and compare the data even among member states of the EU. When systems in other countries are then compared, differences in basic system structures complicate interpretation of the variability in implementation even further. Therefore, as stated above, reported case numbers from the EU and worldwide must only be evaluated in the context of the quality of the surveillance program implemented in the individual countries, and trends must be looked at critically.

Outlook: the rest of the world

There remain many countries with an unknown BSE risk, as materials potentially infected with BSE have been distributed throughout the world (Office International des Epizooties, 2001). In a joint WHO/FAO/OIE Technical Consultation on BSE in June 2001, it was therefore concluded that all countries should evaluate their potential exposure through systematic assessment. As well, it is important for other countries to know the real BSE distribution worldwide in order to minimize import risks. Historically, however, few countries have undertaken a national risk assessment before detection of their first BSE case. In the same joint meeting it was concluded that countries should strongly consider, on the basis of these risk assessments, the use of appropriate BSE tests on target populations and on a sufficient number of animals, i.e. the development of targeted surveillance systems.

In North America, both Canada and the USA have undertaken quantitative risk assessments. The assessment of Canada (Morley, Chen & Rheault, 2003), published just before the first case was detected, concluded that the likelihood of BSE in Canada was extremely low to negligible. The Harvard risk assessment of the USA (Cohen et al., 2001) also concluded that the nation was highly resistant to any introduction of BSE and that BSE was extremely unlikely to become established in the USA. However, the many uncertainties over export data from Europe and lack of internal data (such as the effectiveness of enforcement of measures), combined with general problems in data validity as previously described, make it difficult to make reliable quantitative assessments of BSE risk. The quantitative and mathematically sophisticated nature of these assessments can lend an air of accuracy and validity to the conclusions that, in truth, they do not possess (Gravenor & Kao, 2003). Without good data, reliance on quantitative predictions is potentially dangerous and current quantitative risk assessments need to be interpreted carefully. Although in Canada and the USA some surveillance has been in place for several years, experience shows that it is likely not sufficient, either in population tested or in number of animals tested, to estimate the extent of the problem. Therefore, in June 2004, the USA began a one-year program to test the whole cattle risk population for one year (Anonymous, 2004).

In South America, several countries have been assessed in the GBR. Most of these are GBR I, one is GBR II. Argentina made their first risk assessment in 1991, and has already updated it several times. In the countries not assessed in the GBR, no risk assessment has been undertaken, often BSE is not notifiable, and the implementation of surveillance is in the initial stages (van Gelderen, Gimeno & Schudel, 2003).

In Asia, one survey on BSE concluded that the majority of Asian countries did not perform a risk assessment (Ozawa, 2003). According to Eurostat data, however, many Asian countries did receive significant amounts of MBM, implying that the BSE agent could have reached them. This was confirmed when Japan reported its first case in 2001. In Asia there are still countries in which notification of BSE is not mandatory. In other countries it is notifiable, but few have a compensation scheme for BSE suspects. This is reflected in the negligible number of suspects in most of these countries. In Japan, a quantitative risk assessment was published after the first case was detected (Sugiura, Ito, Yokoyama, Kumagai, & Onodera, 2003), and only in Japan has an intensive surveillance program been introduced.

Australia and New Zealand have been assessed in the GBR process and are currently categorized as GBR I. Both countries have implemented some surveillance.

From most other regions of the world, there is no information available regarding risk assessment and surveillance systems in place. As well, in most countries, only minimal measures are in place and may not be effectively enforced. Control of the amplification cycle, primarily through control of cattle feed, can significantly decrease the risk of BSE spreading within countries.

Conclusions

The evidence accumulated to date indicates that the control measures put in place since 1988, and especially since 1996 in the United Kingdom, have brought the epidemic under control, and there is no logical reason why the consistent decline seen over the past decade should not continue. Also in most of the other EU member states and many countries with reported BSE cases, measures including a targeted surveillance system, have been implemented.

However, differences in the structure and implementation of surveillance systems lead to variability in the amount and quality of the data available from almost all countries, including some countries of the EU. The variability complicates the ability to interpret and compare results.

Many countries have neither a risk assessment nor a surveillance system for BSE in place. These countries might be exporters of potential risk material to other countries, even though they have not been identified as countries affected with BSE. Because, minimal information exists regarding the BSE situation in these countries, their trading partners are subsequently limited in their ability to assess their own risks.

These uncertainties emphasize the need for all countries throughout the world to assess their risk. Implementation of a targeted surveillance system can at least provide basic information on the BSE status of these countries. As well, implementation and enforcement of some economically feasible proactive measures can act to control, or at least suppress amplification, of BSE at whatever level it is present.

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PRELIMINARY RAMAN SPECTROSCOPIC INVESTIGATION OF THE CHANGES IN BEEF STERNOMANDIBULARIS MUSCLE DURING THE THAWING OF MUSCLE STRIPS FROZEN PRE-RIGOR.

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Background

The potential of Raman spectroscopy in the analysis of foods has long been known, mainly due to the high information content (which can be understood in terms of chemical and physical parameters) which can be obtained without sample destruction and on any physical state, even in the presence of water. Very few investigations have been carried out on the application of Raman spectroscopy to the study or analysis of meat since Asher *et al* (Asher, et al 1976) first published a short account of the Raman spectrum of whole muscle. A number of studies in the mid 1980s investigated contraction of single fibres and various purified myofibrillar protein (Pézolet et al 1988). However, much of the work has concentrated on simplified systems and only recently has any work been attempted on bulk meat tissue (Beattie et al, 2004;Brondum et al 2000;Pedersen et al 2003).

The background understanding of the factors governing the Raman spectrum of meat is very limited so that it is important to extend the investigations of intact samples to conditions which are somewhat distant from conventionally processed consumer beef but where the data are easier to interpret. During the rigor process there are biochemical changes due to post mortem glycolysis (eg ATP depletion, lactate accumulation) and also changes in protein conformation due to the formation of actomyosin. Muscles frozen pre rigor when thawed go into rigor at a much faster rate and with a greater degree of contraction than normal rigor (Lawrie 1998). Davey & Graafhuis (1981) used thaw rigor as a technique for the early prediction of ultimate pH, since this was achieved within 10 to 20 minutes of thawing the samples. Thaw rigor provides a potential technique for studying boith the biochemical changes and contraction together by allowing samples to contract whilst thawing and the biochemical changes independent of contraction by securing the muscle to prevent contraction during thawing. The latter procedure should be similar to the normal rigor process where little contraction is observed aas the muscle goes into rigor.

Objectives

To study the changes in Raman spectra as strips of beef *sternomandibularis* muscles frozen pre rigor go into rigor on thawing in conditions which allow and prevent physical contraction occurring.

Materials and methods

Experimental

Samples of *m. sternomandibularis* were dissected from the neck at 30 min *post mortem* from 6 animals. From each muscle sample a number of strips 5 mm thick, 10 mm wide and 60 mm long were cut, wrapped in aluminium foil and placed in liquid nitrogen within 10 minutes of dissection.

Raman Spectroscopy

Raman spectra were recorded using the 785 nm excitation wavelength Raman spectrometer previously described (Beattie et al., 2004). Removal of the 'fluorescence' background was effected in SpectraCalc ™ where the multiple point baseline correction program was modified to allow the positions used for baseline points to be fixed. The initial and final spectra were averaged then subtracted to elucidate the variation between spectra recorded pre-thaw *rigor* and post thaw *rigor*.

The samples rapidly dissected upon removal from the freezer to give a fresh cut surface. Typically recording commenced *ca.* 8-10 min after removing from the freezer, while defrosting was complete *ca.* 12-15 min after

removal. Spectra were recorded for 1 min with a 20 second delay before recording the subsequent spectrum. The precise start time of each spectrum was recorded and every three spectra the length of the muscle was measured. For the thaw *rigor* studies the sample was not moved from its location. Half of the sample strips were loosely clipped on the rotating stage to allow contraction to occur, while half of the strips were firmly clamped at each end to prevent significant amounts of contraction.

The presence of collagen in the beef samples was determined by the band intensities at positions which are known to correspond to various modes of proline and hydroxyproline (Beattie 2002). Any samples showing exceptionally large changes in collagen within the spectra upon contraction were eliminated, to allow clearer interpretation of the changes affecting the myofibrillar proteins. Subtracted Raman spectra were generated to elucidate the changes in the Raman spectrum during thaw *rigor*. For the unclamped sample set this subtracted spectrum was produced by averaging all the samples prior to the onset of contraction, those after contraction had completed and performing a spectral subtraction normalised on the phenylalanine band at 1003 cm^{-1}, which is widely recognised as being insensitive to environmental factors. A residual band was evident at a slightly higher wavenumber positions, but was sufficiently weak to not interfere with the normalisation. For the clamped data set, the spectra recorded at the same times when contraction started and had completed in the unclamped samples were selected for averaging and subtracted as before.

A sample of silverside aged for 21 days (Beattie,et al., 2004) was used to compare the effects of sample orientation relative to the incident beam by mounting the sample with the myofibrillar long axis parallel and perpendicular to the incident beam.

Results and discussion

Figure 1 shows the average Raman spectrum of the pre-thaw *rigor* samples, with some bands of interest marked with a number referring the table of band assignments (Table 1).

The subtracted Raman spectrum from the unclamped data set is shown in Figure 2a. There are large changes in the regions known to be sensitive to the secondary and tertiary structure of proteins, the amide I (1640-1685 cm^{-1}), amide III (1225-1305 cm^{-1}) and ν(C-C) region (880-1020 cm^{-1}). One possible explanation of changes in these regions is typically that there is widespread conformational change within the proteins of the sample. However, there is another explanation which might apply in this particular case due the very high degree of orientation involved in the myofibrillar proteins and that is that the Raman spectra might have changed due to reorientation. It is well known that the Raman spectrum of highly oriented molecules can look different depending on their orientation relative to the incident radiation. Figure 2b shows the subtraction Raman spectrum obtained by subtracting the Raman spectrum of aged beef acquired with the myofibrillar long axis parallel to the beam away from the Raman spectrum acquired with a perpendicular orientation. Comparing Figure 2 a and b shows that there is a striking similarity in many areas, which would suggest that much of the changes occurring in the three regions highlighted above may be arising from reorientation of the myofibrillar proteins.

The average contraction observed was 60 % of the original muscle length, the level of contraction at which the myosin pushes against the Z-disk that hold the actin in place. Since both major proteins (actin and myosin) overlap fully and the anchor proteins are being perturbed, it is reasonable to assume much of the protein will be distorted, causing widespread reorientation with respect to the myofibrillar long axis. Thus it seems reasonable to assign the changes in the amide and ν(C-C) regions to reorientation of the myofibrillar proteins rather than large scale conformational changes.

Figure 2c shows the subtracted Raman spectrum of the clamped samples, and it is readily noticeable that the effect of thaw *rigor* on the amide and ν(C-C) regions is significantly different from the unclamped samples. The amide I and ν(C-C) regions are noticeably weaker for the clamped samples, suggesting a much slighter change in orientation. This is not surprising as only a small amount of overlap occurs between the actin and myosin so only a few myosin S2 heads are reorienting, along with the various other minor proteins involved in the binding of myosin to actin. If the predominant effect had been conformational change, rather than orientation, it would be expected that the spectra of the clamped and unclamped data set would be much more similar. The fit between the orientation subtraction spectrum and the unclamped thaw rigor is expected since the effect of extreme contraction would be that the myofibrillar axes would be buckled and instead of recording the Raman spectrum using a beam which truly perpendicular to the fibre axis we are recording some of the fibres at an angle to that axis.

While there are clear differences between the amide and ν(C-C) regions of the Raman spectra of the clamped and unclamped data sets, there are also some bands which show similar changes between the two data sets. These bands are marked in Figure 2 by the dashed vertical lines. These bands are all ones that are known to be affected the polarity of the environment of the protein. In the clamped data set the $CH_{2\ sc}$ band at 1450 cm^{-1}, which is negatively correlated with hydrophilicity, shows a strong decrease, which indicates a strong increase in the polarity of the protein environment. This result is expected as the massive contraction observed in thaw *rigor* is caused the release of large amounts of calcium salts, which will significantly increase the ionic strength of the myofibrillar matrix, thus the polarity of the proteins' environment. The same band at 1450 cm^{-1} is observed to increase, contrary to the expectation of the environmental changes, but in line with the changes to the orientation of the myofibrils, showing that the reorientation has a stronger effect on that band than the environmental effects.

Conclusions

We have demonstrated that the Raman spectrum of meat is affected by the process of thaw *rigor*. When the sample is unrestrained and able to contract freely, these changes can primarily be attributed to the reorientation of the myofibrillar proteins. When the sample is restrained so that significant amounts of contraction are not possible, it was found that the effect of reorientation was reduced and that the increase in ionic strength during thaw *rigor* affected the environmentally sensitive bands.

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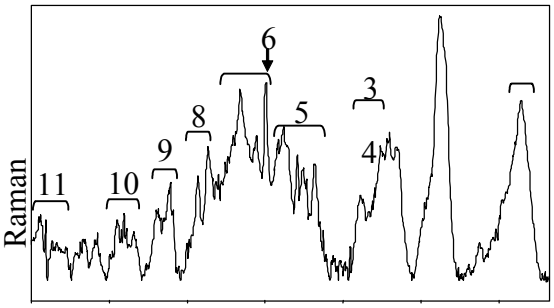

Figure 1 Average Raman spectrum of pre-thaw rigor *m. sternomandibulasris*. Peaks of interest are number as for

No	Band Position/ cm^{-1}	Comments
1	1640-1685	Amide I – COONH.
2	1450	CH_2 scissor.
3	1225-1305	Amide III
4	1260	His, tautomer II.
5	1020-1130	C-C, N stretch.
6	1003	Phe ring stretch.
7	880-1020	C-C stretching bands
8	820-860	Tyr
9	600-800	Cys and Met
10	500-570	Cys-Cys and skeletal
11	410-480	Pro and Hyp bands

Table 1 Assignments of the main peaks of interest in the Raman spectra of meat and muscle. The band numbers refer to the average spectrum of aged pork meat in Figure 1.

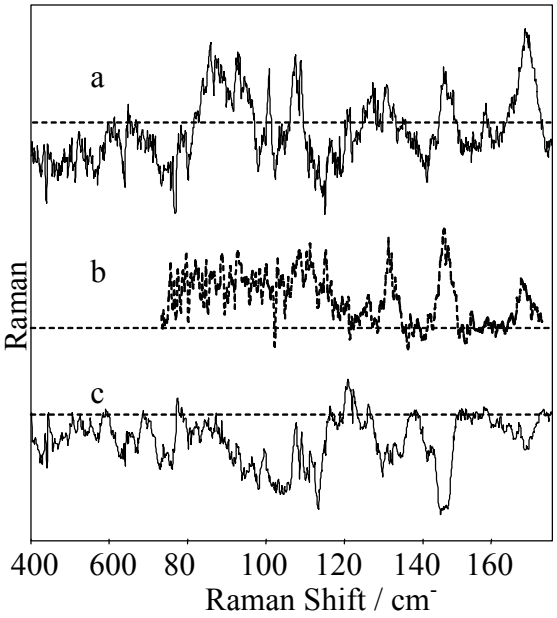

Figure 2 Effect of the development of thaw rigor on the Raman spectrum of beef which has a) not been clamped and c) been clamped. b) subtraction spectrum of parallel and perpendicular spectra of aged beef.

EFFECT OF OUTDOOR REARING SYSTEM ON FAT DEPOSITION AND EATING QUALITY IN ORGANIC HEAVY PIGS

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Background

Over the last years, the interest in organic production has increased considerably in Italy. However, only a small percentage of pigs today are organically produced (about 0,2 % of total slaughtered pigs in 2003). Nevertheless, the organic sector, both fresh meat (loin) and traditional processed products (dry cured ham, salami, etc.), is growing. The possibility of fattening pigs through outdoor system using pasturage field, seems to meet the organic regulation requirements for animal welfare and farmer demand for low cost investments (housing and manure utilisation). Over the last ten years, several studies were published on the effects of outdoor rearing system on growth performance, carcass composition and meat quality traits of fattened pigs. However, almost all research has considered the slaughter animal live weight approximately between 80 to 110 kg. Effects of grazing, physical activity (exploring, rooting, etc), fattening season (cold or hot weather, raining days), soil conditions (type of cover crops, watery mud), on fresh meat quality traits are still not well known. Investigations on eating quality, mainly performed on loins (*M. Longissimus dorsi*), have produced contradictory results, as far as pigs from different rearing (outdoor *vs* indoor) or finishing systems (conventional *vs* organic) have been compared.

Objectives

In the Italian production, more than 80 % of pigs are slaughtered at an average live weight of 165-170 kg (heavy pig, with minimum age of 9 months) for traditional dry cured ham production. The quality traits of fresh ham intended for curing have obviously been considered more important than those of other cuts. However, eating quality profile of fresh loin is also a concern for organic driven consumers.

This study investigates the main eating quality traits of pork loin (*M. Longissimus dorsi*) from outdoor and indoor organically produced heavy pigs.

Materials and methods

Investigation was carried out using a total of 96 samples of pork loin from heavy pigs –castrated male only-, produced in 3 different organic farms, and slaughtered at the same abattoir during 4 trials.

Animals

The pigs were from three-way cross-bred slaughter pigs, where the terminal sire was pure-bred traditional Italian Large White and the dams were crosses between Landrance and Duroc. All piglets came from one herd, organic farm with reproductive outdoor system; after 45 days of farrowing and 55 days of post-weaning, pigs were delivered to the three finishing farms (average live weight 37.7 ± 3.8 kg). Some pigs was stalled in a conventional piggery according to organic specification (indoor rest area with deep straw and outdoor concrete paddock) in groups of 8. The pigs reared outdoors were kept in grass fields (2/3 grass 1/3 wood bush, about 3000 m^2 each group of 8 animals). All pigs were fed restrictedly twice a day with the same commercial organic feed (15.5% crude protein, 0.65% lysine, 1.8% linoleic acid, 12.2 MJ of ME/kg of dry matter) receiving 2.0-2.5-3.0 and 3.5 kg/d of feed approximately at 40-80-110-140 kg of live weight (the study started in May and ended in November 2003). After 3 h of transport and 2h of rest in the lairage, pigs were electrically stunned and slaughtered.

Meat

At the end of slaughter line, the estimation of carcass meat percentage was performed using FOM (Fat o Meter equipment) by measuring subcutaneous fat (twice) and LD muscle depth. Subcutaneous fat thickness of fresh ham was also manually measured in the lateral side at level of *M. Biceps Femoris*. Carcasses were immediately hot boned (approximately 40-45 minutes after slaughter), and the primal cuts were cooled

separately (loins-rib in, shoulders, hams, bellies, back fat, and neck cuts) at 0-2 °C for 24 h (air speed 0.5 m/s), as usual in Italian pig slaughterhouses. pH was measured at 45 min and 24 h by inserting the electrode in the *M. Longissimus dorsi* at the last rib. A 10-12 cm section of LD sample was dissected from each loin (right side), after 24 h cooling (above the last rib), vacuum packed and transported to the lab for analysis. From each sample a slice of 2.5 cm thick was used for cooking loss and shear force measurement, using a Instron equipment with Warner Bratzler device on boiled meat (cooking was complete when central temperature reached 75 °C). Two chops of 2.5 cm thickness were assigned to panel test (8 trained panellists) for sensory analysis on roosted meat, cooked in oven (cooking was complete when central temperature reached 75 °C). The panellists used an 8-point category scale to score the tenderness, juiciness, pork flavour and overall palatability. Both shear force and sensory analysis were performed on fresh meat after 3 days of ageing. A slice of fresh meat was used for chemical determination of intramuscular fat (IMF) and fatty acid composition (Riley et al., 2000).

Statistical analysis

Lean meat percentage, fat thickness, ph, IMF, fatty acid composition and sensory data were analysed by general linear model procedures (SAS procedure GLM). Rearing system, batch and finishing farm were considered as fixed effects. The two-way interaction between rearing system and farm were also tested. Differences between pair-wise combinations of the least square means were tested for significance ($P<0.05$).

Results and discussion

Main results of carcass quality traits are shown in Table 1. Organic pigs finished outdoors had lighter carcass weights, leaner carcasses and less subcutaneous fat deposits (P <0.005). Difference in daily gain and carcass weight between indoor and outdoor rearing system in finishing pigs may be explained with the different amount of energy needed for physical activities (exploring, rooting, etc.) of free ranging pigs, as reported in similar studies (Enfalt et al., 1997; Sather et al. 1997). However, other studies (Beattie et al., 2000; Gentry et al., 2002) found a positive or no effect of rearing system on average daily gain and carcass weight, in outdoor finished pigs. It is clear that the level of physical activity depends on e.g., soil characteristics, presence of mud, raining days and climate conditions (temperature, day light etc).

Table 1. Least square means for carcass and main LD quality traits

	rearing system (RS)			significance	
Trait	indoor	outdoor	SE	farm	farm x (RS)
Carcass traits					
Cold carcass weight (kg)	133.4[a]	127.2[b]	5.65	<0.05	<0.05
Lean meat (%)	50.6[b]	52.2[a]	1.85	n.s.	n.s.
P2 fat thickness (mm)	32.3[a]	30.1[b]	2.12	<0.05	n.s.
Ham fat thickness (mm)	27.7[a]	25.5[b]	1.15	n.s.	n.s.
LD quality traits					
Ph 1	6.37	6.42	0.12	n.s.	n.s.
Ph 24	5.57	5.52	0.08	n.s.	n.s.
Intramuscular fat (%)	3.8[b]	4.1[a]	0.22	n.s.	<0.05
Shear force (kg)	2.8	2.9	0.27	n.s.	n.s.
Cooking loss (%)	28.6	27.4	2.04	n.s.	n.s.
LD sensory analysis					
Tenderness	4.36	4.17	0.16	n.s.	n.s.
Juiciness	4.65[b]	4.88[a]	0.21	n.s.	n.s.
Pork flavour	3.31	3.48	0.13	n.s.	n.s.
Overall liking	3.84[b]	4.12[a]	0.11	n.s.	n.s.

LSM estimates with different superscript, within a row (rearing system) differ, $P < 0.05$; n.s. not significant

All these environmental, management and climate variables may play a critical role in increasing or decreasing of the activities of free range pigs, making the comparison between different studies difficult. During this experiment (from May to November, 2003) high summer-autumn temperatures and a long rainless period, may have reduced the environmental effect on energy consumption.

Significant reduction of backfat in carcasses from outdoor finished pigs was observed, with consequent increasing of estimated lean meat percentage. Lean meat percentage values found in this experiment are not comparable with other findings from similar studies, because of the great difference on carcass weight and specific estimating equation used.

An unexpected significant reduction of subcutaneous fat was found in the ham of the outdoor finished pigs. This may be considered a negative aspect, since the required level (Regulation for typical Italian cured ham, EC origin protected product) is fixed at 20 mm minimum of thickness. However, more data should be recorded to clarify if the intense physical activity of free range could affect the development of thighs (bone length and ham thickness etc.).

Intramuscular fat of outdoor finished pigs was significantly higher than that of the indoor finished. The result is in agreement with Gentry et al. (2002), but conflicts with the findings of Enfalt et al., (1997) and Danielsen et al. (2000). Nevertheless, the high carcass weight and the age of pigs in this experiment, call for great attention on comparative findings from different studies. It is possible that in long fattening period with restricted feed, the age (9-10 months), physical activity and muscles maturity, may play an important role on shifting fat deposition from subcutaneous to intramuscular depots. No significant differences were found concerning pH1 and pH24, Warner Bratzler shear force on cooked meat, or cooking loss between the two rearing system.

Table 2. Fatty acid composition (% least square mean and standard error) in intramuscular fat from LD

	rearing system		
Fatty acid	indoor	outdoor	SE
14:0	1.36	1.43	0.08
16:0	26.18	25.48	0.32
16:1 n-7	2.93	2.72	0.12
17:0	0.15	0.16	0.01
18:0	13.12	12.94	0.27
18:1 n-9	43.86	43.66	0.21
18:1 n-7	3.36	3.47	0.05
18:2 n-6	6.12[b]	7.03[a]	0.23
18:3 n-3	0.74[b]	0.81[a]	0.02
20:0	0.46	0.35	0.09
20:2 n-6	0.54	0.61	0.02
20:4 n-6	0.27[b]	0.36[a]	0.03
SAFA	41.27[a]	40.36[b]	0.27
MUFA	50.69	50.46	0.38
PUFA	7.13b	8.20a	0.31

LSM estimates with different superscript, within a row (rearing system) differ, $P < 0.05$; Identified fatty acids below 0.15% -trace- are not shown

Eating quality can usually be well described by a sensory panel. Juiciness and pork flavour are probably the most important meat quality traits for consumers eating pork. In this experiment, no differences were found for tenderness and pork flavour. However, the panellists found the meat of outdoor finished pigs juicier and more acceptable (overall liking). Several researchers have found no differences in the eating quality of pork when comparing pork from indoor and outdoor rearing (Van der Wall et al., 1993; Jonsall et al., 2000), nor a negative effect on tenderness or juiciness (Enfalt et al., 1997). Jensen and Jakobsen (1996) did, however, report a clear difference in meat quality and sensory quality between loins from organic pigs reared indoors and outdoors. Also Gentry et al. (2002), reported that meat from outdoor finished pigs has higher flavour intensity. In this experiment, better juiciness scores of loins from outdoor finished pigs compared to indoor finished, may be linked with the higher level of intramuscular fat found. No easy comparison is possible from the sensory quality results of this experiment and other similar studies, since slaughter procedure applied in Italy (hot boning and loin dissection from bones, with high risk of cold shortening before rigor mortis) may play a critical role on influencing tenderness and juiciness.

Fatty acid composition in the intramuscular fat of the loins (*M. Longissimus dorsi*) are show in Table 2.

Only small differences in fatty acid composition (%) between the loins of outdoor and indoor finished pigs were found. A slight increase in unsaturated fatty acids was observed for 18:2 n-6, 18:3 n-3 and 20:4 n-6, from outdoor finished pigs, and consequently, SAFA and PUFA were significantly affected. The fatty acid composition of the pork intramuscular fat has been reported to be affected by feed composition (Kouba et al., 2003), breed or genotype, sex as well as carcass fatness (Wood et al., 2003). Furthermore, it has been suggested that the rearing system may affect the lipid composition of pig muscles (Hogberg et al., 2001) when interacting with sex and genotype.

The differences found in this experiment in polyunsaturated fatty acid (18:2 n-6, 18:3 n-3 and 20:4 n-6) composition may due to the intense physical activity of outdoor finishing pigs compared to the indoor groups, with consequent change on lipid classes (neutral and polar lipid) with their respective roles in living animal (Clarke, 2000). However, because outdoor pigs were reared in a large grazing area, the amount and quality of grass daily ingested may have affected the total amount of unsaturated fatty acids in the feed. No significant effect of the type of organic finishing farm was found on the fatty acid composition of IMF.

Conclusions

Overall, results comparing indoor and outdoor pig finishing systems have been variable. Some reasons for this variation include differences in seasonal effects, ground type, grazing area, genotype and final slaughter weight. Organic production system with its specific managing and feeding requirements, may also affect the performance and meat quality of outdoor finished pigs. Changes in carcass composition (leaner of fatter) and fat distribution, in outdoor finished pigs compared to the indoor-finished, may represent a positive or negative aspect depending on final product destination (fresh meat or traditional cured salami).

Manipulation of fatty acid composition, by changing feed composition and feeding regime in outdoor organic pig finishing system, using grass or silages, will have to be carefully tested on large scale experiments. For processed products with long curing, such as dry cured ham (14-16 months of seasoning), increasing of PUFA should be carefully monitored, for increasing risk of fatty acids oxidation and off-flavour development.

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QUANTITATIVE EVALUATION OF CHLORAMPHENICOL CONTENT IN CHICKEN MEAT PRODUCED BY DOMESTIC PRODUCERS WITH ELISA - TEST

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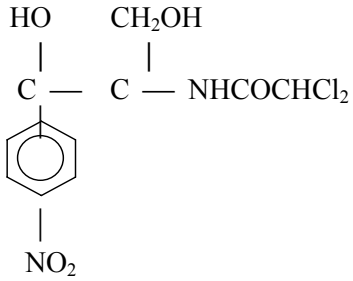
Background

Chloramphenicol is an antibiotic of wide specter. It affects Gram-positive and gram-negative bacteria, rickettsia, spirochete and Chlamydia. Because of its fantastic antibacterial effects and pharmacokinetics it has been used very often in animal treatment.
According to its chemical composition it is a derivate of nitro-phenol, molecular formula is C11H12Cl2N2O5 molecular weight M =323, 13 g/mol. Its full name is D-[-]-threo-2-dichloroacetamido-1-[p-nitro-phenyl]-1, 3-propaniol.

Structural formula:



This compound is very stable up to temperature of 100°C and it is in the wide range specter of pH (2-9). Pure substance is very liposoluble; poorly soluble in water (0, 25%) because of that it is used therapeutically in the form of palminate, stearate and sucinate. Chloramphenicol is metabolized in liver and excreted from body through urine.
Treatment of human beings by chloramphenicol is only recommended in life threatening infections. Misuse of chloramphenicol can lead to: leucopenia, trombocitopenia, irreversible plastic anemia etc. It is fully cumulative toxin because of its structure or toxic metabolites (mainly the ones that contain nitro group).
Due to chloramphenicol negative impact on human's health, treatment of animals whose products meat, milk and eggs are used for human's consumption, is strictly prohibited in EU since 1994. According to regulations, the presence of residues of this antibiotic in food must be monitored continuously in all products of animal origin.
Conventional method (radioimmunology) for quantitative evaluation of chloramphenicol has been switched by new more precise methods as: gas chromatography/MS, liquid chromatography/MS and ELISA-test. These methods are more specific and more sensible with detection level (10^{-12}g/g), or (ng/kg).

Objectives:

During March 2004 the authors of this paper were investigating presence of chloramphenicol content in samples of chicken meat from market of Canton Sarajevo. We expect higher level of self control at our producers and importers**.**

Materials and methods:

Elisa-test is analytical method which can be used for quantitative evaluation of: antibiotics, hormones, pesticides, vitamins etc; in products of animal origin. Authors of this paper were quantitatively evaluating the content of chloramphenicol residues in chicken meat during March 2004 from market of Canton Sarajevo.

Principle of the assay

Assay is based on the antigen-antibody reaction, which is being done in vitro on micro titer plate. Micro titer plate is precoated by fixed antibodies of sheep to rabbit IgG. Chloramphenicol standards or diluted chicken meat samples, chloramphenicol enzyme conjugates and antichloramphenicol antibody is added. Free chloramphenicol and chloramphenicol enzyme conjugate compete for chloramphenicole antibody binding sites (competitive enzyme immunoassay). After incubation time the non-bound (enzyme labeled) reagents are removed in a washing step. The amount of chloramphenicol enzyme conjugate is visualized by the addition of a chromogen substrate. Bound enzyme conjugate transforms the colorless chromogen into a colored product. Reaction of substrate is stopped by addition of stop solution. The color intensity is measured photometrically, where optical density is inversely proportional to the concentration of chloramphenicol in sample**.**

Preparation of sample(chicken meat)

a) Homogenize 10g of chicken meat weight 3g of the homogenized tissue sample and transfer into a glass tube.
b) Add 6ml of ethyl acetate and mix (head over head) for 10 min.
c) After centrifugation (10 min, 2000g)
d) 4ml of the ethyl acetate and evaporated at 50°C
e) The fatty residue is dissolved in 1ml iso-octane/ thrichloromethane (2:3;v/v) and 1ml of dilution buffer is added.
f) Mix with vortex 1min and centrifuged 10 min with 2000g.
g) 50µl portions of the upper layer are pipetted into the test.

Assay procedure

a) Into wells of microtitar plate, parallel have been filed with 50µl of negative probe two wells (B1 ,B2) and standard dilutient from 0,025ng/ml to 2ng/ml(well C1and C2 to well H1 and H2). In the rest of wells fill in duplicate 50µl of prepared sample.
b) In the same order every well (except A1,A2) was filed with 25µl of enzyme conjugate and 25µl of diluted antibodies
c) After a mixing plate was incubated for 1 hour on 4°C.
d) Content of the wells have been washed three times with diluents.
e) Into each well was added 100µl of substrate and incubated 30min on room temperature.

Then 100µl of stop solution was filled in every well and content roughly mixed.

Spectrophotometeric reading

Optical density of generated color was read immediately by "IDEX" spectrophotometer; on wavelength λ=450nm. Blank probe was done against air.

Calculation

a) After we measured values of absorbance for each well and calculated medium value of two parallels, the calculation of parameters has been done %A (absorbance percentage) by following formulae:

$$\%A = \frac{\text{Average value of absorbance (standard or sample)}}{\text{Average value of absorbance of zero standards}} \times 100$$

b) Calibration curve was drawn for standard dilutions of chloramphenicol with known concentration.
c) Values of chloramphenicol concentration in samples (chicken meat) were read from the curve by appropriate values of %A.

Compared results

To compare these results authors have analyzed same samples by diffuse microbiological test using test species bacteria: Bacillus cereus ATCC 11778, Bacillus subtilis ATCC 6633, Staphylococcus epidermidis ATCC12228, Sarcina lutea ATCC9341 and Sarcina lutea ATCC 15957.

Results and discussion

In 25 examined samples of chicken meat from territory of Canton Sarajevo were not detected quantities of chloramphenicol greater than the limits of detection by this method, the Elisa-test (detection limit is 0,02ng/g) Samples (chicken meat) were from domestic producers and also from different foreign producers. The assay results are summarized and presented in table No: 1. Samples are grouped into next order.

a) A1- domestic chicken meat, locality 1
b) A2- domestic chicken meat, locality 2
c) B1- imported chicken meat, locality 1
d) B2- imported chicken meat, locality 2

NOTICE: Because of are professional relations with clients we did not notify producers names.

Table1. Chloramphenicol content in the samples of chicken meat

Group	Location	Number of samples	Chloramphenicol content (ng/g)
A1	1	7	< 0,02
A2	2	6	< 0,02
B1	1	6	< 0,02
B2	2	6	< 0,02

Conclusions

1. Elisa-test through assay has shown as very sensible, reliable and simple analytical method for quantitative analysis of chloramphenicol residues in chicken meat.
2. In 25 examined samples of chicken meat were not detected quantities of chloramphenicol greater than the limits of detection by this method, the Elisa-test (detection limit is 0,02ng/g)
3. Critical moment of Elisa-test for screening chloramphenicol in chicken meat is procedure of preparing sample for assay which is relatively complicated and long lasting.

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FLAVOUR PERCEPTION OF OXIDATION IN BEEF

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Background

Lipid oxidation limits the storage life of meat (Rhee, 1988; Gray *et al*., 1996) through the production of rancid/off flavours but is also important in cooking-induced production of flavour volatiles, both directly and as precursors for the synthesis of other components of meat flavour.

Methods for measuring the effect of oxidation on meat flavour are either direct, ie. taste panel, or indirect by determining the degree of lipid oxidation by measuring oxidation products. The former is expensive, baseline perceptions may vary and they depend on prior experience/conditioning. The latter is usually quicker, cheaper and more reproducible within and between laboratories, but on the other hand, only determines chemical compounds which may or may not be directly involved in the oxidised flavour. Furthermore, widely used assays for rancidity, such as the determination of 2-thiobarbituric acid reacting substances (TBARS) measure a range of compounds. Although the chemical determinations are generally related to the deterioration in meat flavour there are few specific investigations of this relationship.

Ruminant meat is relatively stable in terms of lipid oxidation because of its high proportion of saturated fatty acids relative to polyunsaturated fatty acids (PUFA). However, by feeding cattle with lipids protected against rumen biohydrogenation it is possible to obtain meat with an increased content of n-3 PUFA (Scollan *et al*., 2003) making it more desirable in terms of human nutrition. Protected PUFA with different degrees of unsaturation, such as lipid supplement (C18:2 *n*-6 + C18:3 *n*-3 ratio 1:1) or fish oil (C20:5 *n*-3 and C22:6 *n*-3) can produce meat with a range in oxidative stability, so that knowledge of the shelf-life and flavour of the modified meat becomes important (Richardson *et al*., 2003). This meat with a wide range in oxidative potential can be used to assess the relationship between chemically determined oxidation and the organoleptic assessment of flavour as evaluated by a trained taste panel.

Objectives

The objective of this work was to relate human perceptions of lipid oxidation, as determined by a trained taste panel, to a chemical measurement of oxidation, using meat from animals with a wide range of potential oxidation because of the diet-induced changes in their PUFA composition, and further enhancement by displaying the meat in high oxygen modified atmosphere packages for various lengths of time.

Materials and methods

Meat was obtained from 73 Angus- and Charolais-cross steers raise for different on-going trials on 10 different diets: grass silage (high in C18:3 *n*-3), cereal concentrate (high in C18:2 *n*-6), three diets with 3% added fat consisting of three levels of protected lipid (high in C18:2 *n*-6 and C18:3 *n*-3, ratio 1:1)(PLS) and a control with Megalac ® (relatively saturated)(contPLS) and three diets with three levels of inclusion of protected fish oil (high in C20:5 *n*-3 and C22:6 *n*-3) and a constant amount of unprotected fish oil (PFO), as well as an unprotected fish oil control (contPFO).

48 hours after slaughter, the left loin of each carcass was removed, vacuum packaged, and kept at +1 ^{o}C. Loins of the grass and concentrate fed animals were kept vacuum packaged for an extra 8 days until reaching 10 days of ageing, whereas loins from the rest of the diets were kept for 11 days until reaching 13 days of ageing according to the protocols of their respective projects. After aging, each loin was cut into 2-cm thick steaks, vacuum packaged, frozen, and kept at -18 ^{o}C until analysed.

TBARS and sensory analyses were performed on steaks displayed for 0, 4 or 9 days under simulated retail conditions in modified atmosphere packages (MAP). After frozen storage, samples were thawed at +1 ^{o}C for

24 hours. They were transferred onto a polystyrene tray, covered with a permeable film and enclosed in a plastic transparent bag impermeable to oxygen. The atmosphere in the bag was modified to contain CO_2:O_2 in the ratio of 25:75. Samples were displayed for 4 or 9 days at 4 ^oC under illumination (700 lux 16 h on) until analysed. Samples of day 0 of display were not displayed in MAP, but instead were analysed immediately after the vacuum was broken after thawing to avoid oxidation. These were considered as non-oxidised control samples. TBARS (thiobarbituric acid reactive substances) were analysed by the steam distillation method of Tarladgis *et al*. (1960) and expressed as mg of malonaldehyde per kg of lean muscle. Steaks for sensory analysis were grilled and turned every three minutes for homogeneous cooking until the internal centre temperature reached 74 ^oC measured by a hand-held digital thermometer. Uniform cuboids were cut, wrapped in coded aluminium foil, and kept at 60 ^oC until the sensory evaluation was performed. Sensory assessments were performed by trained taste panel of nine members under controlled conditions, which included booths with red lights to mask colour differences. Panellists received three samples at a time to compare the meat within each animal that had been displayed for 0, 4 and 9 days. Panellists rated typical beef flavour, abnormal and rancid flavours as well as the overall palatability on an unstructured line scale where 0 meant no flavour or 'dislike extremely', and 100 meant very intense flavour or 'like extremely'.

Statistical analysis of panel data was performed by analysis of variance (Genstat 5 Release 3.1) within diet with conditioning time and panellist as factors and panel treated as a block structure. Differences between mean values were assessed post hoc using the least significant difference procedure. TBARS were analysed using the Kruskall-Wallis rank sum test. Spearman's rank correlation coefficients (rho) were calculated on the animal by display means for TBARS and sensory data using Minitab Release 11.

Results and discussion

Table 1 shows the values for TBARS and sensory attributes. Meat oxidation increased throughout the time at display for each of the diets, as shown by the rising TBARS values. The increments were not linear, however, being smaller between days 0 and 4 of display than between days 4 and 9 of display, as lipid oxidation is a free-radical chain reaction (Rhee, 1988). The lowest values and lowest increment in oxidation was produced by meat from the two control diets and the silage-fed animals probably due to the higher proportion of saturated fat in the meat from animals fed the contPLS and contPFO diets, as well as a higher content of vitamin E (Richardson *et al*., 2004) that acts as a powerful antioxidant. Accordingly, meat from animals fed concentrate diet had a low vitamin E concentration and the highest TBARS values. The greater the inclusion of protected PUFA in the diet of the animals, the higher TBARS values were obtained. This was especially observed in PFO diets, where an extra level of inclusion of protected lipid gave a higher oxidation value. In PLS diets, the oxidation of the meat did not increase from PLS2 to PLS3, because the attempt to increase C18:3 deposition in the muscle above PLS2 failed even with a 25% increase in protected lipid in the feed.

Sensory attributes were also influenced by length of display. Positive attributes, such as typical beef flavour or overall palatability, decreased throughout display, whereas negative attributes, such as abnormal or rancid flavours, increased. Although all diets started with similar values, the biggest reduction in beef flavour occurred in the diets producing meat with the most PUFA, especially in PLS2, PLS3, PFO3 and concentrate fed animals, with the lowest beef flavour intensities at day 9 of display. In general, the decrease in strength of beef flavour was more pronounced between days 4 and 9 of display than between days 0 and 4. Byrne *et al*. (2001) suggested that the disappearance of 'meaty' flavour was related to both the 'meaty' compounds degrading and becoming masked by flavour-affecting compounds derived from lipid oxidation. Concentrate, PLS2, PLS3 and PFO3 fed-groups showed also the highest mean ratings for abnormal and rancid flavours, closely related to their higher values of TBARS. Consequently, they received much lower values for overall palatability than the other diets.

Panellists could barely detect rancidity in unconditioned meat (0 days of display) with mean ratings all being less than 2. The lack of oxygen and the use of intact meat during frozen storage avoided the initiation of lipid oxidation (Spanier and Miller, 1996). However, abnormal flavour was perceived from this early stage onwards. Its value was always higher than those of rancidity, implying that rancid was considered an abnormal flavour, but not the only one of such. Negative flavours such as painty, cardboardy, bitter and sour have been described to increase with post-mortem ageing (Spanier *et al*., 1997).

The correlations between these attributes were very high (Table 2). TBARS was a good predictor of the perception of rancidity (rho = 0.84). Panellist preferences were related to the presence of the typical beef flavour (rho = 0.93) and to the absence of abnormal (rho = -0.89) and rancid flavours (rho = -0.84). The development of rancid flavour in relation to TBARS value followed a sigmoidal curve: $y = 0.0553x^3 - 1.122x^2 + 7.3962x + 2.3204$ ($R^2 = 0.62$). The third grade of the curve implied that rancidity is perceived strongly once it reaches the threshold but then, either the perception of the panellists becomes numbed, or the analytical technique fails to discriminate TBARS accurately at high levels of oxidation. The development of beef flavour in relation to TBARS also followed a sigmoidal curve but with a different direction than the previous one: $y = -0.0359x^3 + 0.8276x^2 - 6.2937x + 24.475$ ($R^2 = 0.68$). This means that typical beef flavour gets weaker but there is always a residual beef flavour in the meat throughout display, even if the development of other off-flavours becomes more perceivable. The two curves cross at a corresponding TBARS value of 2.3. From this point onwards, the perception of rancid flavour overpowers the perception of beef flavour. This could be considered as the oxidation limit at which meat could be rejected. Tarladgis *et al*. (1960) and Turner *et al*. (1954) suggested a TBARS of 0.5-1.0 as the threshold value for the detection of off-odour in pork. However, pork has a milder flavour than beef, and therefore, rancid flavours are probably noted at a lower concentration than in beef. Nevertheless, Melton (1985) suggested that oxidised flavours were detectable in beef at TBA numbers of 1.

Conclusions

Oxidation produces a decrease in beef flavour and overall palatability, as well as an increase in abnormal and rancid flavours during retail display of beef. Under the experimental conditions used, a TBARS value of around 2 could be considered the limiting threshold for the acceptability of oxidation-altered flavour in beef. This threshold was clearly higher than that of 0.5-1.0 suggested for pork.

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Acknowledgements

Authors are most grateful to Anne Baker for technical assistance. This work was supported by the EU (QLK1-CT 1999-51147), ABP, MLC, Masterfoods, Sainsbury's and Ministerio de Ciencia y Tecnología, Programa Ramón y Cajal.

Table 1. Values for TBARS and sensory attributes.

DIET	days	TBARS *y*	Beef flav. *z*	Abnormal flav. *z*	Rancid flav. *z*	Ov. Liking *z*
ContPLS	0	nd	26.5 ^{c}	11.7 ^{a}	1.2 ^{a}	26.8 ^{c}
	4	0.24	22.5 ^{b}	23.1 ^{b}	5.4 ^{a}	19.9 ^{b}
	9	0.43	17.1 ^{a}	34.0 ^{c}	14.2 ^{b}	15.9 ^{a}
	sig/sed	***	*1.60*	*2.91*	*3.06x*	*1.91*
PLS1	0	nd	25.5 ^{b}	10.7 ^{a}	1.3 ^{a}	29.4 ^{b}
	4	0.48	22.3 ^{b}	19.5 ^{b}	4.9 ^{a}	22.6 ^{b}
	9	3.78	10.8 ^{a}	42.4 ^{c}	17.8 ^{b}	8.6 ^{a}
	sig/sed	***	*1.57x*	*2.65x*	*3.98x*	*4.35x*
PLS2	0	nd	26.1 ^{c}	15.0 ^{a}	1.7 ^{a}	25.6 ^{c}
	4	0.98	17.5 ^{b}	27.9 ^{b}	11.0 ^{b}	14.6 ^{b}
	9	6.60	7.8 ^{a}	51.1 ^{c}	21.3 ^{c}	5.7 ^{a}
	sig/sed	***	*3.27x*	*5.33x*	*3.97x*	*3.33x*
PLS3	0	nd	26.3 ^{c}	12.6 ^{a}	1.3 ^{a}	27.5 ^{c}
	4	0.82	19.9 ^{b}	22.3 ^{a}	8.0 ^{b}	19.6 ^{b}
	9	6.21	10.1 ^{a}	45.3 ^{b}	18.0 ^{c}	8.3 ^{a}
	sig/sed	***	*2.79x*	*5.74x*	*2.90x*	*3.21x*
ContPFO	0	nd	24.9 ^{b}	13.6 ^{a}	1.7 ^{a}	26.7 ^{b}
	4	0.31	23.4 ^{a}	16.4 ^{a}	4.3 ^{a}	23.2 ^{b}
	9	1.61	16.3 ^{a}	31.6 ^{b}	12.7 ^{b}	13.7 ^{a}
	sig/sed	***	*2.32x*	*3.59x*	*3.12x*	*3.45x*
PFO1	0	nd	22.7 ^{b}	17.3 ^{a}	1.5 ^{a}	21.6 ^{b}
	4	0.49	21.2 ^{b}	16.1 ^{a}	4.3 ^{ab}	19.9 ^{b}
	9	2.14	14.1 ^{a}	36.3 ^{b}	11.4 ^{b}	11.9 ^{a}
	sig/sed	***	*1.53*	*3.00*	*3.73x*	*2.83x*
PFO2	0	nd	23.6 ^{b}	13.5 ^{a}	2.0 ^{a}	24.4 ^{b}
	4	0.56	19.7 ^{b}	20.5 ^{b}	5.2 ^{a}	18.3 ^{a}
	9	3.00	12.4 ^{a}	39.7 ^{c}	18.5 ^{b}	10.2 ^{a}
	sig/sed	***	*1.47*	*3.09*	*4.39x*	*2.63x*
PFO3	0	nd	25.2 ^{c}	13.7 ^{a}	1.2 ^{a}	27.2 ^{c}
	4	1.10	20.4 ^{b}	22.4 ^{b}	6.9 ^{a}	19.4 ^{b}
	9	5.17	9.8 ^{a}	49.5 ^{c}	19.0 ^{b}	7.0 ^{a}
	sig/sed	***	*2.32x*	*3.01*	*5.05x*	*2.95x*
Grass	0	nd	25.5 ^{b}	12.8 ^{a}	0.9 ^{a}	25.2 ^{b}
	4	0.59	18.6 ^{ab}	24.1 ^{ab}	5.3 ^{ab}	16.7 ^{a}
	9	1.16	14.4 ^{a}	31.2 ^{b}	12.1 ^{b}	11.9 ^{a}
	sig/sed	*	*3.57x*	*5.92x*	*3.84x*	*4.12x*
Concentrate	0	nd	25.2 ^{b}	18.2 ^{a}	1.3 ^{a}	26.2 ^{b}
	4	3.15	12.1 ^{a}	38.0 ^{b}	9.5 ^{b}	10.6 ^{a}
	9	6.68	7.7 ^{a}	53.7 ^{c}	21.4 ^{c}	6.05 ^{a}
	sig/sed	***	*2.81x*	*6.52x*	*4.09x*	*3.44x*

y, mg malonaldehyde/kg muscle (medians);
z, 0-100 scale (0-very low; 100-very high) (mean values);
nd = not detected;
a, b, c. Mean values with different superscripts within diet in the same column are significantly different (P≤0.05)
sig/sed significance (TBARS column), mean standard error (MSE=sed) (other columns);
x Interaction between conditioning time and assessor, therefore significance and sed were recalculated.

Table 2. Spearman's rank correlation coefficients between TBARS and sensory attributes (n=216).

	TBARS	Beef flavour	Abnormal flavour	Rancid flavour
Beef flavour	–0.80 ***			
Abnormal flavour	0.82 ***	–0.87 ***		
Rancid flavour	0.84 ***	–0.79 ***	0.83 ***	
Overall palatability	–0.84 ***	0.93 ***	–0.89 ***	–0.84 ***

*** = P≤0.001

PREPARATION AND PROPERTIES OF HAEMOSTATIC POWDER AND FILM

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Background

Blood contains 18-19 g/dl of proteins, of which 7-8% is plasma and 10-12% is globin. Those are very useful and valuable materials. Plasma contains fibrinogen, thrombin and factor XIII which are blood coagulant factors. It has been found there are many valuable biomedical materials. If they can be utilized, it can increase blood value and decrease environmental pollution. In 2003, we extracted fibrinogen and thrombin from porcine blood as a haemostat solution and investigated its effectiveness of clotting. We found it had very effective haemostatic action (Chen et al., 2003), thus, we try to develop other forms of haemostats.

Objectives

This study is to use fibrinogen and thrombin to prepare freeze-dried powder and haemostat film, and investigate the properties of the products.

Materials and methods

Fibrinogen and thrombin are extracted by the methods described by Futami et al. (1984) and Divakaran (1982), respectively. The two extracts are dried by freeze-drying to make powder. Haemostatic films are prepared with the combination of original extract solutions of fibrinogen and thrombin by the ratio of 20:1 and added with 12% of 0.25M $CaCl_2$ and coagulated on the surface of petri dish. The films are dried using freeze-drying, and 40□ oven drying for overnight and for five hours, respectively. The solubility and turbidity of the powder products are analyzed. Thickness, water absorption and swelling rate of films are also measured.

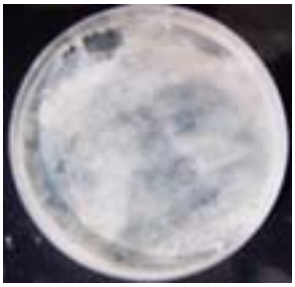
Results and discussion

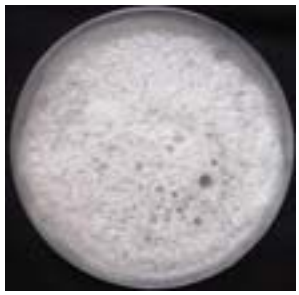
The activity of thrombin, properties and haemostatic function of thrombin and fibrinogen have been studied before (Chen et al., 2003). Figure 1 shows fibrinogen and thrombin powder dried by freeze-drying. The solubility and turbidity for the freeze-dried fibrinogen are 51.2% and 21.5, and for thrombin are 14.35% and 10.1, respectively (Table 1). Figure 2 shows moisten film, oven dried film and freeze-dried film. It is found the thickness and swelling rate of the moisten
film is higher than the others. The moisten film is also more elastic. The water absorption of freeze-dried film is higher than the others and absorbed more water. The picture of freeze-dried film is more porous(Figure 2c). When it is used to arrest the bleeding it may absorbed more blood. In other words, it is more effective on haemostatic action.

fibrinogen

thrombin

Fig. 1 Pictures of freeze-dried powders of fibrinogen and thrombin.





a. moisten film b.oven-dried film c. freeze-dried film

Fig. 2 Pictures of films

Table 1. Solubility and turbidity of freeze-dried powders of fibrinogen and thrombin

Iterms	**Fibrinogen**	**Thrombin**
Solubility(%)1	51.2	14.35
Turbidity(T)2	21.5	10.1

1. Solubility(%)=(protein in supernatant)/total proteinx100.
2. Turbidity=transmittance at 540nm

Table 2.Thickness, water-absorption and swelling rate of haemostatic films

Iterms	Moisten film	Oven-dried film	Freeze-dried film
Thickness(mm)	2.62	1.72	2.29
Water-absorption(%)	6.13	27.14	180
Swelling rate(%)	21	1.16	-30

Water absorption(%)=((Wa-Wb)/Wb)x100 Wa=weight after soaking in water
Wb=weight before soaking
Swelling rate(%)=((Ta-Tb)/Tb)x100 Ta=thickness after soaking
Tb=thickness before soaking

Conclusions

This study is to use fibrinogen and thrombin separated from porcine blood to prepare haemostatic powder and film. The result of film thickness is different due to the methods of preparation. It was found water-absorption of freeze-dried film is higher than the others, and the swelling rate was found higher in moisten film

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EFFECTS OF KID FEEDING WITH A DE-STONED OLIVE POMACE ON THE QUALITY OF RAW AND COOKED MEAT

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Background

Olive oil by-products have been widely employed for animal feeding in many species (Sansoucy, 1985; Molina Alcaide and Nefzaoui, 1996). In Southern Italy, the semi-arid ecosystems hamper the availability of pastures and forages during the year, therefore, the possibility of using agricultural by-products may be quite interesting, both under a zootechnical and economic point of view, on the condition that the products used are of good quality and safe for animal feeding and welfare. The olive oil industry plays an important role in Southern Italy; furthermore, the exploitation of olive oil by-products is even more feasible in a region like Apulia, where olive oil is produced for about 12% of the total world output and small ruminant breeding is particularly practised. Previous reports carried out on lambs have shown that feeding olive cakes positively affects growth performances and meat quality (Lanza *et al.,* 2000; Zumbo *et al.*, 2001; Foti *et al.*, 2003). In this trial we investigated the use of a new type of by-product, i.e. a virgin olive pulp produced by the removal of stones from the olives (Amirante *et al.*, 2002), which has already provided satisfactory results in lamb feeding, as we have described elsewhere (Ragni *et al.*, 2003; Vicenti *et al.*, 2003).

Objectives

The aim of the study was to evaluate the effect of using a de-stoned olive pomace as a feed for kids' diet on meat colour and on the chemical composition, fatty acid profile and tenderness of raw and cooked meat.

Materials and methods

The experiment was carried out at the University farm located in Bari (Apulia, Southern Italy, 41 °N) on sixteen male Garganica kids, weaned at about 50 days of age, divided into 2 groups of 8 subjects each, homogeneous for age and body weight. Kids were fed *ad libitum* for 6 weeks on either a concentrate pelleted diet (control group) or on a diet containing 20% olive pomace (olive pomace group). Diets were planned in order to contain approximately the same amount of protein (16.5%), fat (5.0%) and crude fiber (10.5%). Kids were slaughtered following 12 hours fasting. After 24 hours of refrigeration at 4 °C, the Longissimus lumborum (Ll) muscle was dissected from the right half carcass and split into two pieces, one of which was used raw while the other was cooked in an electric ventilated oven at 180 °C until the internal temperature of 75 °C was reached in the core of the meat sample, recorded by a thermocouple (ASPA, 1996). Meat samples were weighed before and immediately after cooking to determine cooking loss percentages. Colour (L = Lightness; a = redness; b = yellowness) was evaluated only on raw samples using the Hunter Lab system (colourmeter Miniscan XE; D65/10° illuminant). Tenderness was assessed by the Warner Bratzler Shear device system on raw (cylindrical, half an inch of diameter) and cooked (rectangular, 1x1 cm section) meat cores using a universal test machine (Instron 5544). Peak force was expressed as kg/cm^2 and represents the cutting force required to shear perpendicularly to the direction of the fibres. Raw and cooked meat samples were analysed for chemical and fatty acid composition (ASPA, 1996). Lipids were extracted using a chloroform/methanol 2:1 v/v solution (Folch *et al.*, 1957). Fatty acids were methylated using a BF_3/methanol solution (12% v/v) and analysed by gas chromatography (Chromopack CP 9000) using a 60 m silicated glass column with a 0.25 mm internal diameter and 0.2 μm film thickness. The atherogenicity (AI) and thrombogenicity (TI) indexes (Ulbricht and Southgate, 1991) and the PCL/PCE (plasma cholesterol lowering/plasma cholesterol elevating) ratio (Reiser and Shorland, 1990) were also calculated.

Data were processed by analysis of variance using the GLM procedure of SAS (1999/2000). The model adopted took into consideration as main effects the diet and the cooking method and their interaction. Means were compared by Student's t test.

Results and discussion

Meat colour was not affected by the integration of the olive pomace in the diet (Figure 1). Previous studies have documented that olive cakes are rich in unsaturated fatty acids (Sansoucy, 1985), which in meat may increase its susceptibility to lipid oxidation, with consequences on colour, texture, flavour as well as its nutritional value (Ponnampalam *et al.*, 2002). For this reason, olive cake feeding has been accompanied with the administration of antioxidants such as vitamin E to stabilize meat lipid deterioration (Lanza *et al.*, 2000; Zumbo *et al.*, 2001).

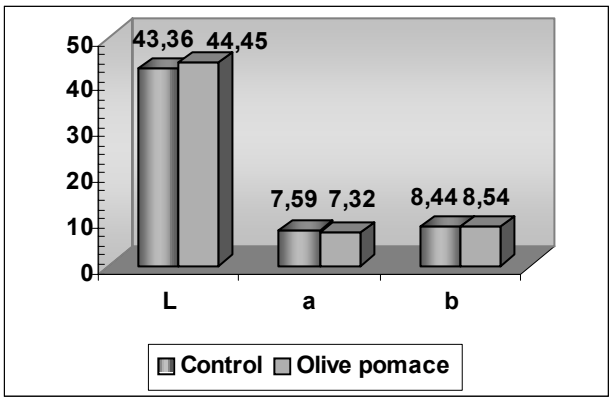

Figure 1. Meat colour of the *Longissimus lumborum* muscle in kids

In this study, similarly to what we (Vicenti *et al.*, 2003) and other Authors (Foti *et al.*, 2003) previously found in lambs, the values of meat colour were not changed by the integration of the olive pomace in the diet.

As for the chemical composition of meat (Table 1), no significant differences between diets were found for raw meat, whereas cooked samples of the olive pomace group displayed a greater moisture content (P<0.05) and significantly (P<0.01) less fat and N-free extract in comparison with cooked control samples. For both the diets administered, cooking determined a decrease of meat moisture in turn of a concentrating effect on fat (P<0.01) and protein (P<0.01) and, although only in the olive pomace group, also on ashes (P<0.01). The olive pomace diet resulted in a significantly (P<0.05) greater cooking loss (16.19%) in comparison with the control group (13.30%). The chemical composition of kid meat obtained in this study was comparable to that reported by Dhanda *et al.* (1999), who studied meat quality characteristics in different goat genotypes.

Table 1. Chemical composition of raw and cooked meat samples and cooking loss (%)

	Control			Olive pomace			Significance of main effects		SED
	Raw		Cooked	Raw		Cooked	Diet	Cooking	
N (samples)	8		8	8		8	32	32	*DF = 28*
Moisture	75.43	**	66.28^{B}	75.05	**	67.18a	ns	**	0.652
Protein	18.97	**	26.12	19.26	**	26.25	ns	**	0.481
Fat	3.29	**	5.20^{A}	3.55	**	4.54^{B}	ns	**	0.327
Ash	1.08	ns	1.12	1.01	**	1.15	ns	**	0.079
N-free extract	1.21	ns	1.27^{A}	1.13	ns	0.87^{B}	*	ns	0.289
									DF = 14
Cooking loss		13.30^{B}			16.19^{A}		*	/	2.227

Differences between diets within raw or cooked meat samples: A, B: P<0,01; a, b: P<0.05. Differences between raw and cooked samples within each diet and significance of main effects: **: P<0.01; *: P<0.05; ns = not significant.

Raw control meat samples showed significantly (P<0.01) more SFA and PUFA in comparison with the olive pomace group, whereas less (P<0.01) MUFA and UFA. In the corresponding cooked samples, the fatty acid class distribution was somewhat reversed. Cooked meat samples of the olive pomace group contained a greater amount of SFA (P<0.01) and a lower proportion of MUFA (P<0.05) as well as UFA (P<0.01) as compared to the control diet.

Within the polyunsaturated fatty acids present in meat, raw samples of the control group, showed a higher (P<0.01) amount of ω6 fatty acids with no difference in the ω3 fatty acid fraction, so that the ω6/ω3 ratio was comparable between the diets for both raw and cooked samples. The PUFA/SFA ratio was markedly better in raw control samples than in the olive pomace ones (P<0.05), while cooking cancelled this difference. However, other studies conducted on goats have reported higher PUFA/SFA values respect to those found by us in this study (Banskalieva *et al.*, 2000), although the differences may be ascribable to the diet, to the slaughtering age and to the goat breed.

With regards to the dietetic properties of meat (Table 3), the atherogenicity index did not differ among diets as far as raw meat samples are concerned, while cooking resulted in a significant increase of the AI in the olive pomace group with respect to the controls, both in the raw (0.62 vs 0.54; P<0.01) as well as cooked ones (0.62 vs 0.52; P<0.01). Within raw samples, the thrombogenicity index was significantly higher in the control group with respect to the olive pomace diet (1.32 vs 1.09, P<0.01), while in cooked samples the trend was completely reversed, with olive pomace samples displaying a thrombogenicity index value of 1.20 against 1.11 obtained for the control group (P<0.05). Moreover, a significant (P<0.01) reduction of the TI value was recorded in control samples after cooking, on the contrary of the olive pomace diet, where cooking markedly increased meat thrombogenicity index (P<0.01).

The control diet improved the PCL/PCE ratio in comparison with the olive pomace feeding treatment, at a level of P<0.05 for raw meat while at P<0.01 for cooked samples. In both feeding treatments the PCL/PCE ratio did not change following cooking.

Table 2. Fatty acid classes in raw and cooked meat samples (%)

	Control			Olive pomace			Significance of main effects		SED
	Raw		Cooked	Raw		Cooked	Diet	Cooking	
Samples (n.)	8		8	8		8	32	32	DF = 28
SFA	44.02A	**	38.97B	39.75B	**	41.45A	*	**	1.157
MUFA	47.67B	**	54.82a	53.82A	ns	53.00b	**	**	1.442
PUFA	8.22A	**	5.50	6.30B	*	5.35	**	**	0.852
UFA	55.90B	**	60.32A	60.12A	*	58.35B	*	**	1.292
ω6	7.15A	**	4.83	5.30B	ns	4.60	**	**	0.726
ω3	1.07	**	0.67	1.00	ns	0.75	ns	**	0.245
ω6/ω3	7.09	ns	7.15	5.98	ns	6.14	ns	ns	1.712
PUFA/SFA	0.19a	**	0.14	0.16b	*	0.13	*	**	0.023

Differences between diets within raw or cooked meat samples: A, B: P<0,01; a, b: P<0.05. Differences between raw and cooked samples within each diet and significance of main effects: **: P<0.01; *: P<0.05.
ns = not significant.

Table 3. Atherogenicity (AI) and thrombogenicity (TI) indexes and PCL/PCE ratio in raw and cooked meat

	Control			Olive pomace			Significance of main effects		SED
	Raw		Cooked	Raw		Cooked	Diet	Cooking	
Samples (n.)	8		8	8		8	32	32	DF = 28
AI	0.55	ns	0.52B	0.54	**	0.62A	**	ns	0.033
TI	1.32A	**	1.11b	1.09B	**	1.20a	**	*	0.067
PCL/PCE	1.34a	ns	1.31A	1.24b	ns	1.19B	**	ns	0.081

Differences between diets within raw or cooked meat samples: A, B: P<0,01; a, b: P<0.05. Differences between raw and cooked samples within each diet and significance of main effects: **: P<0.01.
ns = not significant.

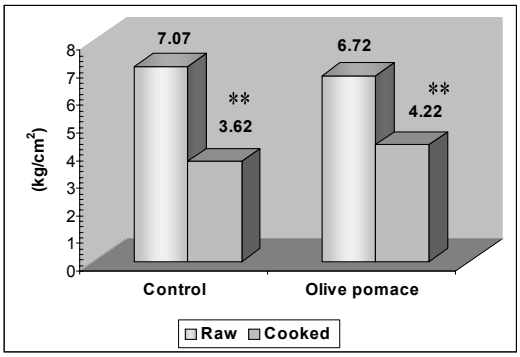

Figure 2. Peak force (kg/cm^2) in raw and cooked *Longissimus lumborum* meat samples
Differences between raw and cooked samples within each diet: ** p<0.01.

Meat tenderness did not seem to be influenced by the diet (Figure 2), neither for raw samples nor for cooked ones. However, cooking improved meat tenderisation, as showed by the significant (P<0.01) decrease of the peak force required to shear meat, both in the control group (7.07 vs 3.62) than in olive pomace meat samples (6.72 vs 4.22).

Conclusions

Based on the findings of this study, we may conclude that the integration of a de-stoned virgin olive pomace at 20% level in the diet of growing goat kids did not influence meat chemical composition, colour and texture. It did, however, provide controversial results in terms of meat dietetic properties, especially following cooking.

Acknowledgments

The research was funded by Project ALBIO (DM MiPAF, n° 441/7303/01 – 28/11/2002). The authors are grateful to Mr. Massimo Lacitignola for his technical collaboration.

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USING DENSITY TO MEASURE MYOFIBRILLAR DENATURATION

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Background

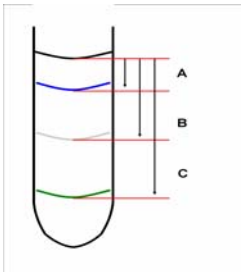
Excessive denaturation of myofibrillar proteins during the post mortem period can have important implications for meat quality. Denaturation of myofibrillar proteins by conditions of high temperature and low pH during, particularly during the pre-rigor period, causes exaggerated shrinkage of the myofibrillar lattice due to reduced electrostatic repulsions between the thick filaments (Offer, et al, 1983. Offer & Knight, 1988). Lattice shrinkage contributes to a paler meat colour, due to increased reflectance, and increased drip loss due to expulsion of fluid from the intra-lattice space. Although the implications of myofibrillar denaturation are well recognised in pork as the PSE condition (Offer & Knight, 1989), similar consequences are reported in beef (Simmons et al, 2000).
Current methods for quantifying myofibrillar denaturation have limitations: direct measurement of the myofibrillar lattice is complex and slow (Offer, et al 1989; Smulders et al., 1990.); reduced myofibrillar solubility is, in our experience, relatively insensitive inconsistent; and myosin ATPase activity is indirect and influenced by unrelated events such as proteolysis (Ouali, 1990). We describe here a simple method to measure denaturation through changes in myofibrillar density.

Objectives

Current methods for measuring directly the changes in myofibrils post mortem are slow and difficult. We set out to develop a simple method of measuring myofibrillar denaturation through changes in myofibrillar density.

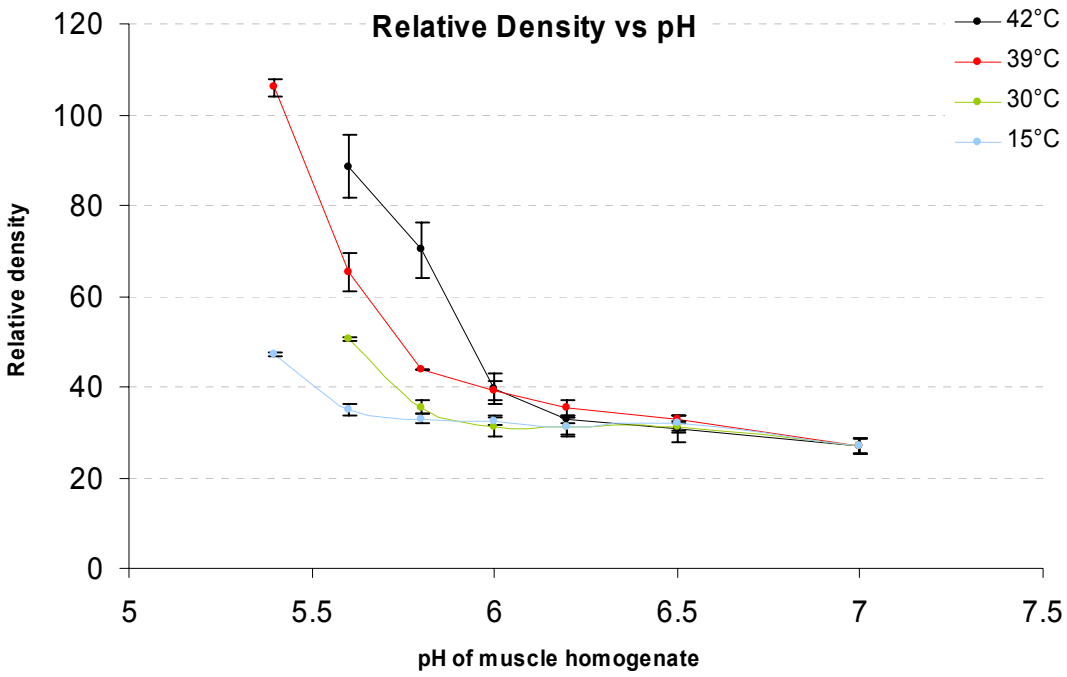
Materials and methods

Beef longissimus samples were held at constant pre-rigor temperatures of 15, 30, 39 and 42°C.
When the pH of the meat reached 7.0, 6.8, 6.5, 6.2, 6.0, 5.8, 5.6 and 5.4; 5 gram samples were taken. These 5 gram samples were Ultra-Turrax homogenised for 20 second at 13500rpm in 30ml of pH 5.3 homogenisation solution, (250mM.Sucrose; 10mM.KCl; 1mM.EDTA, pHed to 5.3 with KOH).
0.5 ml of the homogenate was centrifuged @ 6000g for 2 min and the supernatant discarded. The remaining pellet was washed with 1.0 ml of homogenisation solution, centrifuged and the resulting washed pellet re-suspended in 0.5 ml homogenisation solution.
15 µl of washed, re-suspended homogenate was then thoroughly mixed with 1.5 ml of a 30% Percoll:Sucrose:KCl:Triton (Ratio 29:23:47:1) gradient solution containing 10 µl of Amersham Biosciences density marker beads (mixture of blue (1.042 g/ml) and green (1.063 g/ml)) in the same tube. This mixture was centrifuged at 18,000g for 30 min.
The tube was then carefully removed from the centrifuge and distance from the bottom of the meniscus to "A" Blue marker bead, "B" myofibrils and "C" Green marker bead layers was measured.



Results were expressed as a Ratio of $\frac{B-A \times 100}{C-A}$

Results and discussion



Increasing the pH of the Percoll gradient decreased the density of the myofibrils. A higher pH increases the electrostatic charge on the filaments and, consequently, the lattice spacing (Offer & Knight). The measured myofibrillar density therefore probably reflects the extent to which denaturation events has modified the amount of charge on the myofibrillar filaments.

Varying denaturing conditions were produced by maintaining beef muscle samples in different constant temperatures during the pre-rigor temperature. Because the rate of glycolysis, and hence pH decline, is temperature-dependent, the changes in myofibrillar density during the pre-rigor period were measured at equivalent pH values. The results clearly demonstrate that denaturation depends on the meat pH reaching values below pH 6. Furthermore, a very extreme temperature dependency was evident: although the density at ultimate pH in the 30C samples was significantly greater (?) than the 15C samples, a further 9C increased produced a very dramatic increase in density.[This next sentence assumes that the density at pH 6 is significantly greater at 39 than 42 – exclude otherwise: The significantly greater density at 39C compared with 42C when measured at pH 6 can probably be attributed to the slower rate of pH decline at the lower temperature, thereby allowing more time for denaturation to occur event though the kinetics will be slower at the lower temperature.]

Conclusions

A simple methodology is described to measure changes in myofibrillar density that can be attributed to denaturation events. The procedure can be carried out at any stage in the pre-rigor period, is quantitative and is not affected by proteolytic events.

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SHIFTING SENSORY THRESHOLDS OF PRE-COOKED ENTIRE MALE MEAT USING THE MARINATING TECHNOLOGY.

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Background

The government of Norway has decided that from year 2009 castration of male pigs will no longer be accepted. Entire males are associated with an unpleasant taste and smell largely due to the presence of skatole and androstenone. The political decision in Norway has urged a need for updating the knowledge in selected areas aiming at preventing the occurrence of boar tainted meat in the marked. Such areas are the development of new, robust classification methods at the slaughterhouse, but also achieving more knowledge on processing possibilities has been encouraged.
The most common approach for processing of entire male meat is to comminute the meat and then « dilute » it to an undetectable concentration of androstenone and skatole. This works well for comminuted meat products like fresh and fermented sausages (Bonneau and Squires, 2000). However, it is believed that a substantial fraction of the entire males will be downgraded if the castration ban becomes effective. There is therefore be a need for recipes that can work well also for intact muscles. Needle injection is the most efficient technique for flavour adjustment of intact meat. It has, among other, been suggested as an efficient technology for modifying pre rigor off-flavour (Sindelar et al., 2003) plus characteristic, strong lamb flavour (Young and Ho, 1998).

Objectives

The work reported here was conducted in order to identify marinades that could be efficient with respect to reducing the sensation of boar taint.

Materials and methods

Materials: Neck chops were collected from a commercial slaughterhouse. Only samples having skatole contents > 0.21 ppm in the neck fat were selected. No attention to breed was made. 12 entire males and 4 castrates were selected one-day post-mortem. Small samples were removed for pH, fat content and androstenone determination. Thereafter the necks were vacuum-packed and frozen at -40°C. A few weeks later the samples were thawed, injected, heated and later served for sensory analysis.
Methods:
Marinades for injection: All marinades were laboratory-made based on chosen ingredients. Ingredients are given in Table 1. Water is not listed (adds up to 1000 gram). Other ingredients (see Table 1) were used in smaller quantities and appeared post-sensory testing as not relevant, and brand names were not given. Four marinades were selected among 18 different marinades by a subjective sensory panel for further evaluation by objective sensory profiling (Løvlund, 2002). The criterion of selection was that the marinades should provide tasty meat as well as reduce the sensation of boar taint. Only three of the marinades revealed interesting properties with respect to affecting boar taint, and these marinades are described in Table 1.
Analysis of pH, fat, androstenone and skatole contents: pH was measured with a Beckman ϕ31 pH meter. The fat content was measured on neck meat slices using low field Nuclear Magnetic Resonance (NMR) measurements on homogenised and dried meat (Pedersen et al., 2001). Skatole was determined using an extraction method where skatole is extracted from fat in tris-acetone and then reacted with a colour reagent before spectroscopic quantification (Mortensen and Sørensen, 1984). Androstenone was determined using a fat extraction method followed by the use of a commercial immunoassay kit (Riedel deHaen, Seelze, Germany).
Preparation of samples for sensory analysis: The meat samples were injected with 20% (w/w) increase. Thereafter the meat samples were packed individually in cook-shrink bag and chilled overnight before being

cooked to an internal temperature of 71°C. The samples were chill-stored for 10 days and then re-heated and served to a trained sensory panel. The profile used was the one defined by Dijksterhuis et al. (2000) where it is reported that androstenone relates mostly to urine, and skatole relates mostly to manure. Their profile was modified to include characteristic flavours describing the ingredients of the marinades. The panellists used intensity scores from 1-9; 9 meaning highest intensity score.

Statistics:

The data were analysed using Minitab version 14 (www.minitab.com). The routines used for analysis of variance were: General linear method for modelling and Tukey's test for comparisons.

Table 1. Important ingredients in the marinades injected in the different entire male neck muscles.

Ingredient (in grams pr kg marinade)	Marinade 1	Marinade 2	Marinade 3
Salt (NaCl)	40.0	59.4	52.8
Sodium nitrite	0.24	0.36	0.32
Ascorbate			1.6
Phosphate (E451, E450)(as P_2O_5)	14.3	18.8	16.9
Soy sauce –reduced salt (Kikkoman Corp.)	250.0	-	-
Dextrose	12.8	16.9	17.5
Fructose	3.7	3.7	3.3
Liquid smoke (Wright's, USA)	3.7	9.9	-
Garlic powder (E.H.Woree, Germany)	-	-	2.2
Oregano, oleoresin (Kalsec, USA)	-	-	0.5
Paprika, extract (Chr.Hansen, Spania)	-	1.2	-
Others	Lemon pepper	-	Tomato, onion and bacon flavour

Results and discussion

The 16 animals used were characterised as shown in Table 2. The table shows that a wide range of androstenone (A) and skatole (S) values was obtained. The correlation between A and S was low (r=0.34). To most consumers sensory threshold values for S and A of entire males would be above 0.2-0.25 mg/kg and 0.5-1.0 mg/kg, respectively (Bonneau and Squires, 2000).

Table 2. Characteristics of the entire males and castrates used.

Samples (no)	Weight (kg)	pH	Fat range (%)	Androstenone (mg/kg)	Skatole (mg/kg)
Entire males (12)	70.6-186.1	5.64-6.22	5.0-12.4	0.8 - 4.0	0.23-0.68
Castrates (4)	72.1- 86.2	5.76-6.15	13.3-18.9	0.08-0.10	0.03-0.06

The manure taste related most strongly and significantly to skatole while the urine taste related most strongly and significantly to androstenone. This agrees with previous investigations (Dijksterhuis et al., 2000). The taste and smell attributes of manure or urine were correlated (r > 0.89), and only the taste attributes were therefore chosen for presentation here.

Figure 1 shows the taste attributes with the larger standard deviations; i.e. for those taste attributes the panellists used the intensity scale to a larger extent than for other attributes.

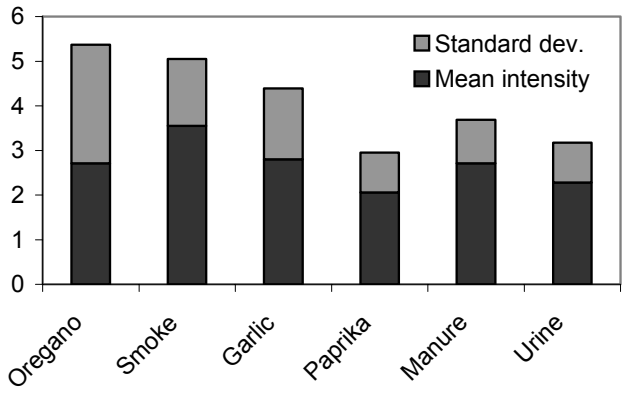

Figure 1. Mean intensity and standard deviation of different important sensory taste attributes for the 16 neck chops used (4 marinades tested).

Table 3. The intensity scores for manure and smoke taste for the 16 neck chops.

	Taste attribute	Castrates	S: 0.21-0.3 ppm	S: 0.3- 0.4 ppm	S: 0.4 -0.68 ppm
Marinade 1	**Smoke** **Manure**	**2.8** **2.1**	**2.6** **2.4**	**3.0** **2.4**	**2.6** **2.9**
Marinade 2	**Smoke** **Manure**	**6.0** **1.8**	**6.1** **1.8**	**6.0** **2.0**	**5.5** **2.3**

Marinades 1 and 2 were good at reducing manure taste. There was a significant ($p < 0.05$), and negative correlation between smoke taste and manure taste; i.e. the presence of liquid smoke appeared to be important with respect to reducing the taste of manure. Marinade 2 was significantly better ($p = 0.02$) at reducing the taste of manure than was marinade 1. Paprika also tended to reduce taste of manure but appeared much less successful compared to liquid smoke (results not shown). There was no significant difference between each skatole group. However, for marinade 1 the highest skatole group (S = 0.4-0.68 ppm) tended ($p = 0.06$) to have a higher taste of manure compared to the castrates. It was observed (not shown) that even for the castrates the panellists never rated these samples with intensity score exactly equal to 1.0 for taste of manure. The taste attribute manure therefore appeared somewhat difficult to exclude for all samples even for castrates.

The tastes of oregano, smoke and garlic were clearly detected, when these ingredients were added to marinades.

Marinade 3 gave the lowest mean intensity for taste of urine. Table 4 shows that the mean sensation of urine was reduced for marinade 3 compared to marinade 1 ($p = 0.008$). It was not possible to identify one single ingredient in marinade 3 that related significantly to androstenone level. The castrates had significantly lower taste of urine than the group that contained samples between 1-2 ppm of androstenone ($p < 0.03$). For the urine taste attribute the panellists gave intensity scores close to 1.0 for castrates, in particular for marinade 3.

Table 4. The intensity scores for oregano and urine taste for the 16 neck chops.

	Taste attribute	Castrates	A: 0.5 - 1 ppm	A: 1-2 ppm	A: 2-4 ppm
Marinade 1	**Oregano** **Urine**	**1.0** **1.6**	**1.3** **2.2**	**1.1** **2.5**	**1.3** **3.2**
Marinade 3	**Oregano** **Urine**	**6.3** **1.2**	**7.6** **1.7**	**7.7** **1.9**	**7.5** **2.3**

Conclusions

The sensory sensation of boar taint can be reduced by the addition of specific flavour components. Taste (or odour) of manure can be substantially reduced/eliminated by the use of liquid smoke. A marinade dominated by oregano and garlic flavour can reduce the sensation of urine taste.

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PERFORMANCES AND MEAT QALITY OF LEPRINO VITERBESE BREED RABBIT, BRED WITH THREE DIFFERENT HOUSING SYSTEM.

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Background

Rabbit meat consumption is important in Italy, Spain and France and in these Country different breeding systems and diets were studied, besides the increased interest in animal welfare have spurred the study of less intensive rearing systems (Dalle Zotte, 2002).
Many investigations have been carried out to study the effects of alternative housing systems on performances, meat quality and welfare of rabbit, but the results have often in conflict, (Di Lella et al. 1999, Dal Bosco et al. 2002); besides some hybrids don't adapt to extensive system, while the Leprino Viterbese has shown good performances. The Leprino Viterbese is a hybrid obtained crossing meat breeds (70 % New Zealand White; 10% Belgian Hare; 10% local breed; 5% English Lup) and Viterbo local breed. Its name derives from particular colour of fur and morphological characters that remember hare.
This hybrid is peculiar for its rusticity that allows it to be raised in extensive conditions and for the image of healthiness transmitted to the consumer by its expect.

Objectives

The aim of this work is to investigate the effects on the meat quality of three housing systems and compare the performances of Leprino Viterbese with industrial hybrid bred in intensive system.

Materials and methods

The experiment was carried out on 68 male rabbits, 51 of Leprino Viterbese and 17 of industrial hybrid, intensively reared in cages (Hi). The Leprino Viterbese animals were divided in three housing type: 17 open air (Va), 17 on the turf with mobile cages(Vt) and 17 intensively reared in cages (Vi).
The Leprino Viterbese animals, after weaning, were randomly assigned to the three experimental groups and fed the same growth food.
The industrial hybrids (Hi) were reared with the same food given to Leprino Viterbese rabbits, and the experimental animals were chosen randomly in an industrial farm which bred, in the same time and intensively, the Vi rabbits.
The animals were slaughtered at the same live weight, reaching in the average 2480 g of live weight at 89 days of age.
The carcasses were aged for 24 hours at 3°C, then they were weighed without liver and dissected to separate and to weigh the *longissimus thoracis et lumborum* muscle (LD), the thigh, the kidney fat and shoulder fat.
The thighs were dissected in order to estimate percentages of lean meat, bone and fat. On thigh the s*emitendinosus* muscle weight and length and femur length were determined.
To perform the physical and chemical analysis, both LD were divided in five samples for water holding capacity (WHC), shear force (WBS) on raw and cooked meat, colour and chemical analysis.
WHC was performed using the Grau and Hamm method (Hamm 1986).
Shear force was measured on raw and cooked meat (in water bath at 75°C for 30'), by a Warner Bratzler device mounted on Instron 1011, on 3 cores for each animal of square section 1x1 cm (Chrystall et al. 1994).
Colour coordinates were measured at four points on the surface of muscle split in horizontal. After exposure to oxygen for 1 hour, lightness (L*), redness (a*), yellowness (b*) were determinate with CIEL*a*b* System and from the colour coordinate, hue angle (H = arctang b*/a*) and chrome ($C=(a^{*2}+b^{*2})^{1/2}$) were calculated according to Cassens *et al. (*1995), using D65 illuminant with spectrophotometer Minolta CM-2600d. In the same time visual reflectance spectra (R%) between 360-740 nm (by steps of 10 nm) were measured, in this paper only the points with significantly differences among groups were referred.
The analysis of variance with GLM procedure of statistical package of SAS using a monofactorial model (housing types) was performed. The analysis included also contrast test for differences between genotypes

within the same housing type (intensive breeding, Vi *versus* Hi) and between extensive and intensive breeding (Va and Vt *versus* Vi and Hi). Finally correlation between WHC, WBS and visual reflectance spectrum was performed.

Results and discussion

Carcass weight was similar for the groups (1406.4 g in the average), because the animals have been slaughtered at fixed live weight (2500 g about). The groups, fed with same diet, reached the prefixed weight at similar age (89 days in average). Also if carcass had similar weight subcutaneous and internal sites showed different fat deposition (table 1), in fact industrial hybrid (Hi) had low fat in perirenal deposition (9.14 g), compared to the others, particularly to Va group, that showed the highest value (16.01 g), in addition to the housing effect, the two genotypes (Leprino Viterbese and industrial hybrid) had different perirenal fat. The Leprino Viterbese is more precocious than the other and therefore it tends to fatten more, while the rabbits reared extensively didn't show slower maturity as reported in in Margarit et al. (1999), when comparing classic cages with mobile cages on grass. The animals bred on open air (Va) showed a highest development on *longissimus thoracis et lomborum* weight (86.84 g), differing significantly only by Vt group (77.89 g), while the rabbits bred in intensive way had intermediate values and they didn't differ significantly from the two extensive groups. The LD weight, however was lower than that reported in Pla et al. (1996) for different genotypes. The greater body development of the group Va was confirmed also by the thigh weight, but differed significantly only by Hi (193.9 g vs 185.5 g) and was similar to the other Leprino Viterbese groups. Difference between genotypes within the same housing type was noticed, but with low significance (P=0.09). The length of femur showed the housing effect, in fact the animals bred in intensive way have developed less leg bone (9.16 cm vs 8.88cm of Va and Vt groups vs Vi and Hi), mainly due to the less physical activity.
The dissection of the thigh didn't show high differences for meat and bone percentage, but rabbits intensively bred were significantly leaner compared to the others(2.27% in average between Vi and Hi, versus 3.01% in average between Va and Vt of total fat). The general carcass fattiness of the rabbit extensively bred probably depends of genotypes and climatic influence too (Dalle Zotte, 2002).
Meat quality (table2) was influenced particularly by housing types; in fact only the water holding capacity showed little difference (P=0.07%) between the genotypes bred in intensive way.
Difference was relevant in WHC between Hi group and the others bred in an extensive way (19.68% vs 14.26% average between Va and Vt), the value of Vi was intermediate. Shear force on raw and cooked meat showed similar trend among groups. Generally the LD of rabbit bred in intensive system were significantly more tender (1.66 kg in average between the two groups in raw meat and 1.65 kg in cooked one), while Vt group was tougher than others (2.13 kg and 2.25 kg for raw and cooked meat respectively).
Animals of Hi group showed highest lightness (Dal Bosco et al., 2002; Hernàndez et al., 1998), significantly different by Vt group (56.79 vs 54.68) probably, latter group integrated the diet with blades of grass and the meat became less light and more red also if had the lower value of Hue than other groups.
The visual reflectance spectra had different trends among groups near the 470 nm and 560 nm, isobestic point for deoximyoglobin and oximyoglobin, end in Soret pick (420nm) as reported in figure 1 and in table2.

At 420 nm the curves showed different trends for intensive system compared to extensive system, while in 470nm and 560 nm significantly differences were found only between Vt and Hi.
Limited correlation was found between WHC and different data of visual spectrum reflectance (Figure 2), while the correlation coefficients were significant in all considered points after 450 nm between WBS on cooked meat and reflectance value at different wavelength, reaching maxima values after 610nm.
Also for the rabbit the spectrum of reflectance can discriminate the groups and indicate the difference in physical quality of meat.

Conclusions

The housing system influences the carcass and meat quality. Particularly the meat of industrial hybrid is different compared to Leprino Viterbese bred on the turf with mobile cages, latter therefore shows distinct characteristics of rusticity required by consumer that consider this characteristics correlated with healthiness and animal welfare.

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Acknowledgements

Work supported by Region Lazio Project, PRAL 2002 " Valorizzazione delle risorse del settore cunicolo del Lazio mediante tecnologie opto-informatiche per la determinazione morfo-ponderale qualitativa e l'analisi comportamentale del coniglio da carne" Prof. Finzi University of Tuscia (Viterbo).

Table 1 - Performances at dissection of rabbit carcass

	Carcass weight (g)	Perirenal fat (g)	LD weight (g)	thigh weight (g)	Femur lemgth (cm)	Meat %	Bone %	Fat %
Va	1410.6	16.01a	86.82 a	193.9a	9.09ab	78.8	17.8	3.31 a
Vt	1408.5	13.46a	77.89 b	191.9ab	9.24a	78.9	18.4	2.72ab
Vi	1399.3	14.25a	83.06 ab	192.3ab	8.78b	79.4	18.3	2.31b
Hi	1408.1	9.14b	83.79 ab	185.5b	8.98ab	79.6	18.2	2.24b
Means	1406.4	13.23	82.87	190.9	9.02	79.2	18.2	2.65
Root MSE	76.20	4.901	12.471	11.76	0.577	1.79	1.42	1.095
Vi *vs* Hi	ns	**	ns	0.09	ns	ns	ns	ns
Va, Vt *vs* Vi, Hi	ns	*	ns	ns	*	ns	ns	**

NOTE different letters mean significantly differences for P<0.05;

Table2 - Physical quality and colour on *Longissimus thoracis and lomborum*

	WHC	WBS (kg) on raw	WBS (kg) on cooked	L*	C	H	420nm	470nm	560nm
Va	13.93^{b}	1.95^{ab}	1.91^{ab}	55.01^{ab}	10.42	80.35^{ab}	7.66^{b}	27.04^{ab}	19.83^{ab}
Vt	14.59^{b}	2.13^{a}	2.25^{a}	54.68^{b}	10.33	79.58^{b}	7.65^{b}	25.29^{b}	19.12^{b}
Vi	15.42^{ab}	1.63^{b}	1.58^{b}	55.77^{ab}	9.39	85.00^{a}	8.59^{a}	26.29^{ab}	19.85^{ab}
Hi	19.68^{a}	1.78^{b}	1.71^{b}	56.79^{a}	9.30	85.17^{a}	8.88^{a}	27.37^{a}	21.05^{a}
Means	15.64	1.88	1.86	55.81	9.86	82.52	8.19	26.51	19.96
Root MSE	4.093	0.392	0.536	2.766	2.470	8.036	1.164	2.824	2.360
Vi *vs* Hi	0.07%	ns	ns	ns	ns	ns	ns	ns	ns
Va, Vt *vs* Vi, Hi	ns	***	***	ns	ns	**	**	*	ns

NOTE different letters mean significantly differences for P<0.05;

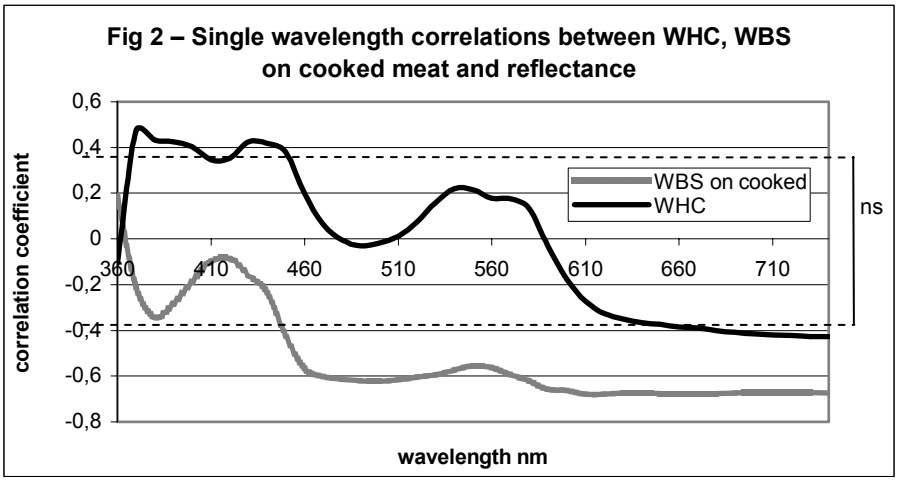
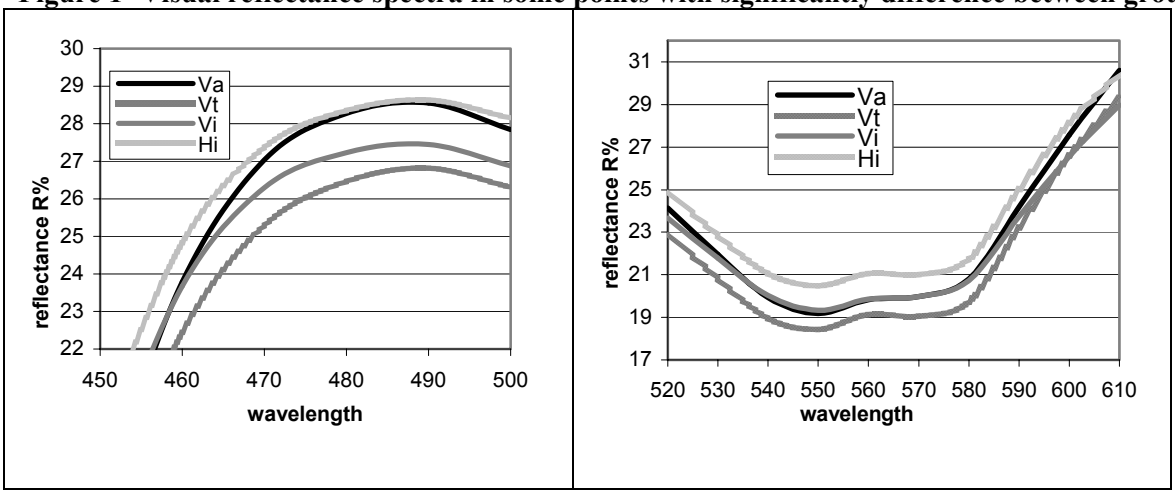


Figure 1- Visual reflectance spectra in some points with significantly difference between groups



LEVEL OF GAMMA RADIATION IN SOME MEAT PRODUCTS MARKETED IN UPPER EGYPT

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Background

Gamma radiation is electromagnetic radiation similar to light but of much higher energy. The wavelength of gamma rays is much shorter than that of visible light. Gamma rays are emitted inradioactive decay along with alpha or beta radiations (FAO, 1994). Radioactive contamination arises from both natural and artificial sources, the latter having assumed greater importance since the Second War World (Gracey *et al*., 1999). Radionuclides are readily transferred to the human population through domestic grazing meat producing animals, which are effective collectors of contamination from various vegetative sources (Gilbert *et al*., 1989, McGee *et al*., 1993), from the atmosphere (MAFF, 1994), from soil (Andersson *et al*., 2001) or from nuclear accident (Jones, 1989 and Prohl *et al*., 1989). After Chernobyl nuclear accident, the contamination of beef by radionuclides was found up to 120 Bq/Kg in 1987 (Hrusovsky *et al*., 1989). Also, it was found that the radionuclides in all examined imported lamb meat and 17 % of roast beef samples were 82 and 4 Bq/Kg,respectively (Marouf *et al*. 1991).

Objectives

The purpose of this study was to determine the level of gamma rays in some selected imported as well as locally produced meat products marketed in Upper Egypt.

Materials and methods

A total of 105 locally produced and imported meat products were collected to detect the levels of gamma radiation. Preparation and digestion of samples were applied according to the technique recommended by Gajan and Larry (1972). All prepared samples were first screened with a Geiger Muller apparatus for primitive evaluation of gamma radiation. Scaler ratemeter type 6-90 was used for the measurement of the levels of gamma radiation in each sample as count per minute (cpm). At the same time, the radiation of the background (air) was measured and subtracted from the sample reading, where the excess was converted to Becquerel unit per kilogram (Bq/Kg) (WHO, 1994).

Results and discussion

Radionuclides, which undergo significant gastrointestinal absorption by man and animals, are those of greatest concern in food chain transference. These radioactive contaminants are also readily transferred to animal products, such as milk and meat, which are then consumed by man (FAO, 1994). It was observed that the differences between the highest levels of gamma radiation in the background were lower than the maximum permissible limits recommended by Council Regulation ECC No. 1707/86. These limits were, in terms of maximum permitted levels of caesium-134 and caesium-137, 370 Bq/Kg for milk and infant feeds, and 600 Bq/Kg for other food (MAFF, 1994). It was observed that the imported canned luncheon meat from Palestine was had the most radiation (299.50 Bq/Kg), the canned beef imported from France (238.67 Bq/Kg) being the second. Lower results were recorded by many investigators, such as Hrusovsty *et al*. (1989) who found caesium radionuclides in canned meat products were up to 70 Bq/Kg, Battiston *et al*. (1991) who reported that the concentration of total caesium in muscle reached a maximum value of 156 Bq/Kg, as well as Marouf *et al*. (1991) who recorded that all imported lamb meat samples were contaminated with caesium-137 at the level of 82 Bq/Kg. The canned luncheon imported from Palestine had gamma radiation levels close to the minimum permissible limits (300 Bq/Kg) reported by FAO (1994)

Conclusions

Although most of the locally produced meat products in Egypt are manufactured from imported meat, the examined imported canned beef and canned luncheon had higher gamma radiation levels in comparison to those measured in the locally produced canned beef and canned frankfurters. Such observations may be attributed to different sources of contamination by gamma radiation, or other isotopes emitting gamma.

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Table 1. Mean values of gamma levels (cpm) in locally produced canned meat and some meat products samples in comparison to background (air)

Type of sample	N	Mean gamma in samples	Mean gamma in background
Canned beef	20	179.50	177.50
Canned frankfurter	5	179.83	175.16
Beef burger	5	169.83	175.16
Beef kofta	5	170.33	175.16
Beef luncheon	5	176.83	174.83
Beef sausage	5	167.17	175.16
Minced beef meat	5	163.33	175.16

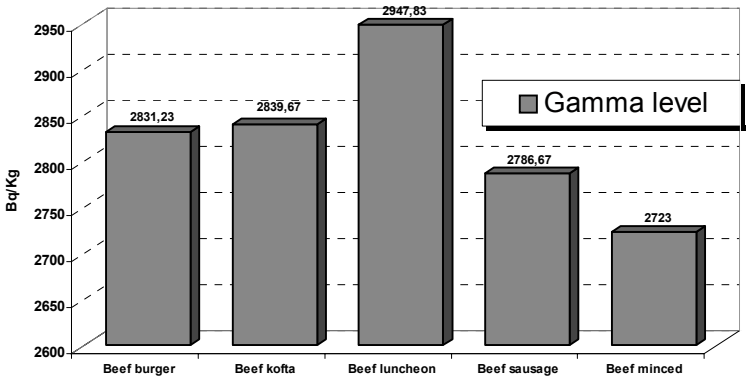

Figure 1. The level of gamma radiation in some local meat products.

Table 2. Differences between Highest levels of gamma (cpm) in locally produced canned meat and some meat products samples in comparison to background (air)

Type of sample	Highest level of gamma in sample (cpm)	Highest level of gamma in BG (cpm)	Difference (±) (cpm)	Difference (Bq/Kg)
Canned beef	208.65	200.15	+ 8.50	141.67
Canned frankfurter	202.06	193.00	+ 9.06	151
Beef burger	183.73	193.00	- 9.27	0
Beef kofta	183.93	193.00	- 9.07	0
Beef luncheon	196.27	192.30	+ 3.97	66.17
Beef sausage	179.80	193.00	- 13.20	0
Minced beef meat	173.27	193.00	- 19.73	0

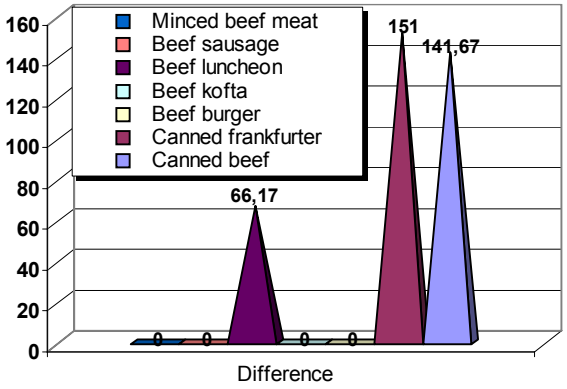

Figure 2. The difference in radiation between some local meat products with respect to the assay background.

Table 3: Mean gamma levels (cpm) in imported canned meat products samples in comparison to background (air)

Type of sample	N	Origin	Mean gamma in samples	Mean gamma in background
Canned beef	30	Brazil	189.50	184.67
Canned beef	10	France	178.67	174.33
Canned luncheon	10	Palestine	197.17	186.17
Canned luncheon	5	Holland	193.17	187.83

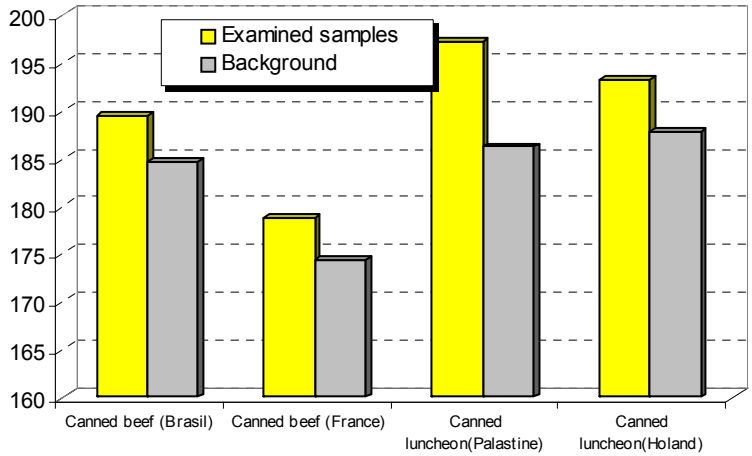

Figure 3. Mean levels of gamma (Bq/kg) in some imported canned meat in comparison to the background (air).

Table 4: Differences between the highest level of gamma radiation (cpm) in imported canned meat products samples in comparison to background (air).

Type of sample	Origin	Highest level of gamma in sample (cpm)	Highest level of gamma in BG (cpm)	Difference (±) (cpm)	Difference (Bq/Kg)
Canned beef	Brazil	201.60	192.45	+ 9.15	152.50
Canned beef	France	208.07	193.75	+ 14.32	238.67
Canned luncheon	Palestine	209.07	186.10	+ 17.97	299.50
Canned luncheon	Holland	197.20	187.85	+ 9.35	155.83

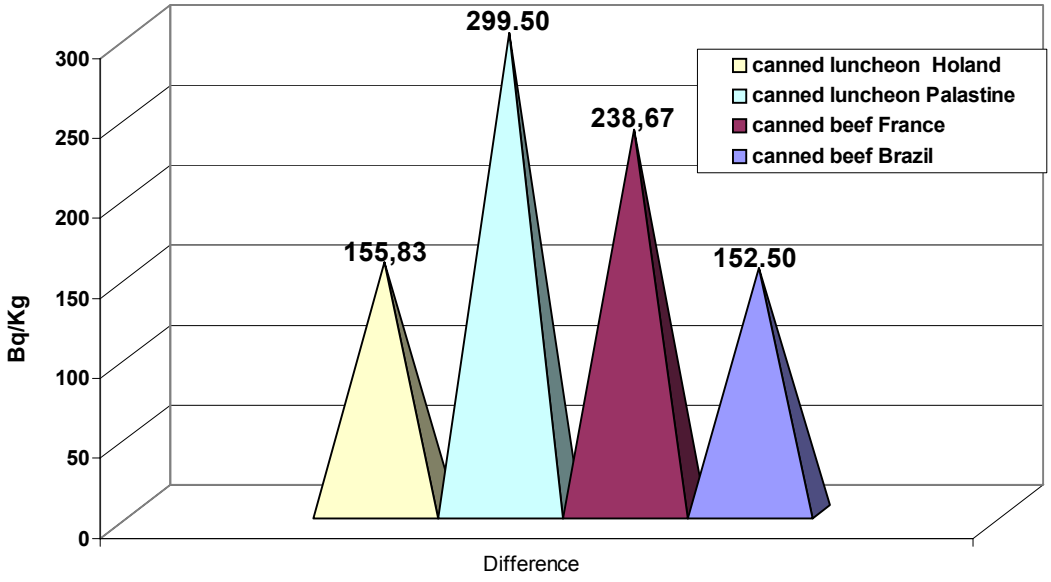

Figure 4. The difference between measured imported canned meat products and background measured in the assay

EFFECT OF CURRENT, VOLTAGE, FREQUENCY AND CARCASS TEMPERATURE ON IMPEDANCE MEASUREMENTS OF LAMB CARCASSES

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Background

Over recent years the livestock industry has changed from being production oriented to being consumer driven. Because people demand leaner meat, it is important to determine body composition in a non-invasive, objective and practical manner (Marchello *et al*., 1992). Bioelectrical impedance measures the resistance and reactance of a constant current as it passes through a biological mass and is related to the proportions of fat and lean. This arises because muscle and fat have different electrical properties (Marchello *et al*., 1999). Lean tissue is a highly conductive substance composed mostly of water containing electrolytes (~ 75%) (Swatland, 1984). Fat is essentially anhydrous and serves as an insulator, exhibiting impedance to the flow of an applied electrical current (Berg *et al.*, 1998). By measuring the resistance to an alternating current passed through the tissues, between electrodes separated by a known distance, the proportions of fat and lean can be estimated. The source electrodes introduce an alternating current at the base of the measuring object. The detecting electrodes measure the voltage drop due to the circuit at anatomical reference points (Bohuslavek et al., 2000). The most common BIA method involves two pairs of electrodes. The positive and negative electrodes of each pair are positioned a short distance from each other. A larger distance separates the two pairs. This four electrode or tetrapolar measurement is essential to eliminate electrode and field distribution problems associated with two electrode measurements (Bohuslavek *et al.,* 2000). Because BIA is safe, inexpensive, portable, rapid, easy to perform, and requires minimal operator training (Kushner, 1992), it would be likely to find favour in the industry. Early work on the application of BIA to measuring body or carcass composition used simple equipment developed for use on humans and operating at fixed current, voltage and frequency. Tong et al. (2001) showed that for pork carcasses impedance readings were dependent on frequency and temperature, but not on current and voltage. Furthermore, they showed that the prediction of carcass composition was improved when impedance measurements were taken at a number of frequencies. In designing a practical device for use on a lamb slaughterline it is important to choose the best combination of current, voltage and frequency and to be aware of the effect of temperature on impedance.

Objectives

The objective was to determine the effect of current, voltage, frequency and carcass temperature on BIA readings of lamb carcasses.

Materials and methods

Data on 49 lamb carcasses of varying fatness were used in this study. A laptop computer equipped with an Agilent 82357A USB/GPIB interface adapter was used to control a 4-electrode Hewlett Packard 4284A Precision LCR Meter (Agilent Technologies, www.agilent.com) to measure electrical impedance of hot carcasses, at 4 levels of alternating current (0.4mA to 1.4mA in steps of 0.3mA), 4 levels of voltage (100mV to 550mV in steps of 150mV), and 15 frequencies (8kHz to 200kHz at irregular intervals). The cranial transmitting electrode was placed proximal to the fifth cervical vertebrae. The caudal transmitting electrode was inserted into the *gastrocnemius* muscle, proximal to the Achilles tendon. The two receiving electrodes were placed 5cm cranial and 5cm caudal respectively to the transmitting electrode. Hot carcass measurements of Rs and Xc were recorded after carcass dressing, and impedance was calculated. Using the same apparatus and terminal placement, the electrical impedance of 15 carcasses was measured at deep muscle temperatures of 39, 35, 30, 25, 20, 15 and 0°C.

Results and discussion

Impedance is a measure of how current is slowed or stopped as it passes through a material. In biological systems, electrical conduction is related to water and ionic distribution in the conductor. Because fat-free mass (FFM), which includes the protein matrix of adipose tissue, contains virtually all of the water and conducting electrolytes in the body, conductivity is far greater in FFM than fat mass (Pethig, 1979). The hypothetical relationship between impedance and electrical volume was proposed by Nyboer et al. (1943) who first demonstrated that electrically determined biological volumes are inversely related to impedance (Z), resistance (R), and reactance (Xc) where,

$$Z = \sqrt{Rs^2 + Xc^2} \ .$$

Therefore, from the Rs and Xc readings from the LCR impedance (Z) was calculated. The effect of current and voltage on impedance can be seen in Figures 1 and 2. The straight lines clearly indicate that bioelectrical impedance did not change significantly over 4 alternating currents (Fig. 1) or 4 voltage levels (Fig. 2). Frequency did, however, affect impedance. As frequency increased from 8 to 200kHz, impedance significantly decreased (Fig. 3).

As carcasses were chilled from 39 to 0°C, impedance increased significantly from 200 to 380Ω (Fig. 4). This confirms that bioelectrical impedance measurements are temperature dependent. However, if impedance measurements were taken at the same point on the line, this temperature effect would be unimportant, as carcasses would be within 2°C of each other.

All these results, while preliminary, are in strong agreement with those found by Tong et al., (2001) who carried out similar work using pig carcasses.

Conclusions

These results suggest that the use of any current or voltage level in the ranges examined in this study would be appropriate to measure bioelectrical impedance in lamb carcasses. However, as frequency increased from 0 to 200kHz, impedance decreased, suggesting that there may be an optimum frequency for measuring impedance in lamb carcasses. Finally, bioelectrical impedance measurements are temperature dependent, but the effect is probably small enough to be unimportant for measuring carcasses at a fixed point on the kill line. Further work includes the investigation into the use of BIA to accurately predict saleable yield and fat-free-lean weight through carcass dissection and compositional analysis.

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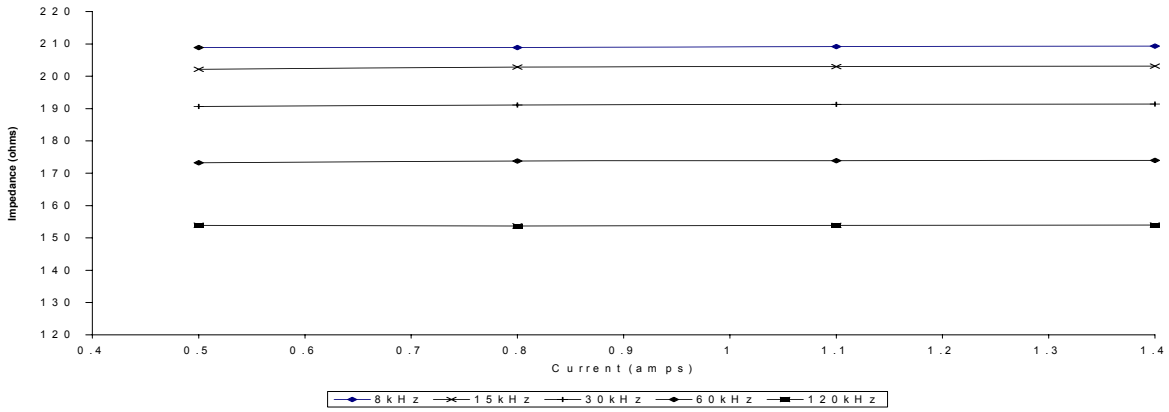

Figure 1. Effects of current

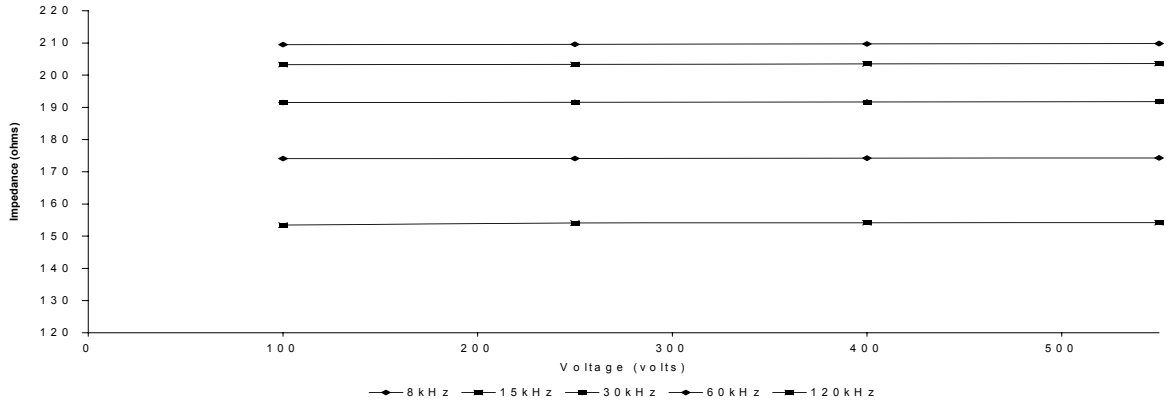

Figure 2. Effects of voltage

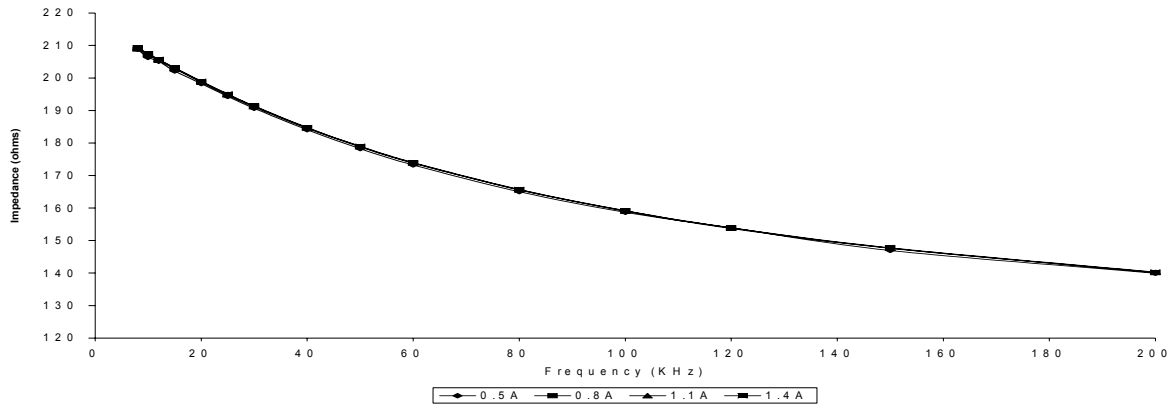

Figure 3. Effects of frequency

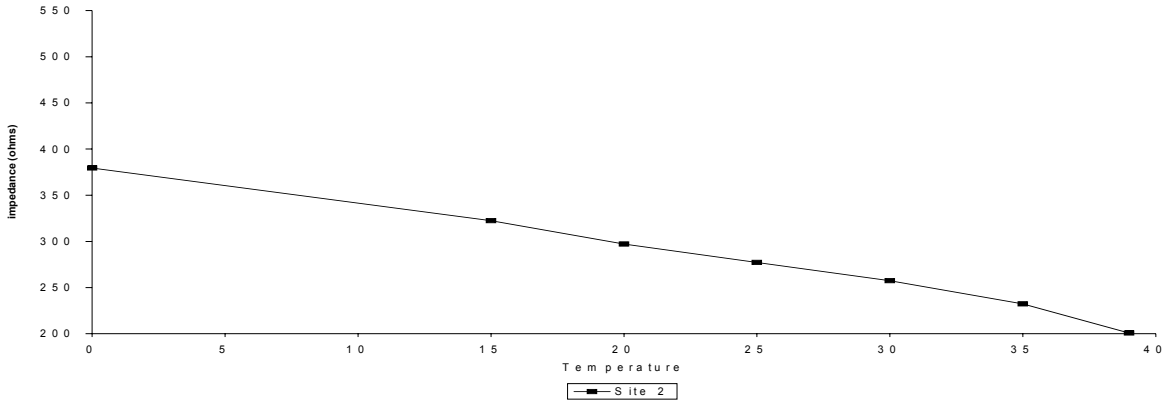

Figure 4. Effect of carcass temperature

THE AUTOFOM AND CVT-2 FOR PREDICTING SALABLE MEAT YIELD IN PORK CARCASSES

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Background

In Canada, the introduction in 1968 of a value-based system involving carcass weight and a ruler measurement of backfat thickness for grading pork carcasses and further refinements during the 80's (reflectance probe grading and the introduction of muscle thickness) have had a dramatic impact on the Canadian pork industry (Fortin, 1989). With these light reflectance probes (measurements of fat thickness lateral to the mid-line and the addition of muscle thickness into the prediction equations) the level of accuracy and precision improved (RMSE: from 2.6 % for the ruler to 2.1-2.2 % for the reflectance probes). Concurrent to the Canadian industry efforts to improve current grading technologies and implement new ones (Fortin et al., 2003), similar efforts were undertaken in Europe and the US. From these efforts, a new generation of grading instruments were developed. Ultrasound instruments such as the AutoFom (SFK Technology A/S, Herlev, DK) and CVT-2 (AUS, Ithaca, NY, US) are now commercially available for grading pork carcasses.

Objectives

The objective of this study was to evaluate under Canadian conditions the next generation of grading instruments which have become commercially available since the introduction in Canada in 1986 of the light reflectance probes, namely: the AutoFom (SFK Technology A/S, Herlev, DK) and the CVT-2 (AUS, Ithaca, NY, US). In addition, the reflectance probe HGP2 (Hennessey Grading Systems Ltd, Auckland, NZ) was utilized as the baseline instrument.

Materials and methods

Traditionally, grading instruments have been evaluated on the basis of the accuracy and precision of their respective equation to predict lean yield (lean and yield being defined in numerous ways). Calibration of a given instrument was done on a sample of carcasses deemed representative of the population onto which the prediction equation was going to be applied. Statistical parameters such as RMSE (Root Mean Square Error) and R^2 were used to assess these equations. Based on these parameters, that instrument was then certified for use in a national grading system if it met certain standard performance criteria. The over-riding assumption being that these equations generated from a sample of carcasses deemed representative of the population would perform as well when applied to the general population of pigs. However, in most cases, that assumption was never verified.

Hence, in this study, two independent sets of carcasses were used to evaluate the ultrasound instruments: one data set (calibration data set) to calibrate the instruments and a second data set (validation data set) to validate the fore-mentioned calibration.

The calibration data set and the validation data set consisted of 194 and 72 carcasses, respectively (Table1). Sampling was stratified by fat thickness. The boundaries for the middle category were defined as the Canadian population mean ± 0.50 standard deviation.

Two ultrasound instruments (CVT-2 System [3.5 MHz, 125 mm scanning guide], AUS, Ithaca, NY, US; and AutoFom, SFK Technology A/S, Herlev, DK) were evaluated. The reflectance probe HGP2 (Hennessey Grading Systems Ltd, Auckland, NZ) was used as the baseline instrument. For each instrument, the same operator was used for the entire study. For the CVT-2 and AutoFom, the operators were trained by the respective suppliers. The following carcass measurements were recorded: **AutoFom**: scan of the carcass as per supplier's instructions, **CVT-2**: fat thickness (average of five measurements) and muscle (*m. longissimus*) depth (average of five measurements) measured over a distance of 125 mm near the last rib, 5

cm lateral to the exposed surface of the mid-line. The muscle depth was defined as the distance between the fat-muscle interface to ribs. **HGP2**: fat thickness and muscle (*m. longissimus*) depth, 7 cm lateral to the exposed surface of the mid-line between the 3rd and 4th last ribs.

The day following slaughter, the left side of the carcasses was dissected as per the cutting procedure described by Fortin et al. (2003). Salable meat yield was defined as: 100*{(lean in picnic, butt, loin, tenderloin and ham) + belly (skinless, trimmed) + side ribs} / weight of cold side.

For the calibration phase, the models for predicting salable meat yield for the HGP2 and CVT-2 were obtained by Multiple Linear Regression analysis (Statistical Analysis System version 8.2, SAS Institute, Cary, NC, USA). The AutoFom calibration model was generated by Partial Least Squares analysis in which variables selection was done using a cross-validation procedure with 20 groups of 9-10 carcasses. The software program UNSCRAMBLER 7.6 (Camo, Trondheim, Norway), was used to generate the AutoFom calibration model.

For the validation of the calibration models, the following parameters were examined: 1) systematic bias defined as $Bias_s = 3(\lceil - Y_m) / n$ where $\lceil$ is salable meat yield predicted by the calibration model, Y_m the salable meat yield determined by the actual cutout of the side and n the number of carcasses, 2) proportional bias defined as $Bias_p = 1- b$ where b is the slope of the linear regression of predicted salable meat yield ($\lceil$) on measured salable meat yield (Y_m) [$\lceil = a + bY_m$], 3) the Mean Squared Prediction Error ($MSPE_V = 3(\lceil - Y_m)^2 / n$) and 4) and the Standard Error of Prediction ($SEP_V = \{3(\lceil - Y_m)^2 / (n–1)\}^{½}$).

Results and discussion

In Table 2 are the measurements obtained from the various grading instruments under investigation. For the AutoFom, however, the values of the fat and muscle measurements derived from the processed scan images and then used to generate the calibration models were not provided by the manufacturer. For the HGP2 and CVT-2, the following calibration model for predicting salable meat yield was selected:

Salable Meat Yield = a+ b*(fat thickness) + c*(muscle depth).

A recent Canadian study (Pomar et al., 2001) also showed that a linear calibration model for predicting yield was adequate. The calibration model for the AutoFom, provided by SFK Technology A/S, DK, is comprised of 36 variables (27 fat thickness and 9 muscle depth measurements).

The calibration parameters R^2 and RMSE assigned to each prediction model associated with the HGP2, CVT-2 and AutoFom are shown in Table 3. The CVT-2 prediction model compared favourably with the HGP2; RMSE: 1.57 % vs. 1.56 %, respectively, but was slightly better than the equation proposed for AutoFom; RMSE: 1.68 %. For each of the three instruments, all measurements retained for inclusion into their respective models were recorded over the loin muscle region: HGP2, CVT-2 and AutoFom. Consequently, it might then be argued that the high correlation between fat thickness measurements over the loin region or between muscle depth measurements over the loin region completely negated the advantage of using several variables, albeit statistically significant, over and above one fat thickness measurement and one muscle depth measurement over the loin region.

In Table 4, the validation parameters for the prediction of salable meat yield for the HGP2, CVT-2 and AutoFom are presented. For all instruments, the $bias_p$ was significantly different from zero ($P<0.01$). The AutoFom exhibited a slightly more pronounced $bias_p$ (0.39) compared to the CVT-2 (0.21); the HGP2 was intermediate (0.30). Figure 1 illustrates the respective relationship between Predicted salable meat yield and measured salable meat yield. With respect to a perfect theoretical relationship (solid line) of "Predicted salable meat yield vs Measured salable meat yield" ($Y = a + bX$ where $a = 0$, $b = 1$, $bias_p = 0$, and $R^2 = 1$) the fitted relationship (broken line) attests to an overestimation of the predicted salable meat yield for low measured salable meat yields (fat carcasses) and to an underestimation of the predicted salable meat yield for high measured salable meat yields (lean carcasses). Although a proportional bias was detected, no systematic bias ($bias_s$; $P>0.05$) was detected for all three instruments (Table 4). Standard Error of Prediction (SEP_V), another indicator of how well a prediction equation performs, was the lowest for the CVT-2 (SEP_V: 1.621),

the highest for the AutoFom (SEP_V: 2.052) and intermediate for the HGP2 (SEP_V: 1.833). Insofar as for the prediction of salable meat yield, the validation procedure revealed a slight advantage for the CVT-2 (a less pronounced $bias_p$, no $bias_s$ and a lower SEP_V).

Conclusions

With the introduction in the early to mid-eighties of reflectance probe grading, the level of accuracy and precision in predicting yield, one of the key components of any grading systems, was dramatically improved. Since then, numerous attempts to improve accuracy and precision have been made: the development of ultrasound-based instruments being one of them. In this study, two ultrasound instruments, CVT-2 and AutoFom, were evaluated and compared with the reflectance probe HGP2, the probe traditionally used in Canadian studies as the baseline probe.

Calibration and validation procedures showed that, if one was to base the assessment of these ultrasound instruments strictly on the precision and accuracy for predicting salable meat yield, the improvement over the baseline reflectance probe HGP2 would be considered rather minimal, particularly for the AutoFom. However, the major advantage of these ultrasound instruments is that they are non-invasive. Furthermore, the AutoFom, being already fully automated and requiring minimal human intervention, can easily be integrated into the operation of a processing plant in order to fully use the information generated at the time of grading.

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Table 1. Number of carcasses: calibration data set and validation data set.

	Calibration data set (n=194)		Validation data set (n=72)	
Fat thickness [a]	Gilts	Barrows	Gilts	Barrows
Less than 17.3 mm	73	16	24	8
17.3-21.0 mm	25	40	12	13
Greater than 21.0 mm	11	29	4	11

[a] Fat thickness measured with the HGP2 (Hennessey Grading Systems Ltd, Auckland, NZ)

Table 2. Means and Standard Deviations of fat thickness and muscle depth for the Calibration data set and Validation data set.

	Calibration data set		Validation data set	
Variables	Mean	Standard deviation	Mean	Standard deviation
HGP2 [a]				
3/4 LR Fat thickness (mm)	17.93	3.79	1.10	4.23
3/4 LR Muscle depth (mm)	52.07	5.95	52.42	6.41
CVT-2 [b]				
Average fat thickness (mm)	18.70	4.46	19.06	5.36
Average muscle depth (mm)	57.04	5.35	57.41	5.40
Salable meat yield (%)	59.81	3.12	59.68	3.74
Warm carcass weight (kg)	59.81	3.12	59.68	3.74

[a] Hennessey Grading Systems Ltd, Auckland, NZ [b] AUS, Ithaca, NY, US

Table 3. Calibration parameters (R2 and RMSE) for the prediction of salable meat yield and AutoFom.

Grading Instrument	R^2	RMSE [d]
HGP2 [a] (Fat [**] and Muscle [**])	0.74	1.56
CVT-2 [b] (Average Fat [**] and Average Muscle [**])	0.75	1.57
AutoFom [c] (36 variable model)	0.75	1.68

[a] Hennessey Grading Systems Ltd, Auckland, NZ [b] AUS, Ithaca, NY, US [c] SFK Technology A/S, Herlev, DK [d] RMSE: Root mean square error. Variables in models for HGP2, CVT-2 and UltraFom 300: [**] P<0.01, [*] P<0.05

Table 4. Validation parameters for the prediction of salable meat yield and the relationship of predicted salable meat yield ($\hat{}$) on measured salable meat yield (Ym): HGP2, CVT-2 and AutoFom.

			$\hat{} = a + b_* Y_m$				
Instrument	$Bias_s$ [d]	$Bias_p$ [d]	Intercept a	Slope b	SEP_V	$MSEP_V$	R^2
HGP2 [a]	-0.03(0.22[e]) [NS]	0.30 [**]	15.50 (2.901 [e])	0.70 (0.048 [e])	1.833	3.312	0.70
CVT-2 [b]	-0.01 (0.19) [NS]	0.21 [**]	12.71 (2.707)	0.79 (0.045)	1.621	2.590	0.79
AutoFom [c]	0.003 (0.24) [NS]	0.39 [**]	23.51 (2.798)	0.61 (0.047)	2.052	4.152	0.61

[a] Hennessey Grading Systems Ltd, Auckland, NZ b AUS, Ithaca, NY, US c SFK Technology A/S, Herlev, DK d NS P>0.5; ** P<0.01 e Standard error

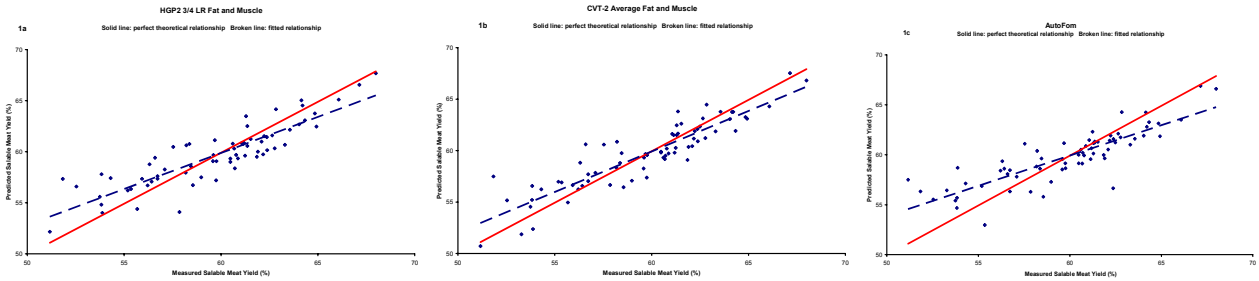

Figure 1. Validation of the HGP2, CVT-2 and AutoFom calibration models for predicting salable meat yield: relationship between predicted salable meat yield and measured salable meat yield.

REGULATION OF TASTE-ACTIVE COMPONENTS OF MEAT BY DIETARY PROTEIN LEVELS

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Background

Dietary nutrients play a significant part in determining growth rate and meat yield. It is known that the compositions of protein and total amino acids of meat are constant with respect to feeding treatment, hence the meat taste is considered to be constant in that regard as well. However, the relationship of taste components of meat with nutrients is not fully elucidated, and there have been few reports on the effect of feeding treatments on taste-active components of chicken meat. Previously, restricted feeding and dietary low metabolizable energy levels decreased the free Glu content of meat, and the meat taste was deteriorated. Therefore, meat taste can be affected by diets. In this study, the increase in free Glu and sensory score of meat by the diet was studied.

Objectives

In this study, the relationship of dietary crude protein (CP) levels with taste components in chicken meat extract was studied, focusing in particular on the taste-active components. Three experiments were conducted, Experiment 1: meat type chickens were fed graded CP level diets for 10 days, and the meat composition, free amino acids and ATP metabolites were measured. Experiment 2: meat taste was evaluated by a sensory panel. Experiment 3: taste components and Glu-related enzymes of meat were measured at 0, 3, 5 and 10 days.

Materials and methods

Experiment 1: The 14-day-old female Cobb strain broiler chickens were divided into four groups of twelve chicks. The chicks were fed CP 17.6, 26.4, 30.8 and 35.2% diet. All the chickens were kept in individual wire cages. Free amino acids and ATP metabolites of the pectoral meat extract were measured. Experiment 2: Two sensory evaluations, paired comparison test and Scheffe's paired difference test, were conducted between meats of the CP17.5% and 30.8% groups. Experiment 3: The 14-day-old broiler chicks were fed CP 30.8% diet for 10 days. At day 0, 3, 5 and 10, free Glu and Glu-relate enzymes in muscle were measured. For the investigation of Glu regulation mechanism, glutamate dehydrogenase (GDH), kidney type glutaminase (KGA), glutamine synthetase (GS) and alanine transaminase (ALT) activities were measured by enzymatic methods (Bergmeyer, 1974).

Results and discussion

In Experiment 1, free Glu of meat significantly increased in the high CP diet, while 5'-inosinic acid (IMP) was constant. Because free Glu content of meat were above the taste threshold value of Glu, these variations were considered to have affected the taste. In the sensory evaluation (Experiment 2), the taste of the meat of high CP diet group was found superior ($P<0.01$) to that of the control group, especially in overall preference, thickness and umami taste. These results suggested that, dietary CP levels could affect taste-active components, especially free Glu. In experiment 3, after 3 and 5 days on high CP diet, free Glu of muscle was higher than that of 0 day, and tended to decrease after 10 days. KGA activity, with respect to the CP 30.7% diet, was lower than that of the control ($P<0.05$). There were no differences in GDH, GS and ALT activities. KGA was considered to contribute to the free Glu increase. Feedback inhibition of GA activity by high Glu concentration at day 5 may have affected the free Glu concentration of muscle at day 10.

Conclusions

Free Glu and sensory scores of chicken meat increased by dietary CP levels. Because the variation of the taste component improved the taste of meat, the feeding regime may be one of the important factors affecting

the taste of chicken meat. We thus conclude that short-time feeding of high CP diet, especially for 3 to 5 days, may well be an appropriate measure to improve the taste of meat.

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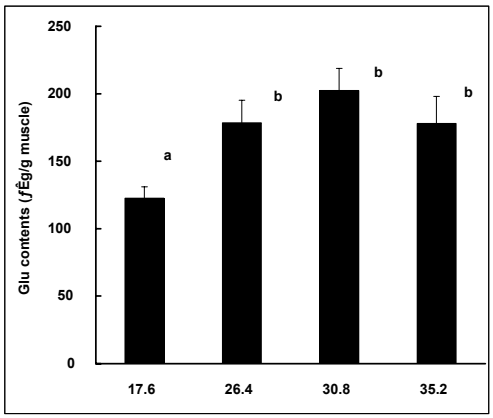

Figure 1. Effect of dietary CP levels on free Glu contents of pectoral meat extract. Values expressed as means+SE, n=6 chicks per each group. (P<0.01)

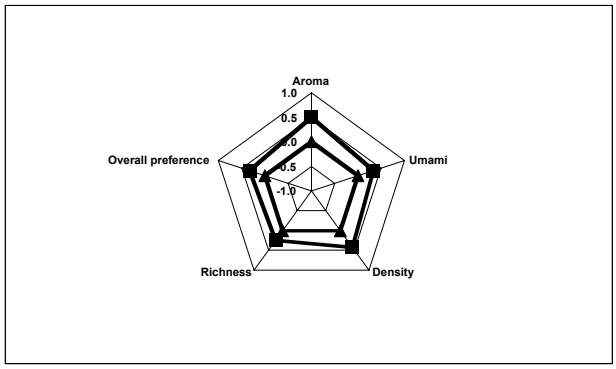

Figure 2. Scheffe's paired difference test between pectoral meat extracts with respect to the CP 17.6 and 30.8% diets.

EARLY PREDICTION OF CARCASS YIELD GRADE BY ULTRASOUND IN KOREAN HANWOO CATTLE

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Background

Real-time ultrasound instruments have been shown to be an accurate predictor of carcass 13th-rib fat thickness and longissimus muscle area (LMA) in beef cattle (Perkins et al., 1992; Robinson et al., 1992; Song et al., 2002). Smith et al. (1992) found that correlation coefficients between live animal ultrasound and carcass measurements of BFT and LMA varied from 0.81 to 0.82 and from 0.43 to 0.63, respectively. Research has also shown that accuracy is highly dependent on the technician and level of experience that the person possesses (McLaren et al., 1991). However, limited information has been published on the accuracy or precision of these systems (Brethour, 1994; Herring et al., 1998). Since 1992, the Korean Livestock Cooperatives Federation has provided a beef grading system for quantifying meat yield and quality factors by subjective evaluation. The possibility of using ultrasound to precisely and accurately estimate carcass measurements in live animals might be beneficial to the beef industry, allowing it to move away from the current practice of pricing cattle on pen averages towards a value-based marketing system.

Objectives

The objective of this study was to evaluate early prediction of yield grade by using ultrasound measurements.

Materials and methods

Two hundred and twenty three Korean Hanwoo cattle of 18, 21 and 24 months of age were ultrasonically scanned by Super-eye Meat (FHK Co. Ltd., Japan) with an electric linear probe (2 MHz frequency: 27 X 147 mm) at the 13th rib of their left longissimus muscle. Vegetable oil was used on the probe to obtain adequate acoustic contact. Scanned images were obtained using double frame display capabilities of the equipment. A transducer guide was used to minimize error that might occur due to animal back line curvature or the overlapping step required to produce one complete image of the longissimus muscle. The resulting ultrasound images were recorded on portable personal computer and later viewed on a display monitor to estimate both back fat thickness (BFT) and longissimus muscle area (LMA) using computer software (Image-Pro Express, Media Cybernetics, USA). In this study, ultrasonic estimate was compared to carcass value for increased prediction accuracy by two prediction methods: regression and decision tree. Regression techniques (SAS, Ver. 8.1; 2000) were used to evaluate the best-fit equation to explain variation in retail yield components (BFT, LMA and live weight (LW)) from ultrasonic. Decision trees that have too many nodes have great probability of prediction error when it is applied to new data. Thus, inappropriate branches were removed from the decision tree, and the remaining tree structure was used as a model for prediction.

Results and discussion

Differences in the ultrasonic and actual carcass measures in BFT and LMA are presented in Table 1. Ultrasonically measured value indicated thinner back fat and smaller longissimus muscle area. Regression equations for prediction carcass yield grade at different months of age are presented in Table 2. Table 3 presents the prediction accuracy of the regression method on the mean yield grade, which was predicted with 73.1% accuracy at 18 months, 83.2% at 21 months and 89.2 % at 24 months of age. However, different grades (A-C) were not predicted equally well at all ages, yield grade A being most predictable. Prediction accuracy was 75.2% for yield grade A and 72.3% for yield grade B at 18 months, 88.9% and 69.2% at 21 months, and 92.8% and 81.5% at 24 months, respectively. By using the decision tree method for carcass yield grades at 24 months of age, 75.8%, 84.8% and 91.0% of prediction accuracy were obtained at 18, 21 and 24 months of age, respectively. Also, if live weight was unknown (as in small farms), the decision tree method enabled prediction only using ultrasonic measurements (BFT and LMA).

Conclusions

The results of the present study suggest that the decision tree method predicted the yield grade of meat with 91% accuracy. The results obtained on image texture suggest that interfacing a computer with an ultrasound system may improve accuracy and precision of the procedure of estimating carcass quality. Such technology might thus serve as a useful tool and predicting aid in mechanical meat grading.

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Table 1. Carcass traits of Hanwoo steers by ultrasonic and carcass measures

Items	Age (months)			
	18	21	24	24
	----------	ultrasound	----------	carcass
LW[1)] (kg)	376.70±65.28[c]	470.64±59.63[b]	544.27±53.16[a]	-
CW[2)] (kg)	-	-	-	313.05±36.50
BFT[3)] (㎜)	3.21±2.06[c]	5.05±2.56[b]	6.59±3.07[a]	6.94±3.38[a]
LMA[4)] ( )	53.13±9.14[d]	64.04±8.76[c]	71.45±9.33[b]	75.88±7.81[a]

[1)] Live animal weight, [2)] Carcass weight, [3)] Back fat thickness, [4)] Longissimus muscle area.
[a,b,c] Means with different superscripts in the same row are significantly different ($p<0.05$).

Table 2. Regression equation for prediction carcass yield grade at 18, 21 and 24 months of age

Age	Regression equation
18 months	$Y = 69.6967 - 0.5487\times UBF^{***} + 0.0387\times ULMA^{***} - 0.00323\times LW$
21 months	$Y = 69.2308 - 0.4793\times UBF^{***} + 0.0420\times ULMA^{***} - 0.00045\times LW$
24 months	$Y = 69.3991 - 0.4419\times UBF^{***} + 0.0403\times ULMA^{***} - 0.00024\times LW$

Yield grade A : $69\leq Y$, B : $66\leq Y<69$, C : $Y<66$.
* $p<0.001$.

Table 3. Prediction accuracy of carcass yield grade at 18, 21, and 24 months of age by the regression method

YGC[1)]	YGU[2)]	18 months		21 months		24 months	
		n	Accuracy	n	Accuracy	n	Accuracy
A	A	115	75.2%	136	88.9%	142	92.8%
	B	38		17		11	
	C	0		0		0	
B	A	18		20		11	
	B	47	72.3%	45	69.2%	54	81.5%
	C	0		0		0	
C	A	0		0		0	
	B	4		0		1	
	C	1	20.0%	5	100.0%	4	80.0%
Total		223	73.1%	223	83.2%	223	89.2%

[1)] Carcass yield grade, [2)] Ultrasonic yield grade.

Table 4. Prediction accuracy of carcass yield grade at 18, 21, and 24 months of age by the decision tree method

YGC	YGU	18 months		21 months		24 months	
		n	Accuracy	n	Accuracy	n	Accuracy
A	A	124	81.0%	138	90.2%	142	92.8%
	B	29		15		11	
	C	0		0		0	
B	A	22		19		8	
	B	43	66.2%	46	70.8%	57	87.7%
	C	0		0		0	
C	A	0		0		0	
	B	3		0		1	
	C	2	40.0%	5	100.0%	4	80.0%
Total		223	75.8%	223	84.8%	223	91.0%

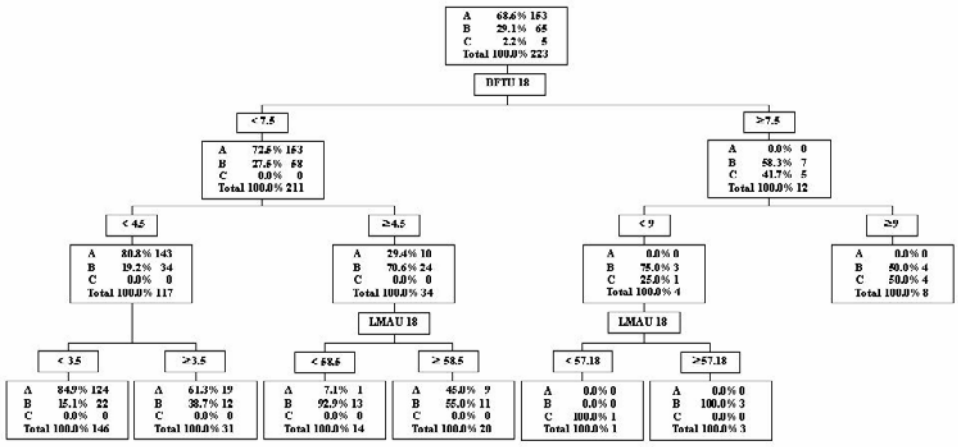

Fig. 1. Distribution pattern of carcass yield grade at 18 months of age by the decision tree method

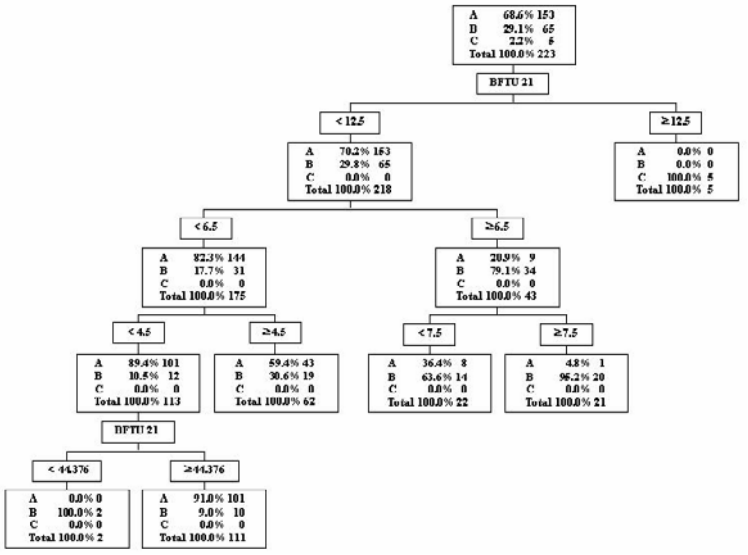

Fig. 2. Distribution pattern of carcass yield grade at 21 months of age by the decision tree method

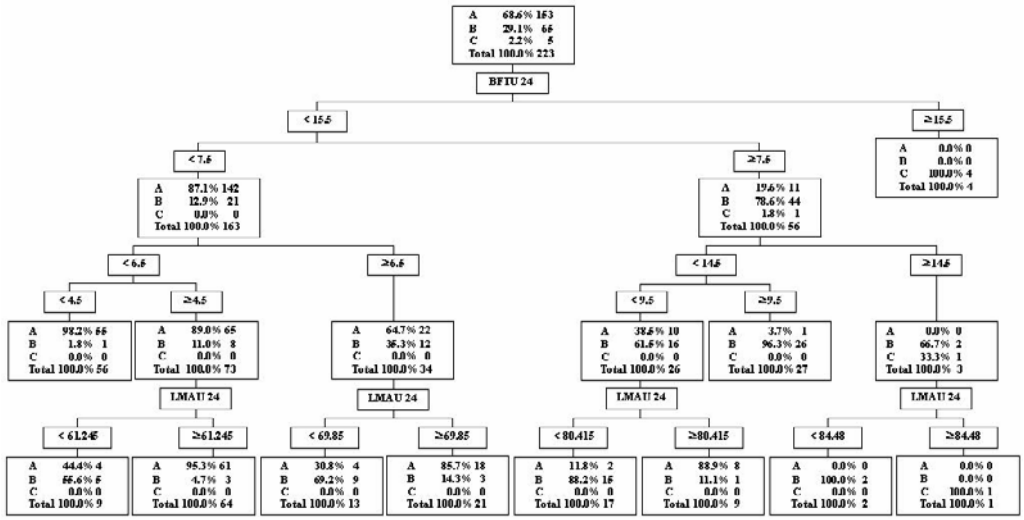

Fig. 3. Distribution pattern of carcass yield grade at 24 months of age by the decision tree method

REGULATION OF TASTE-ACTIVE COMPONENTS OF MEAT BY DIETARY BRANCHED-CHAIN AMINO ACIDS

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Background

Regulation of taste active components of meat is important for the improvement of meat quality. It has been generally believed that it is possible to regulate aroma and storage stability by diet, while regulation of meat taste is difficult. In previous study (2001), we suggested that the main taste-active component of meat, free glutamate (Glu) content, could be controlled by dietary restriction and metabolizable energy. However, there were no reports about the effects of other dietary factors on the taste components of meat.

Zhou and Thompson (1996) reported that glutamate dehydrogenase (GDH) activity in the muscle was regulated by branched-chain amino acids (BCAA) *in vitro*. BCAA, leucine, valine and isoleucine, are known as essential amino acids, and mainly metabolized in the muscle, not in the liver. Therefore, we considered that dietary BCAA may affect the Glu contents of meat *in vivo*.

Objectives

In this study, the effect of dietary BCAA, especially leucine (Leu), level on meat quality was studied. The meat quality was estimated by free amino acids, ATP metabolites and sensory evaluation. As a result, the Glu level and sensory score were changed by dietary Leu level. Then, the Glu-related enzyme activities in muscle were measured to elucidate the regulatory mechanism of Glu in the muscle.

Materials and methods

28-day-old female Cobb strain broiler chickens were divided into 4 groups. Leu contents of experimental diet were 70, 100, 130 (control) and 150% of the NRC (1994) requirement of Leu. Because commercial diet include 130-150% of NRC requirement, we considered Leu130% as control. The 4 groups of chickens were kept on these diets *ad libitum* for 10 days. On day 11, all chickens were slaughtered, and breast muscles (*M. Pectoralis superficialis*) were taken for analyses. Concentrations of free amino acids and ATP metabolites in meat extract were measured by HPLC. Sensory evaluation was carried out with 12 trained panellists using paired difference test and Scheffe's paired comparison test. For the investigation of the mechanism of Glu regulation, the muscle GDH, glutaminase (GA) and glutamine synthetase (GS) activities were measured by enzymatic methods (Bergmeyer, 1978).

Results and discussion

On the Leu70% diet, growth performance, weight gain, feed intake and feed efficiency of chicken decreased significantly compared to those on the other diets ($P<0.05$). Therefore, we considered that Leu70% diet was inappropriate in meat production.

The muscle amino acid analysis showed that free Leu contents increased with an increase in dietary Leu level. On the other hand, free Glu contents of the Leu100% diet increased by 34% compared to that of the control (Leu130%) ($P<0.05$). With a decrease in dietary Leu levels, free Glu of muscle tended to increase. There were no differences in the 5'-inosinic acid contents of muscle in all groups.

In sensory evaluation, all panellists found a difference in meat taste between Leu100% and the control ($P<0.01$) in the paired difference test. Furthermore, compared to the control, the Leu100% group got a significantly higher score in overall preference, chicken like taste and umami taste in Scheffe's paired comparison test ($P<0.01$). On the other hand, there were no differences between groups in aroma and taste intensity. These results suggest that the taste of meat in Leu100% group was superior to that in the control group.

The GDH activity of Leu100% and 150% groups was lower than that of control ($P<0.05$). In contrast, the GA activity decreased with an increase in dietary Leu level ($P<0.05$). There were no differences in the GS activity in each group. From these results, we considered that the GDH and GA activities contribute to the regulation of the free Glu content of muscle by dietary Leu level.

Conclusions

From these results, we conclude that the dietary Leu level affected the free Glu contents in muscle. The decrease in dietary Leu induced an increase in the free Glu of muscle. In sensory evaluation, the taste of meat improved with low dietary Leu level. The muscle activities of GDH and GA were also affected by the dietary Leu level. We conclude that dietary Leu level regulates the free Glu content in muscle, and the taste of meat can be improved by low dietary Leu level.

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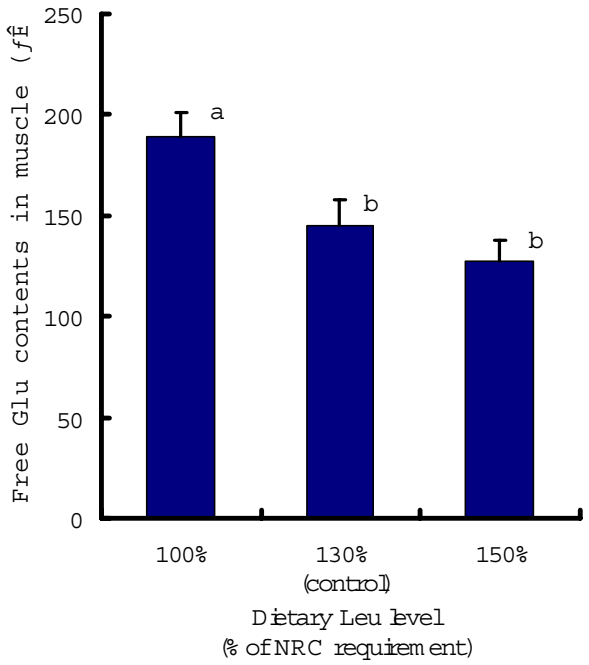

Figure 1. Effect of dietary Leu levels on free Glu contents in chicken breast muscle.
Values expressed as means ± SEM (n = 6).
Bars with different superscripts[a,b] are significantly different, $P < 0.05$.

EFFECTS OF DIETARY LINSEED OIL ON FATTY ACID COMPOSITION OF LAMB MEAT

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Background

Fatty acids are involved in various "technological" aspects of meat quality. Since they have very different melting points, variation in fatty acid composition has an important effect on firmness and softness of the fat in meat. Moreover, interest in meat fatty acid composition stems from the need to find ways to produce healthier meat, which has a higher ratio of polyunsaturated (PUFA) to saturated (SFA) fatty acids and a more favourable balance between ω-6 and ω-3 PUFA (Wood *et al.,* 2003).

The differences in the physiological effects on humans of ω-6 and ω-3 PUFA were unknown until 1980. Nowadays, evidence from epidemiological, clinical and biochemical studies demonstrates that ω-3 PUFA exert a protective effect against some common cancers such as breast, colon and perhaps prostate (Rose and Connolly, 1999), reduce some types of cardiovascular disease, improve rheumatoid arthritis and inflammatory bowel diseases and minimize episodes of rejection (Alexander, 1998).

Nutritionists have focused on the type of PUFA and the balance in the diet between ω-3 PUFA formed from α-linolenic acid (C18:3) and ω-6 PUFA formed from linoleic acid (C18:2) (Williams, 2000). The ratio of ω-6/ω-3 PUFA is considered a risk factor in cancers and coronary heart disease, especially in the formation of blood clots leading to a heart attack (Enser, 2001).

Some international agencies (British Nutrition Foundation, 1992; Department of Health, 1994) concerned with human health have considered the benefits of dietary long chain ω-3 PUFA, establishing a ω-6/ω-3 ratio of less than 4 to improve human health status. Consequently, there have been many attempts to manipulate feeds in order to increase the ω-3 fatty acid content in rabbits (Lopez-Bote *et al*., 1997), poultry (Chanmugam *et al.,* 1992), pigs (Enser *et al.,* 2000; D'Arrigo *et al.,* 2002a, 2002b) and ruminants (Ponnampalam *et al.,* 2002a, 2002b).

Ruminant meats are a relatively good source of ω-3 PUFA, due to the presence of C18:3 in grass. In Mediterranean areas where grazing lands are scarce and on farms where livestock is reared in sheds, it is possible to increase ω-3 PUFA content of ruminant meats by feeding animals on grain-based diets including whole linseed.

Objectives

The present study observed the effects on the fatty acid composition of lamb meat when linseed oil was used in feed.

Materials and methods

Twenty male Gentile di Puglia lambs were weaned at the age of 45 days and immediately divided into two homogeneous groups of 10. Control group lambs were fed on hay and commercial concentrated feed containing soybean oil (30 g kg^{-1}); experimental group lambs on hay and commercial concentrated feed containing linseed oil (30 g kg^{-1}). Energy, protein and fibre contents of both diets were the same.

Lambs were slaughtered at 100 days. The carcasses were refrigerated at 4°C for 24 hours. *Longissimus lumborum* (Ll) muscles were taken from right half-carcasses. Representative sub-samples were taken from Ll muscles and divided into two pieces. One piece was cooked in a ventilated electric oven at 180°C until an internal endpoint temperature of 75°C was reached in the geometric centre of the meat cut. The

temperature was recorded by a thermocouple (Hanna Instruments) inserted into the meat sample placed at the centre of the wire rack (ASPA, 1996).

Raw and cooked meat samples were homogenized in a grinder to perform chemical analysis (ASPA, 1996). Lipids were extracted from both raw and cooked samples according to the method suggested by Folch *et al*. (1957) using a chloroform/methanol 2:1 (v/v) solution. Fatty acids were methylated using a BF_3/methanol solution (12% v/v) and analysed by gas chromatography (Chromopack CP 9000) using a 60 m silicated glass column with a 0.25 mm internal diameter and 0.2 μm film thickness.

The atherogenicity and thrombogenicity indexes (Ulbricht and Southgate, 1991) and the PCL/PCE (plasma cholesterol lowering/plasma cholesterol elevating) ratio (Reiser and Shorland, 1990) were also calculated.
Analysis of variance was carried out on the data using the GLM procedure of SAS. The model considered diet, cooking and their interaction as main effects. Means were compared using Student's t test (SAS, 1999/2000).

Results and discussion

Feed containing linseed oil did not modify the chemical composition of raw and cooked meat when compared to feed containing soybean oil, as found also for 75-day-old lambs (Caputi Jambrenghi *et al*., 2004a). Regardless of diet, cooking lowered the moisture content of meat and increased the levels of protein, ash and N-free extract (P<0.01), without any alteration to the ether extract content (Table 1).

Table 1. Chemical composition of raw and cooked meat (% wet matter)

	Control group		Experimental group		SED	Significance of main effects	
	Raw	Cooked	Raw	Cooked	(DF = 36)	Diet	Cooking
N (samples)	10	10	10	10			
Moisture	75.13^{M}	65.98^{N}	74.99^{M}	65.74^{N}	2.494	ns	**
Protein	19.14^{N}	26.79^{M}	18.84^{N}	26.55^{M}	1.158	ns	**
Ether extract	3.49	3.43	3.94	4.12	1.464	ns	ns
Ash	1.06^{N}	1.70^{M}	1.10^{N}	1.67^{M}	0.142	ns	**
N-free extract	1.18^{N}	2.10^{M}	1.12^{N}	1.92^{M}	0.243	ns	**

Differences between raw and cooked meat within each group: M, N: P<0.01
Significance of main effects: **: P<0.01; ns: not significant

Feed containing linseed oil brought about an increase in the C18:3 ω-3 content of the raw (1.54 *vs* 0.86; P<0.01) and cooked meat (1.12 *vs* 0.42; P<0.01), thus confirming the results obtained with 75-day-old lambs (Caputi Jambrenghi *et al*., 2004b), and in the C22:6 ω-3 level of the cooked meat (0.14 *vs* 0.01; P<0.01) (Table 2).

It also increased total PUFA in the cooked meat (7.24 *vs* 5.96; P<0.01), total ω-3 in the raw (2.12 *vs* 1.64; P<0.01) and cooked meat (1.94 *vs* 0.86; P<0.01), and lowered the ω-6/ω-3 ratio of the cooked meat (2.88 *vs* 6.34; P<0.01) bringing it to below 4, as recommended by the Human Nutrition Society (Carnovale and Marletta, 1997).

On the cooked meat, feed containing linseed oil raised the PUFA/SFA ratio (0.15 *vs* 0.13; P<0.01), bringing it closer to the 0.45 level recommended by the Department of Health (1994), as well as lowered the thrombogenicity index (1.43 *vs* 1.55; P<0.01).

Cooking reduced the level of C18:3 ω-3 (P<0.01) in meat produced with either feed. However, cooking increased the level of C22:6 ω-3 in the experimental group meat (0.14 *vs* 0.06; P<0.01), unlike the control group meat where the level was lowered (0.01 *vs* 0.14; P<0.01).

Cooking had a positive effect on meat produced with the linseed oil feed regarding total SFA (47.62 *vs* 49.68; P<0.01), total MUFA (45.14 *vs* 42.36; P<0.01), total UFA (52.38 *vs* 50.32; P<0.01), the UFA/SFA ratio (1.10 *vs* 1.01; P<0.01), the thrombogenicity index (1.43 *vs* 1.54; P<0.01). Cooking had a negative

effect on meat produced with the soybean oil feed regarding total PUFA (5.96 *vs* 7.66; P<0.01), total ω-3 (0.86 *vs* 1.64; P<0.01), the ω-6/ω-3 (6.34 *vs* 3.83; P<0.01) and PUFA/SFA ratios (0.13 *vs* 0.16; P<0.01).

Conclusions

The use of linseed oil in the production of 100-day-old lambs does not modify the chemical composition of the meat. It improves, however, the dietary characteristics, especially in cooked meat, increasing the C18:3 ω-3, C22:6 ω-3 and total ω-3 contents and improving the ω-6/ω-3 and PUFA/SFA ratios and the thrombogenicity index.

Table 2. Fatty acid composition of raw and cooked meat (% total fatty acids)

	Control group		Experimental group		SED	Significance of main effects	
	Raw	Cooked	Raw	Cooked	(DF = 36)	Diet	Cooking
N (samples)	10	10	10	10			
C10:0	0.30	0.28	0.32	0.34	0.102	ns	ns
C12:0	0.46	0.38	0.34	0.48	0.164	ns	ns
C14:0	4.52	4.22	4.14	4.54	0.679	ns	ns
C16:0	24.16^{BN}	25.16^{aM}	25.36^{AM}	24.44^{bN}	0.760	ns	ns
C16:1	1.88^{N}	2.78^{AM}	1.54^{n}	1.98^{Bm}	0.412	**	**
C18:0	16.98^{M}	15.14^{N}	17.78^{M}	15.96^{N}	0.950	**	**
C18:1 ω-9 trans	6.06^{A}	6.48^{A}	5.12^{B}	5.18^{B}	0.633	**	ns
C18:1 ω-9 cis	33.08^{N}	34.66^{M}	33.44^{N}	35.08^{M}	1.025	ns	**
C18:2 ω-6 cis	4.92^{M}	4.18^{N}	4.54^{M}	3.70^{N}	0.586	*	**
C18:3 ω-6	0.26^{M}	0.12^{BN}	0.26^{n}	0.36^{Am}	0.112	**	ns
C18:3 ω-3	0.86^{BM}	0.42^{BN}	1.54^{AM}	1.12^{AN}	0.231	**	**
C20:1 ω-9	0.64^{am}	0.40^{n}	0.36^{b}	0.44	0.253	ns	ns
C18:2 conj cis	0.08	0.08^{B}	0.16	0.26^{A}	0.120	**	ns
C18:2 conj trans	0.12	0.06^{B}	0.04^{N}	0.26^{AM}	0.160	ns	ns
C20:3 ω-6	0.34^{B}	0.26	0.54^{A}	0.40	0.182	**	ns
C20:3 ω-3	0.06	0.02^{B}	0.01^{N}	0.22^{AM}	0.112	*	**
C20:4 ω-6	0.06	0.08	0.04	0.06	0.068	ns	ns
C20:5 ω-3	0.42^{M}	0.20^{N}	0.32^{M}	0.14^{N}	0.126	*	**
C22:5 ω-3	0.04	0.10	0.01^{N}	0.14^{M}	0.089	ns	**
C22:6 ω-3	0.14^{AM}	0.01^{BN}	0.06^{BN}	0.14^{AM}	0.065	ns	ns
Other acids	3.08^{N}	3.58^{M}	3.04	3.26	0.422	ns	**
Total SFA	48.18^{Bm}	47.06^{n}	49.68^{AM}	47.62^{N}	1.259	*	**
Total MUFA	44.16^{AN}	46.98^{AM}	42.36^{BN}	45.14^{BM}	0.998	**	**
Total PUFA	7.66^{M}	5.96^{BN}	7.96	7.24^{A}	0.948	**	**
Total UFA	51.82^{An}	52.94^{m}	50.32^{BN}	52.38^{M}	1.259	**	**
Total ω-6	6.02^{m}	5.10^{n}	5.84	5.30	0.956	ns	*
Total ω-3	1.64^{BM}	0.86^{BN}	2.12^{A}	1.94^{A}	0.304	**	**
ω-6/ω-3	3.83^{N}	6.34^{AM}	2.78	2.88^{B}	1.438	**	**
UFA/SFA	1.08^{An}	1.13^{m}	1.01^{BN}	1.10^{M}	0.055	**	**
PUFA/SFA	0.16^{M}	0.13^{BN}	0.16	0.15^{A}	0.022	ns	**
Atherogenicity index	0.82	0.80	0.84	0.82	0.076	ns	ns
Thrombogenicity index	1.51	1.55^{A}	1.54^{M}	1.43^{BN}	0.076	ns	ns
PCL/PCE	1.02	0.99	0.98	1.01	0.073	ns	ns

Differences between diets: A, B: P<0.01; a, b: P<0.05
Differences between raw and cooked meat within each group: M, N: P<0.01; m, n: P<0.05
Significance of main effects: *: P<0.05; **: P<0.01; ns: not significant

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VACUUM COOLING OF COOKED MEATS

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Background

Meat is chilled immediately after slaughter. Most of the subsequent operations in the cold chain are designed to maintain the temperature of meat. Cooking is very common operation in the production of many meat products. The aim of any cooking process for meat/meat products is to ensure the destruction of vegetative stages of any pathogenic microorganisms. However, there is always the possibility that the cooking process will not kill some microorganisms that produce spores or that the food can become recontaminated. Therefore, microbiologists recommended that the temperature of the meat should be rapidly reduced, especially from approximately 60 and 5℃, to prevent multiplication of exiting or contaminating bacteria. Rapid cooling is also desirable with cooked products to maintain quality by eliminating the overcooking that occurs during slow cooling.

Some European countries have some similar food safety regulations. Both Irish (Food Safety Advisory Committee, 1991)and UK (Department of Health and Social Security, 1989) government guidelines recommend that cooked meat joints should be cooled from a core temperature of 74℃ to below 10℃ in 2.5h. Chinese government has also made the similar regulations in meats and cooked meats processing (China GB 12694-90). A rapid cooling treatment after cooking can minimize the growth of surviving organism (Burfoot, D, Self, K. P, Hudson, W. R., Wilkins, T. J, & James, S. J. 1990).

Vacuum cooling as rapid evaporative cooling method has been extensively used as an effective method to remove field heat and thus to extend shelf and improve quality for many types of horticultural and floricultural products such as lettuce (Haas & Gur, 1987; Rennie, Raghavan, Vigneault, & Gariepy, 2001), mushroom (Atkins 974), and cut flowers (Sun & Tadhg. Brosnan, 1999). Research has indicated that vacuum cooling can meet both European and USA guidelines. Wang and Sun (2002 part1; 2002 part2; 2004) have developed a mathematical model for describing the vacuum cooling process of large cooked meat joints.

Objectives

In this current study, the previous model is modified and the modified model is further used to analyze the performance of vacuum cooler and the vacuum cooling process of cooked meats.

Mathematical models

Vacuum cooling process includes two fairly distinct phases: (a) the removal of the air in the vacuum chamber from the atmospheric pressure to the saturation pressure at the initial temperature of cooked meats, and (b) the drop of the pressure in the vacuum chamber continuously to the final vacuum pressure. The maximum pressure in the chamber, which can cause water in cooked meats to boil, is the saturation pressure of the water vapour at the initial temperature of cooked meats. The relationship for the saturation pressure of the water vapour in cooked meats and the temperature of cooked meats is determined by (Wang & Sun 2002 part1):

$$P_{sat} = \exp\left(23.209 - \frac{3816.44}{T_K - 46.44}\right) \quad (1)$$

The total pressure in the vacuum chamber is the sum of the partial of air and water vapour:

$$P_{vc} = P_a + P_v \quad (2)$$

The decrease rate of total vacuum pressure in the chamber can be calculated by:

$$\frac{dP_{vc}}{dt} = -\frac{S \cdot P_{vc}}{V_f}$$

(3)

In developing the heat and mass transfer models, cooked meats are assumed to be cylindrical in shape with the internal cooling generation due to evaporation. The assumptions of the mathematical models developed can be summarized as follows:

- The initial temperature and moisture content of cooked meats are constant;
- Cooked meats is homogeneous and isotropic;
- The thermal properties of cooked meats are constant;
- The governing equations for the heat and mass transfer are considered as transient.

With the above assumptions, the differential equation for the transient heat transfer can be expressed as:

$$\rho C \frac{\partial T}{\partial t} = \frac{\partial}{\partial r}\left(\lambda \frac{\partial T}{\partial r}\right) + \frac{\lambda}{r}\frac{\partial T}{\partial r} + q_v$$

(4)

The initial and boundary conditions for Eq. (4) are:

$$t = 0 \quad , \quad T = T_0$$

(5)

$$t > 0 \quad , \quad r = 0 \quad , \quad \frac{\partial T}{\partial r} = 0$$

(6)

$$t > 0 \quad , \quad r = \frac{D}{2} \quad , \quad -\lambda \frac{\partial T}{\partial r} = \sigma \cdot \varepsilon \cdot (T_{sf}^4 - T_{vc}^4) + q_{sf}$$

(7)

Under vacuum, the heat released from cooked meats to the cooling medium by convection is negligible. The radiative heat transfer is considered. In Eq. (4), q_v is the inner heat per unit volume generated due to water evaporation, q_v can be given by:

$$q_v = -h_{vg} \cdot \dot{m}_v$$

(8)

In Eq. (7), the evaporation heat per unit surface area, q_{sf} can be expressed by:

$$q_{sf} = \frac{D}{4} \cdot q_v = -\frac{D}{4} \cdot h_{vg} \cdot \dot{m}_v$$

(9)

During vacuum cooling, water evaporation occurs from cooked meats, the evaporation rate pre unit volume of cooked meats can be calculated by:

$$\dot{m}_v = \frac{4}{D} h_m (P_{sat} - P_{vc})$$

(10)

Where D is the diameter of the cylindrical cooked meats; P_{sat} and P_{vc} are respectively given in Eq. (1) and Eq. (2). The boiling coefficient, $h_m = 8.4 \times 10^{-7}\, kg \cdot Pa^{-1} \cdot m^{-2} \cdot s^{-1}$ (Wang & Sun 2002 part2).

In the models, the thermal properties of cooked meats are assumed to be a constant. However, the thermal conductivity and specific heat of cooked meats can be expressed as functions of compositions. The thermal properties strongly depend on the water content of products. The relationships between the thermal properties and water content of products have been summarized as follows (Sweat, 1986):

$$\lambda = 0.148 + 0.493w$$

(11)

$$C = 1.381 + 2.93w$$

(12)

Models validation

The raw meats are brought from the local supermarket. Then, the meats are cooked through the oven (Type of oven is RF-P130Y, China). The diameter of cooked meats is 80 mm. The samples are weighed by the electric balance and weight transducer. A set of T-type copper/constantan thermocouples with an accuracy of ± 0.1℃ are used to record the temperature distribution of cooked meats and the temperature of vacuum chamber. The core temperature and the surface temperature of cooked meats are measured respectively during vacuum cooling. The vacuum pressure in the vacuum chamber is measured through the pressure gauge. The data were obtained through the data acquisition system (Agilent 34970A, Agilent Technologies, USA). The free volume of the vacuum chamber is $0.02\,m^3$, and the vacuum pump speed is $14.4\ m^3/h$. The ambient temperature is 20 ℃. The diameter of cooked meats is 40 mm. Thermal conductivity is 0.5055 $W \cdot m^{-1} \cdot K^{-1}$. Specific heat is 3505.5 $J \cdot kg^{-1} \cdot K^{-1}$. Density is 1093 $kg \cdot m^{-3}$.

Results and discussion

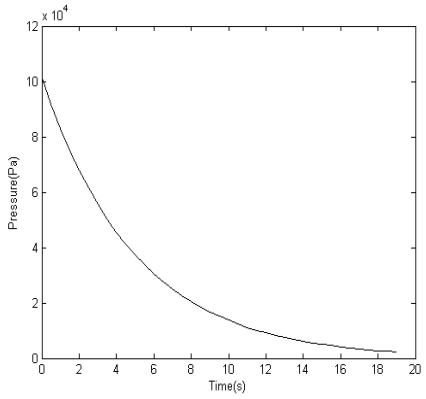

Fig. 1. The vacuum pressure in the vacuum chamber during the first cooling phase

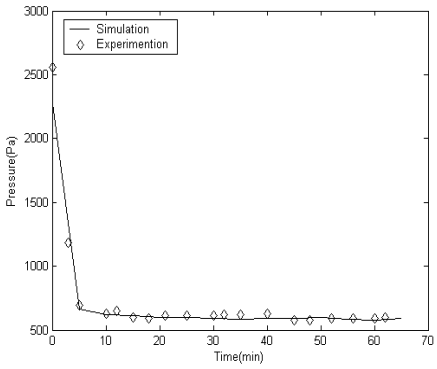

Fig. 2. The variation of vacuum pressure during the final cooling phase

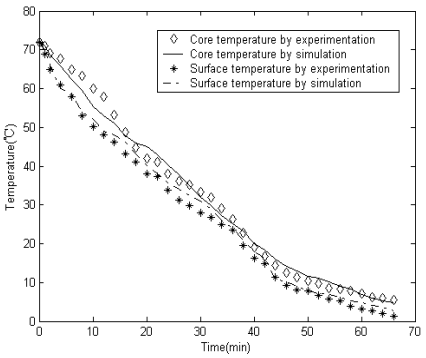

Fig. 3. The variation of temperatures during vacuum cooling

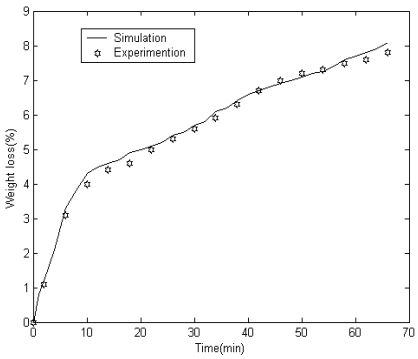

Fig. 4. The variation of weight loss during vacuum cooling

During vacuum cooling, it is assumed that the initial temperature of cooked meats is homogeneous. The curves of vacuum pressure in the vacuum chamber are shown in Figs. 3 and 4. It can be seen that the vacuum pressure in the vacuum chamber ranges from 101325 to 2272.2 Pa in Fig. 3. The pumping time to the flash point is only 19 seconds, which is called the first cooling phase. There is hardly cooling effect in the first phase. Then, the vacuum pressure is reduced from 2272.2 to 620 Pa. The variation of vacuum pressure during the second cooling phase is shown in Fig. 4. It can be seen that the predicted pressure agrees with the experimental value well. However, it can be also found that the experimental pressure can occurs some fluctuations shown in Fig. 4, which is due to the air leakage. Because the defined vacuum pressure, 620 Pa, is maintained by opening the bleeding valve of the vacuum chamber. After the flash point, water in cooked meats begins to evaporate. Therefore, the cooling effect occurs, and the temperature of the cooked meats can

reduce because of evaporation of water. It is during the second cooling phase that the cooked meats are cooled until the defined temperature of the cooked meats is reached.

Fig. 5 shows the surface and the core temperature profiles of the cooked meats by experimentation and simulation. The cooked meats are cooled from 72℃ to 5℃ within 70 min. Generally, the conventional cooling methods, such as slow air cooling and air blast cooling, are about form 6 to 10 hrs. Therefore, vacuum cooling is a rapid cooling method compared with the conventional cooling methods. It is shown that the theoretical simulation results agree with the experimental data well in Fig. 5. The differences of temperatures between the simulation and the experimentation are within 5℃. During the simulation of vacuum cooling, the specific heat, the practical thermal conductivity and the density of cooked meats are assumed to be constant. However, the thermal properties of cooked meats vary during the cooling phase. Consequently, the deviation between the simulation and the experimentation occurs. At the same time, because of the inner thermal conductivity of cooked meats, it can be found that the surface temperature is lower than the core temperature during the cooling phase. The final surface temperature of cooked meats is 1.2℃, and the final core temperature of the cooked meats is 5.5℃. During the cooling stage, the temperature of the cooked meats is above 0℃ to avoid freezing in the surface of cooked meats, which can be seen in Fig. 5.

When the vacuum pressure in the vacuum chamber reaches the saturation pressure, the water in cooked meats will boil. Therefore, evaporation of water occurs during the vacuum cooling, which leads to the reduction of weight of the cooked meats, as is shown in Fig. 6. The variation of weight loss during vacuum cooling is shown in Fig. 6. The simulated weight loss result is 8.1%, and the experimental weight loss value is 7.8%. The simulation result of weight loss of cooked meats agrees with the experimentation data. The deviation between the simulation and the experimentation is about 4%.

Conclusions

This paper describes the vacuum cooling of cooked meats. A modified mathematical model is developed to analyze the performance of vacuum cooler and the vacuum cooling process of cooked meats. The model is based on the assumption that the thermal properties of cooked meats are constant. The mathematical model is solved by the Crank-Nicolson method. The model is used to predict the variation of the vacuum pressure in the chamber and the temperature and weight loss profiles of the cylindrical cooked meats.

In order to validate the model, the cylindrical cooked meats are cooled by vacuum cooling from 72℃ to 5℃. The cooling time is only about 70 min, which indicates that vacuum cooling is a rapid cooling method compared with the conventional cooling methods, because the conventional cooling methods are about 6~10 hrs. The experimental data are compared with the simulated results, it is found that the differences of the temperature between the simulation and the experimentation are within 5℃, and the deviation of weight loss between the simulation and the experimentation is 4%. Anyway, the simulation results agree with the experimental data well, which indicates that the developed modified model can predict the variation of the pressure in the vacuum chamber, the temperature and weight loss of cooked meats.

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CHEMICAL PROPERTIES OF THE MEAT AND BLUBBER OF THE CAPE FUR SEAL (*ARCTOCEPHALUS PUSILLUS PUSILLUS*)

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Background

The Cape fur seal is harvested commercially in Namibia on the south-west coast of Africa. At present, there is a large demand for the hides of the pups and bulls, but the rest of the carcass is processed to carcass meal, which was traditionally used to supplement the diets of ruminants. Due to the occurrence of BSE in previous years, there has been a decrease in the use of animal by-products in any animal feed. Therefore, an alternative use of the meat is desirable. As the meat has shown to be a healthy, lean meat which contains high amounts of macro-minerals such as calcium and phosphorous, as well as iron and selenium (Robinson, 1996), there is reason to process the meat into a human food source. This would make the harvesting a more acceptable occurrence, as it would mean that the entire carcass of the animal would be utilised to its full extent and that this would be a supply of nutritious meat to the consumer.

There has been very little research done on the meat qualities of marine mammal meat, and none could be sourced on the meat of the Cape Fur Seal (*Arctocephalus pusillus pusillus*). There is some literature available on other pinnipeds, their physiology, geography, environment and life cycles. Some of it is comparable to the Cape fur seal, but as the Cape fur seal is a sub-species of sea lion, there are characteristics, which do not compare to other marine mammals.

Fish oil capsules have become a sales success in the pharmaceutical industry and a well-established supplement in the diet of many people around the world. The omega-3 fatty acids in fish oil have been proven to have a prophylactic action on thrombosis as well as the hardening of arteries. Experiments have shown that the consumption of whale and seal oils leads to a less aggressive immune system and that it also influences the viscosity and coagulative properties of the blood (NAMMCO, 1998).

There are already a few seal products, such as leather products as well as meat products, available elsewhere in the world, which are sold commercially. Seal meat is canned, or processed into salami as well as other products. The seal oil is widely used in the cosmetic industry as an ingredient in medical products. The leather from the hides of mostly male animals is used to make briefcases, purses, wallets and other clothing items. In Namibia, shoes are already being produced successfully from the hides of harvested bulls. The fur of the pups is used in the clothing industry and is used in coats, hats and boots.

The Cape fur seal is harvested commercially each year in Namibia, and this sustainable harvesting is destined to continue. The current culling numbers are 30 000 pups per year and 3000 bulls. The harvesting is done according to regulations and is strictly monitored.

Objectives

Maximising these seal resources would benefit Namibia as well as the rest of the world. Information is needed on the dress out percentages, chemical characteristics of the meat and blubber and how these would influence any processing methods used in a production line. The age and gender of the animals used for production purposes will also play a role, and this also requires research and information. The present investigation determines the proximate chemical composition of the *Pectoralis* muscle and the blubber of mature male bulls as well as pups.

Materials and methods

Ten Cape fur seal pups, approximately eight months old, of both sexes as well as ten Cape fur seal bulls, between two and four years of age were used for this investigation. As selective harvesting is not allowed,

the pups were not separated into different gender. All pups were of similar age, all at point of weaning. The bulls were also of similar age although none were sexually mature.

The animals were harvested using standard procedures. This includes being stuck in the heart to bleed within 30 seconds of being stunned by clubbing. The animals were eviscerated and the hide removed. At this point 100g samples of meat was collected from the *Pectoralis* muscle as well as 100g of blubber from the ventral side of the carcass. Samples were collected in separate plastic bags and marked according to animals, vacuum packed and frozen at –5°C once the meat samples had all reached room temperature (26°C).

Fat extraction

Fat was extracted using samples that had been defrosted. Samples were homogenised in a blender and a chloroform:methanol (2:1) extraction was used (Lee *et al*, 1990).

Protein determination

Dried samples, excluding fat and moisture were used and ground in a mortar with a pestle till a fine powder was obtained. An amount of 0.1mg was weighed off per animal and inserted into a foil wrap designed for the Leeko protein analyser. The protein was determined as Nitrogen, which was multiplied by 6.25 to determine the protein concentration in the sample.

Moisture determination

Standard procedures were used in the determination of moisture content of the sample. 2.5g of wet sample was desiccated in an oven at 100°C for twelve hours. The dried sample was weighed and the moisture content determined (AOAC, 1990)

Results and discussion

It was noted that the carcasses of seal pups dress out to very low percentages (49%) compared to the carcasses of other species such as cattle, sheep and pigs. The dress out percentage in this case, includes the head as well as the flippers. All viscera is removed as well as the hide. This is because at present, the whole carcass is used for the production of carcass meal, and no particular cuts are required. If one were to remove the head and flippers as is done with other seal species, this value would decrease even more. It can bee seen from the dressing that most of the energy taken in with their diet goes towards insulation in the form of a thick, snow-white layer of blubber which covers most of the underneath of the tail. In the bulls, these dress-out percentages would be higher as more muscle has been formed in order to move around in the search of food. As the pups were at the point of weaning, none of them would have had to dive deep or swim far to find a source of food, therefore, their bodies had not had time to get accustomed to maintaining high levels of oxygen in their muscles. This was clear to see from the light colour of their meat compared to the dark red colour of the meat of the adult animals. Their staple diet had consisted of milk till then, and thus no other food sources, *e.g.* fish, would have been consumed to affect the colour or flavour of the blubber or meat.

Meat samples were taken from the *Pectoralis* muscle of freshly culled carcasses of ten cape fur seal pups and ten cape fur seal bulls. Blubber samples were taken from the same animals. All pups were approximately eight months of age, whilst the bulls were between two and four years old. As seen in Table 1, the meat of the pups contained a higher percentage fat (4.2g/100g) than that of the bulls (2.4g/100g), but a very similar percentage of protein (23.2g/100g). The blubber samples of the bulls contained a higher percentage of protein (26.6g/100g) than that of the pups (14.6g/100g) but a lower fat percentage (67.1g/100g) than that of the pups (77.2g/100g). Seal meat could therefore be considered as a healthy food commodity due to its lean meat content with most of the fat being blubber and thus stored sub-cutaneously. The moisture content of both the meat as well as the blubber of pups as well as bulls is very similar.

Table 1. Mean values of the chemical composition of the meat and blubber of 10 Cape fur seal pups and 10 Cape fur seal bulls.

	Meat			Blubber		
	Moisture%	Protein %	Fat %	Moisture%	Protein %	Fat %
PUPS	73.0±1.6	23.2±1.5	4.2±1.7	8.0±1.9	14.6±3.0	77.2±1.9
BULLS	74.2±1.6	23.5±1.5	2.4±0.8	5.9±1.6	26.6±4.3	67.±5

Conclusions

Countries with concessions to harvest seals, in this case Namibia, could benefit from full utilisation of the animal. The chemical composition of the meat shows that it is lean meat with a very high protein content and that most of the blubber lies subcutaneously, as in pork. This makes it nutrient dense meat for the consumer to use as an alternative source of red meat.

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METABOLIC MECHANISM OF COMPENSATORY GROWTH IN PIGS

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Background

It has been known for decades that compensatory growth occurs in pigs following a period of feed restriction. Recent studies have shown that compensatory growth also results in more tender meat (Kristensen et al. 2002). However, it is still unclear why compensatory growth would lead to an increase in meat tenderness. It has been hypothesized that the improved tenderness is a result of increased post mortem protein degradation as a consequence of elevated protein turnover during compensatory growth.

Objectives

The aim of the present study was to investigate changes in the proteome of the muscle due to compensatory growth in pigs by analyzing muscle samples taken at slaughter and 48 hours after slaughter.

Materials and methods

Sixteen female pigs were divided into two groups, that either had free access to the feed (control) or were fed restricted from d 28 to d 80, after which had free access to the feed (compensatory). The pigs were slaughtered at d 140. Meat tenderness was analyzed by a sensory panel. Muscle samples from longissimus dorsi were taken at slaughter and 48 hours after slaughter. The muscle samples were homogenized in 0.1M Tris pH 8.0 added protease inhibitor (complete, Roche). The samples were centrifuged 20min, 25000xg, 4ºC and the supernatant was used for two-dimensional gel electrophoresis (2DE). Eleven cm, pH 4-7 IPG strips and 8-16% gel (Criterion, Bio-Rad) were used for the first and second dimension, respectively. The proteins were visualized with silver staining. ImageMaster 2D Platinum software v5 was used for image analysis and the 2D gels were analyzed in two groups, one containing the samples taken at slaughter and the other the samples taken 48 hours after slaughter. ANOVA (SAS) was used to analyze effect of compensatory growth. The proteins of interest were identified with the use of a MALDI-TOF-TOF instrument (4700 Proteomics analyzer, Applied Biosystems).

Results and discussion

The sensory analysis showed that compensatory growth resulted in a significant ($p<0.05$) increase in meat tenderness compared to meat from control pigs.

At slaughter, eight different proteins were found affected by compensatory growth of the pigs (see fig. 1). The stress proteins HSP70 and HSP27 that both are believed to participate in the organization and protection of the myofibrils (Liu and Steinacker, 2001) and may also have an important role in stabilization of the myofibrils post mortem. Therefore, it can be speculated that these proteins also have an impact on meat tenderness. Changes of proteins Enolase 3 and Glycerol-3-phosphate dehydrogenase, both part of the glycolytic pathway, were also observed. These changes are probably a consequence of regulation in the energy metabolism as the pigs showing compensatory growth have a higher protein turnover compared to control pigs. Changes in the *in vivo* glycolytic metabolism may also lead to changes in the post mortem metabolism. It is well established that the post mortem metabolism has an effect on meat tenderness and the relation between compensatory growth and meat tenderness could be a consequence of changes in the glycolysis. The relation of aldehyde dehydrogenase E2, aldehyde dehydrogenase E3 and biphosphoglycerate mutase to compensatory growth is unclear.

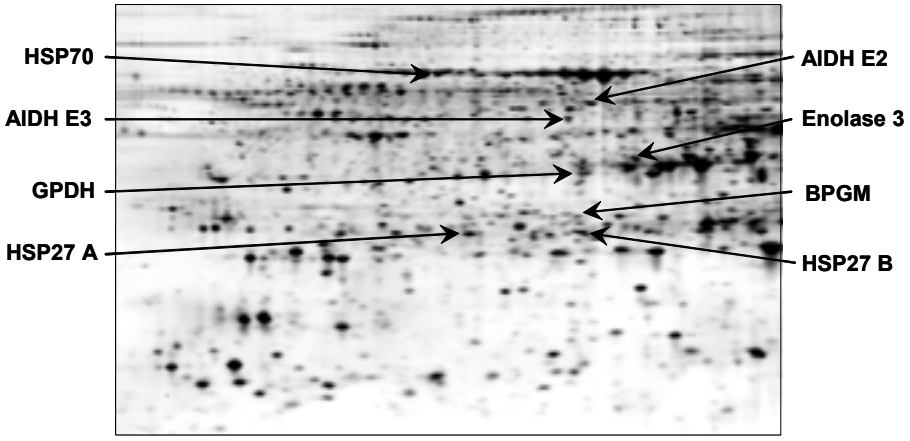

Figure 1. Effect of compensatory growth at slaughter. The arrows show the identified protein changes between pigs showing compensatory growth and kontrol. Heat AlDH E2; Aldehyde dehydrogenase E2. AlDH E3; Aldehyde dehydrogenase E3. GPDH; Glycerol-3-phosphate dehydrogenase. BPGM; 2,3 biphosphoglycerate mutase.

Figure 2A shows that the proteins found to be affected by compensatory growth at slaughter all have higher intensity when the pigs were fed ad libitum. It was presumed that the intensity of some effected spots would be higher in muscle samples from pigs that were fed compensatory as a consequence of the elevated protein turnover. The intensities of the proteins do not necessary reflect the activity of the identified enzyme, as the protein spots could also be an inactive isoform. Furthermore, only the sarcoplasmic protein fraction was used in the present study. Another explanation could be that mainly the amount of myofibrillar proteins increases during compensatory growth, and as a result, the relative amount sarcoplasmic proteins will decrease. However, compensatory growth did not affect the protein concentration of the sarcoplasmic protein extracted from the muscle, and the analysis of the sarcoplasmic protein fractions with 1D gel electrophoresis showed no effect on the relatively small amount of actin and myosin. Finally, the same intensity pattern was not observed in the samples taken 48 hours after slaughter (Fig. 2B).

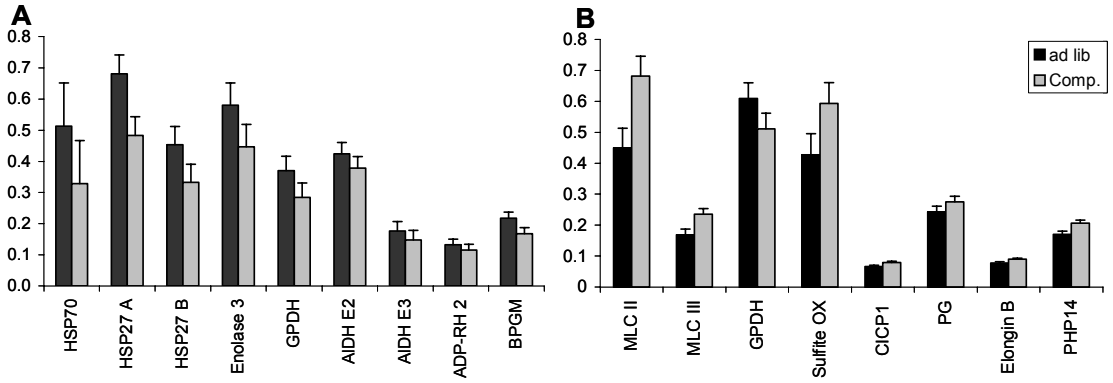

Figure 2. Intensity at slaughter (A) and 48 hours post mortem (B) of proteins that were found to be affected by compensatory growth. AlDH E2; Aldehyde dehydrogenase E2. AlDH E3; Aldehyde dehydrogenase E3. GPDH; Glycerol-3-phosphate dehydrogenase. BPGM; 2,3 biphosphoglycerate mutase. ClCP 1; Chloride intracellular channel 1. Sulfite OX; Sulfite oxidase. MLC II; Myosin light chain II. PHP14; Phosphohistidine phosphatase 14. MLC III; Myosin light chain III.

Muscle samples of longissimus dorsi taken 48 hours post mortem were also analyzed (Fig. 3) as post mortem protein changes have a great influence on the tenderness of meat. A hypothesis of the influence of compensatory growth on meat tenderness has been that it was a consequence of increased post mortem protein degradation. Therefore, it was presumed that the intensity of some protein fragments would be affected by compensatory growth. However, the proteins that were found affected by compensatory growth

at 48 hours post mortem were all full-length. Hence, the results from the present study could not confirm the hypothesis. It was found that the intensity of both Myosin light chain (MLC) II and III were affected by compensatory growth 48 hours after slaughter. MLC have an important role in the structure of the muscle cell, and it has previously been reported that MLC II is related to tenderness (Lametsch et al. 2003). However, it is not clear if the impact MLC has on tenderness is a consequence of post mortem proteolyses or modification (Lametsch et al. 2003). Recently, it was reported that MLC is dephosphorylated *post mortem* and that the dephosphorylation is related to the *post mortem* metabolism (Morzel, M. et al. 2004). The mitochondrial protein sulfite oxidase and the two nuclear proteins elongin B and chloride intracellular channel 1 increase in intensity 48 hours after slaughter as an effect of compensatory growth. An explanation of this observation could be that the number of mitochondria and satellite cells is increased as a consequence of the increase in protein turnover, and that the proteins from these two organelles leaked into the sarcoplasma *post mortem*. The effect of compensatory growth at 48 hours after slaughter on protein gamma and phosphohistidine phosphatase 14 is unclear.

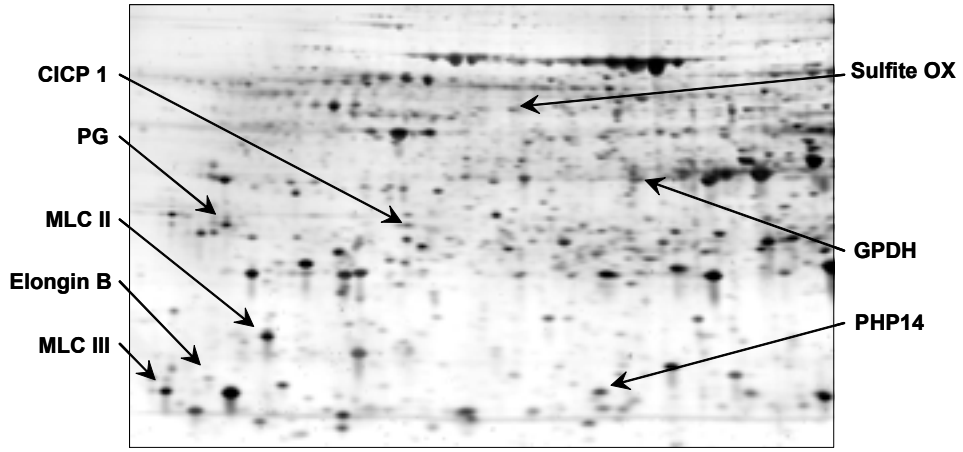

Figure 3. Effect of compensatory growth 48 hours after slaughter. The arrows show the identified protein changes between pigs showing compensatory growth and kontrol. ClCP 1; Chloride intracellular channel 1. Sulfite OX; Sulfite oxidase. GPDH; Glycerol-3-phosphate dehydrogenase. MLC II; Myosin light chain II. PHP14; Phosphohistidine phosphatase 14. MLC III; Myosin light chain III.

Conclusions

The results of the present study could not confirm the hypothesis that the impact of compensatory growth on meat tenderness is a consequence of increased post mortem protein degradation. Compensatory growth affected the at-slaughter level of the two stress protein HSP70 and HSP27. At 48 hours post mortem the protein isoforms myosin light chain II and III showed increased intensity in the sarcoplasmic fraction. It can be speculated that these changes could have an effect on the meat texture. Further studies are needed to clear this and reveal the precise effect of compensatory growth on the activity of the identified protein.

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MEAT QUALITY OF HEIFERS AS INFLUENCED BY GRAZING, FINISHING FEEDING AND CARCASS SUSPENSION METHOD

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Background

In Sweden, the beef production is depending on the seasons. The summer allows grazing and the winter requires indoor housing and feeding. Swedish beef production is based on forage feeding with or without finishing feeding complemented with grain. In order to maintain biodiversity and an open landscape, a production system including the use of semi-natural grasslands is of great importance. Today, within EU, farmers receive subsidies for the commitment to keep semi-natural grasslands open, which will contribute to maintain beef production in Sweden. This production strategy will possibly give a higher proportion of animals slaughtered directly after the grazing period to avoid a second indoor period, which could affect the meat quality.

Steen and Kilpatrick, (1995) showed that finishing feeding at 80% of *ad libitum* intake gave a higher carcass weight while maintaining a constant carcass fat content. That could be of importance since the interaction between those two parameters is in focus in the specifications from the retailers. The consumer is mostly interested in the sensory parameter of tenderness, although beef palatability also depends on juiciness and flavour (Huffman *et al.*, 1996). The great variation in beef quality can be explained by the fact that tenderness and other quality attributes are highly dependent on breed, gender, age, physical activity and rearing system of the animal (Harper, 1999). Lundesjö *et al*. (2002) diminished the variation in meat quality of beef by the use of Pelvic suspension.

Objectives

The aim of this study was to compare the sensory and technological quality of meat from grazing heifers on different indoor feed intensity and slaughter age. A further objective was to study the effect of pelvic suspension.

Materials and methods

Two groups of heifers, of at least 75% Charolais (n = 41) or Aberdeen Angus (n = 40) breed respectively, were used. The experiment had a factorial design, where two levels of indoor feed intensity and two levels of slaughter age were used. All animals had one indoor period followed by a grazing period. Half of the heifers were slaughtered at 18 months of age directly after grazing, whereas the other heifers were slaughtered at 22 months of age after a second indoor period. During the indoor periods the two groups were divided and subjected to two different feed intensities (low and high). For Charolais the low intensity was *ad libitum* intake of grass/clover silage and the high intensity was *ad libitum* intake of silage combined with 2 kg of mixed grain (65% oat and 35% barley). For Aberdeen Angus the low intensity was 80% of *ad libitum* intake of silage and the high intensity was *ad libitum* intake of silage.

The left side of each carcass was rehung from the pelvic bone, while the right side was left hanging in the Achilles tendon. The whole *M. longissimus dorsi* from both sides was vacuum-packed and aged for 7 days at +4 ^{O}C. The pH-value was measured and samples for intramuscular fat analysis (IMF), sensory evaluation and instrumental tenderness (Warner-Bratzler (WB)) measurements were frozen at –20 ^{O}C. Additional samples for sensory analyses and WB measurements were aged for another 7 days before being frozen. Freezing and cooking losses were calculated from the WB samples. A selected and trained panel performed a descriptive sensory analysis. Statistical evaluation was performed using the Procedure Mixed in SAS (Ver. 8e, SAS Institute Inc., Cary, NC, USA).

Results and discussion

The production results from this experiment have been described elsewhere (Hessle *et al.*, 2004). It has previously been showed that instrumental tenderness is improved in different genders by finishing feeding or by intensive compared with more extensive feeding (Bowling *et al.*, 1977; Bowling *et al.*, 1978; Schroeder *et al.*, 1980; Dubeski *et al.*, 1997; Hoving-Bolink *et al.*, 1999a). There are also studies that show no difference in shear force depending on finishing feeding (Harrison *et al.*, 1978; Bruce *et al.*, 2004). This study indicated no significant improvement in shear force for heifers on finishing feeding but a significant improvement with pelvic suspension (Table 1), which agrees with Lundesjö *et al.* (2002). For Charolais heifers, there was an improvement in instrumental tenderness for pelvic suspended sides at 7 days, but no differences could be seen after additional ageing to 14 days. Angus heifers had an improvement in instrumental tenderness for pelvic suspended sides after 14 days of ageing.

For the Charolais heifers, the high feed intensity had positive effects on tenderness with a 23% decrease in bite resistance and a 14% increase in tenderness compared with less intensive feeding. The high feed intensity also led to lower freezing and cooking losses after 7 days of ageing, but not after 14 days of ageing. The pH value was lower in meat from animals of the high feed intensity. The results agree with Hoving-Bolink *et al.* (1999a) who found that intensive feeding resulted in a better eating quality than less intensive feeding. For the Angus heifers, a significant decrease in stringiness was observed for the high feed intensity ($P = 0.008$) together with a slight decrease in acidity ($P = 0.06$). This could be due to finishing feeding after grazing inducing an overall improvement in meat quality, and the level of feeding would be of less importance. Vestergaard *et al.* (2000) showed that extensively fed young bulls, after a finishing period of 10 weeks, showed higher instrumental tenderness as well as sensory scores for tenderness, taste and juiciness, reaching the level of intensively fed young bulls. In the present study did higher carcass weight and higher scores for carcass conformation did not lead to an economic advantage in comparison with the lower slaughter weight (Hansson and Hessle, 2003).

Suspension method combined with slaughter age significantly affected most traits. For the evaluated sensory traits in meat from both the Charolais and Angus heifers only few traits were improved after postponed slaughter until the age of 22 months (Figure 1 and 2). This is contradictory to Harrison *et al.* (1978), where all sensory traits improved after a longer feeding period. However, in the present study, suspending the carcasses by the pelvic bone at the slaughter age of 18 months gave the best eating quality. No additional improvement in meat quality was found at the higher slaughter age in Achilles suspended carcasses except for a higher marbling score. This confirms the results by Hoving-Bolink *et al.* (1999b), where a significant increase in intramuscular fat content did not result in better sensory characteristics. Generally a high marbling score is believed to improve the flavour and juiciness of beef, as Harrison *et al.* (1978) found for grain fed beef. However, Bruce *et al.* (2004) concluded that pasture-finished beef had the highest overall quality because of increased tenderness and juiciness despite the fact that the pasture-finished beef had a lower intramuscular fat content. Of interest in the present study was the decrease in visible marbling in meat from pelvic suspended heifers when slaughtered at 18 months of age in comparison with Achilles suspended carcasses at the same age. In Angus heifers, this was also accompanied by a slight decrease in fatty taste ($P = 0.07$).

Conclusions

An extended rearing period with finishing feeding and/or high indoor feed intensity will probably lead to an improved sensory quality of meat from heifers. However, the effect of pelvic suspension exceeded the other factors by improving the meat quality of heifers slaughtered directly after grazing to the level of finishing fed heifers. Pelvic suspension can therefore be recommended in order to produce beef with a consistent quality.

Acknowledgements

The authors are grateful for the financial support by the Mistra program FOOD 21.

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Table 1. Carcass and meat quality traits in Charolais and Aberdeen Angus heifers (18 and 22 months) suspended in the Achilles tendon or by the pelvic bone

	Charolais Heifers					Aberdeen Angus Heifers			
	Finishing time, Suspension					Finishing time, Suspension			
Quality trait	18 mo, achilles	18 mo, pelvic	22 mo, achilles	22 mo, pelvic	*P*	18 mo, achilles	18 mo, pelvic	22 mo, achilles	*P*
Carcass weight, kg	256	------	331	------	0.001	199	------	240	0.001
Conformation*	7.3	------	9.0	------	0.001	6.1	------	6.3	n.s.
Fat*	5.9	------	9.9	------	0.001	7.2	------	10.3	0.001
IMF, %	1.7	------	2.4	------	0.001	2.3	------	4.2	0.001
Shear force, 7 d	32.8^{a}	30.2^{bc}	30.8^{ac}	30.1^{ac}	0.075	------	------	------	------
Freez loss, 7 d, %	6.8^{a}	5.8^{b}	9.5^{c}	7.1^{a}	0.001	------	------	------	------
Cook loss, 7 d %	23.4	22.0	23.2	21.9	0.148	------	------	------	------
Shear force, 14 d	38.2	29.6	30.5	32.4	0.256	35.0^{a}	28.1^{bc}	30.4^{ac}	0.065
Freez loss, 14d %	6.3^{ac}	5.6^{bd}	7.0^{a}	6.2^{cd}	0.006	4.6^{a}	3.8^{b}	6.6^{c}	0.001
Cook loss, 14d %	19.0^{a}	19.4^{a}	20.3^{ac}	21.7^{bc}	0.006	18.1	18.0	16.9	0.322

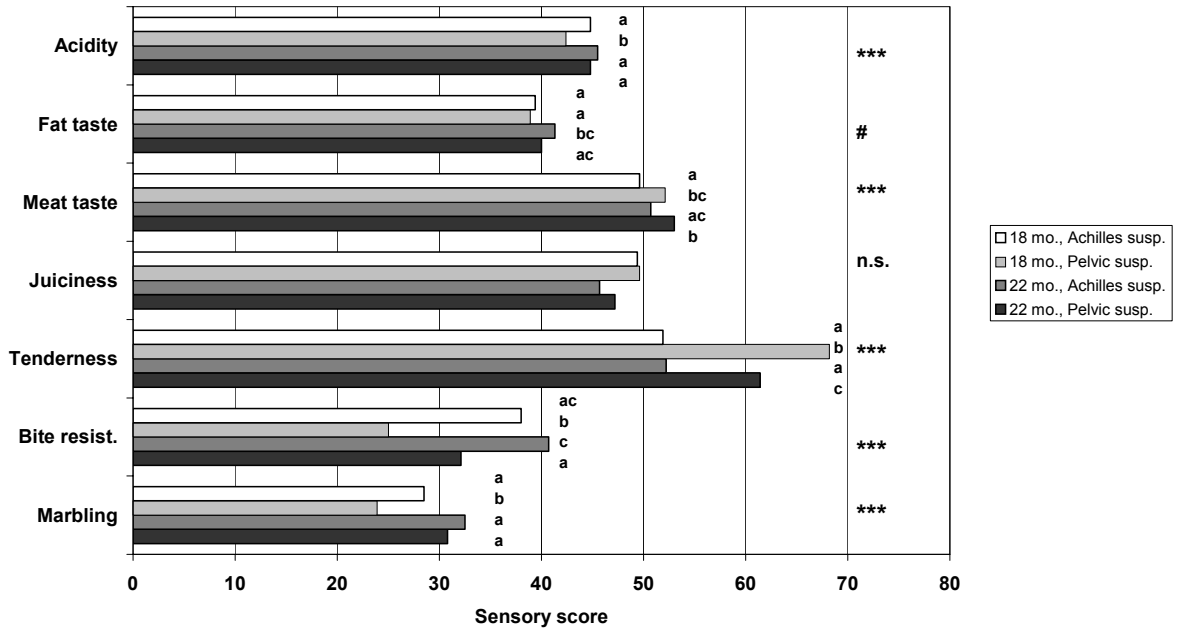

Figure 1. Sensory characteristics in Charolais heifers. Sensory scores within attribute with the same letters are not significantly different (p>0.05). Level of significance: n.s = p > 0.10; # = p≤ 0.10; *** = p≤ 0.001.

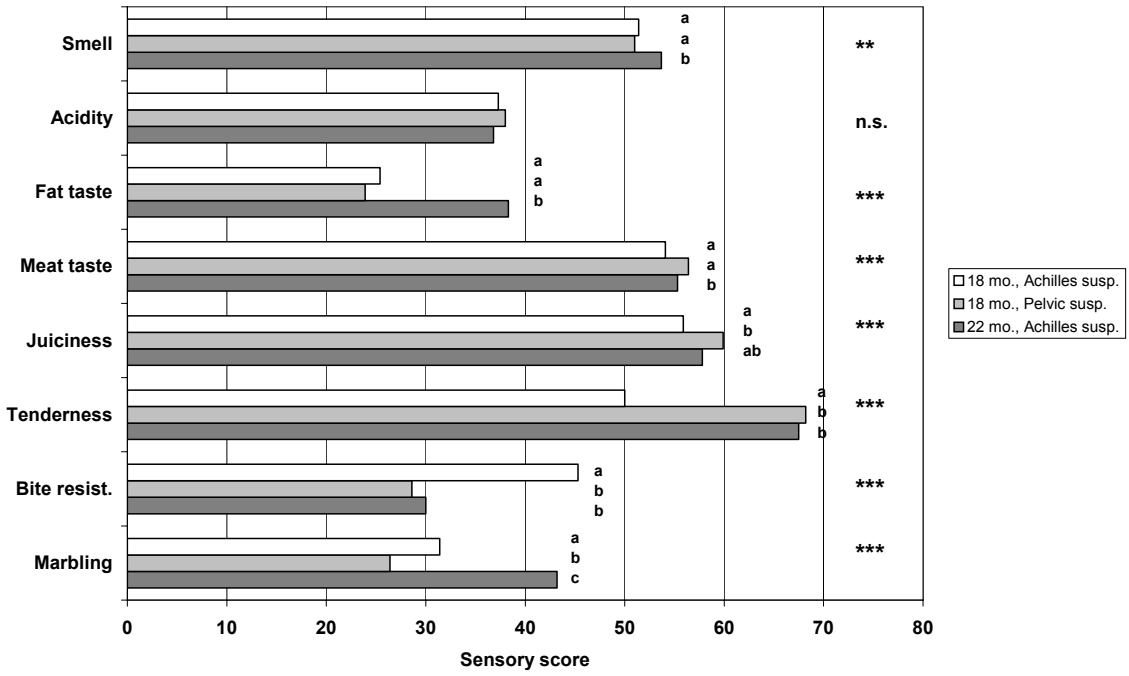

Figure 2. Sensory characteristics in Aberdeen Angus heifers. Sensory scores within attribute with the same letters are not significantly different (p>0.05); Level of significance: n.s = p>0.10; # = p≤0.10; ** = p≤0.01 *** = p≤0.001.

QUALITY CHARACTERISTICS OF MEAT FROM PODOLICA AND CROSSBRED CALVES REARED ON FEED CONTAINING GRAPE SKINS

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Background

During the last few years, an acceleration in the disappearance of livestock breeds has occurred, due to the replacement of traditional rearing systems with simplified industrial production systems. The Rio de Janeiro "International Bio-diversity Conference" formulated proposals to contrast this trend, consisting of a series of operations and incentives aimed at recovering breeds in danger of extinction.

Podolica cattle are an indigenous Italian genotype, which recently received the status of "breed" with the establishment of a Genealogical Register. The intrinsic features of this breed merit particular attention, and consist of great adaptability to difficult climatic and environmental conditions, together with the quality and dietary characteristics of its meat. Farming of Podolica cattle is concentrated in the "inland" areas of southern Italy (Apulia, Abruzzo, Basilicata, Calabria, Campania and Molise), with about 100,000 head (AIA, 2002). This breed still contains several local varieties and up until a few years ago was used almost solely for heavy farm work. With the advent of mechanisation, its importance disappeared and numbers fell constantly. The outstanding flavour and appearance of Podolica meat have earned it the "5R" grade of certified origin, the same as four other indigenous national genetic types (Chianina, Marchigiana, Maremmana and Romagnola). The ability of this breed to exploit foodstuffs with a low nutritional content may be used in order to exploit alternative food sources such as agri-industrial by-products. In Italy, where a great amount of animal feed is imported from abroad, there is a growing interest in these by-products for livestock feed, because they are inexpensive and easy to obtain. Furthermore, the following advantages derive from their use: - reduced pollution due to lack of environmental impact; - reduced production costs to the breeder; - a considerable alternative supply of nutrition; - probable improvements in the flavour and appearance, physical and chemical characteristics of the animal products (D'Urso et al., 1984; Bosi et al., 1985). The choice of by-products is vast, and grape skins are of particular interest because of their suitable chemical composition (12.76% raw protein, 7.73% fat, 46.70% N-free extracts) low cost and, in Apulia, wide availability. Grape skins have already been tested on lambs without negative repercussions on productive performances, or quantity and quality traits. Moreover, when this by-product was added to lamb feed together with grape-seed oil and safflower oil, it determined a higher level of unsaturated and monounsaturated fatty acids (Chiericato and Rioni, 1983; Bittante et al., 1985; Bosi et al., 1985; Vicenti et al., 1996; Ragni et al., 1997; Ragni et al., 1998; Vicenti et al., 1997).

Objectives

The study aims to evaluate the influence of a feeding treatment containing 20% grape skins on the chemical composition and on the fatty acid profile of meat from thoroughbred and crossbred (Marchigiana x Podolica and Chianina x Podolica) Podolica calves.

Materials and methods

24 male calves were used for this study, 8 Podolica (Pod), 8 Chianina x Podolica (Ch x Pod) and 8 Marchigiana x Podolica (Mar x Pod), weaned at about 6 months. Each genotype was divided into 2 groups, with each group containing 4 animals. The animals were fed a diet made of feed (about 60% of the dry matter of the diet) and of hay from permanent pasture (about 40% the dry matter of the diet). One group of each genotype (Test) was fed a control feed with no grape skins, whereas the other groups (Grape skin) received a feed containing 20% grape skins. Table 1 shows the chemical composition of the feeds, carried out in accordance with A.S.P.A. regulations (1980). The calves were slaughtered at a live weight of 450 kg to 500 kg, when breeders and the local market consider them mature. The *Longissimus dorsi* (LD) and *Biceps femoris* (BF) muscles were removed from all carcasses and assessed for pH, using a glass electrode at slaughter. Samples of both the muscles examined were evaluated for meat colour and tenderness. A representative sample was used for chemical analysis (ASPA, 1996) and fatty acid profile. Lipids were

extracted according to the 2:1 chloroform-methanol method described by Folch *et al.* (1957), whereas the acidic profile was assessed using a Chromopack CP 9000 gas chromatograph. Meat colour was estimated by the Hunter Lab system using a colorimeter (illuminant D 65), which measures the values of Lightness (L), Redness (a) and Yellowness (b) by making 5 readings for each meat sample, approximately 2.5 cm thick. Tenderness was measured using a Warner Bratzler shear device applied to an Instron 5544 and expressed as the cutting force (kg/cm^2) required to shear perpendicularly to the direction of the fibres half an inch diameter cylinders of raw meat, taking three measurements for each muscle per subject. The atherogenicity and thrombogenicity indexes were calculated accordingly to Ulbricht and Southgate (1991). The PCL/PCE (plasma cholesterol lowering/plasma cholesterol elevating) ratio was also determined (Reiser and Shorland, 1990). Data were analyzed for variance using the GLM procedure of SAS (1999/2000).

Results and discussion

The effect of diet on the physical parameters (Tab. 2) of the LD and BF muscles was limited, since it had a significant effect only on the lightness index ($P<0.05$) and the shear force WBS ($P<0.01$) of the LD muscle. Genotype had a significant effect only on the yellowness index b ($P<0.05$) and tenderness ($P<0.01$) of the LD muscle, and on the pH ($P<0.05$) of the BF muscle. Considering the effect of the interaction diet x genotype, it is seen that Pod bullocks fed on grape-skins present LD muscle which tends to be less light ($P<0.05$) and the BF muscle with less red and yellow intensity ($P<0.05$ and $P<0.01$). The degree of acidity of the LD muscle was not influenced by genotype and diet, unlike the BF muscle; the BF muscle from Mar x Pod bullocks fed on grape-skins presented the highest pH values ($P<0.01$ and $P<0.05$). However, the type of feed produced different effects among the three genotypes regarding the shear force of the LD muscle. The feed containing grape-skins significantly ($P<0.01$) increased the hardness of the Pod meat when compared to the Ch x Pod and Mar x Pod meat. The use of grape-skins caused significant variations in the chemical composition of the meats (Tab. 3). Both diet and genotype produced considerable differences in the content of water, fat ($P<0.01$ and/or $P<0.05$) and protein. The combined effect diet x genotype gave rise to effects on the water content of meat from animals fed on the grape-skin diet. In the Ch x Pod and Pod bullocks this was significantly reduced ($P<0.01$ and $P<0.05$). The protein content of the meats from the different genotypes was influenced by the type of diet. In particular, the grape-skin diet led to a reduction in the protein content of meat from bullocks. The fat content of meat was also influenced by both genotype and feed, since the grape-skin diet caused a highly significant increase ($P<0.01$) in the lipid content of Pod meat. The acid composition of the fat (Tab. 4) was affected by the type of diet, with significant variations in the contents of saturates, unsaturated, monounsaturated and ω3, the unsaturated/saturated ratio and the thrombogenicity index. The genotype also has a significant effect on all parameters of acid composition of fat, except for the ω3/ ω6 ratio. The diet x genotype interaction has also shown significant variations in almost all the parameters considered. It can be seen how the grape-skin diet favoured ($P<0.01$ and/or $P<0.05$) a greater accumulation of unsaturated and monounsaturated fatty acids in the Pod bullocks, and reduced the accumulation of saturated fatty. The ω6 and ω3 contents were affected in different ways by the grape-skin diet, which reduced both acids in the Pod bullocks ($P<0.01$and $P<0.05$). Finally, the effect of the grape-skin diet was pronounced on the dietetic indexes. Meat from Pod bullocks showed, mostly on Grape skin diet, better thrombogenic index ($P<0.01$) than the other groups.

Conclusions

The experimentation showed that the use of feed containing 20% grape-skins in the diet of different genotypes of meat bullocks generally produced good quality meat and dietary characteristics. With reference to the Podolica breed, an indigenous genotype typical in Southern Italy, it was seen that a grape-skin diet produced meat with a higher content of unsaturated and monounsaturated fatty acids, reduced saturated acids, and led to an improvement in the dietary indices considered beneficial for human health.

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Table 1. Chemical composition of grape skin and diets (% on dry matter)

	Diet		
	Grape skin	Test	Grape skin
Moisture	3.00	8.80	8.20
Protein	12.76	17.03	17.06
Fat	7.73	4.92	4.73
Ash	9.13	8.44	8.21
Fiber	23.68	10.98	9.87
N-free extract	46.70	58.63	60.13

Table 2. Color, pH and tenderness parameters

	Diet x Genotype								
	Test			Grape skin					
N (samples)	4	4	4	4	4	4		P	SED
Variables	Ch x Pod	Mar x Pod	Pod	Ch x Pod	Mar x Pod	Pod	Diet	Genotype	FD=18
LD L	28.59 ^{B}	28.70 ^{a}	29.95	32.42 ^{a}	32.31 ^{a}	28.65 ^{a}	*	n.s.	2.404
a	9.50 ^{B}	13.23 ^{a}	9.69 ^{a}	11.40	10.00	9.95	n.s.	n.s.	2.302
b	8.10 ^{A}	8.45 ^{a}	6.39 ^{a}	8.35 ^{a}	7.20	6.77	n.s.	*	1.146
BF L	31.23	31.56	31.38	31.99	30.83	28.48	n.s.	n.s.	2.467
a	10.11	9.63 ^{aa}	10.94	11.88 ^{a}	12.91 ^{Aa}	8.58 ^{Ac}	n.s.	n.s.	2.039
b	7.28 ^{ABC}	6.29 ^{cd}	7.39 ^{aac}	7.53 ^{a}	7.56 ^{a}	5.87 ^{d}	n.s.	n.s.	0.844
LD pH	6.49	6.48	6.33	6.49	6.59	6.58	n.s.	n.s.	0.284
BF pH	6.21 ^{cd}	6.37 ^{ac}	6.02 ^{Aad}	6.19 ^{ac}	6.55 ^{Aa}	6.20 ^{ac}	n.s.	*	0.231
LD WBS (kg/cm^2)	7.50 ^{A}	5.55 ^{C}	5.50 ^{C}	5.60 ^{C}	5.60 ^{C}	6.25 ^{A}	**	**	0.153

$^{A, B, C, D}$: P<0.01; $^{a, b, c, d}$: P<0.05

Table 3. Chemical composition of meat (%)

	Diet x Genotype								
	Test			Grape skin					
N (samples)	4	4	4	4	4	4		P	SED
Variables	Ch x Pod	Mar x Pod	Pod	Ch x Pod	Mar x Pod	Pod	Diet	Genotype	FD=18
Moisture	70.78 ^{A}	71.03 ^{A}	66.66 ^{Ca}	68.10 ^{B}	70.34 ^{A}	65.55 ^{CDb}	**	**	0.624
Protein	23.40 ^{a}	22.74	23.83 ^{Aa}	21.89 ^{b}	21.66 ^{Bb}	21.62 ^{Bb}	*	*	0.991
Fat	4.36 ^{D}	4.78 ^{D}	8.20 ^{B}	8.07 ^{B}	6.23 ^{C}	10.00 ^{A}	**	**	0.586
Ash	1.05 ^{a}	1.03 ^{a}	0.91	0.96	0.84 ^{b}	0.93	n.s.	*	0.110
Undetermined	0.40 ^{B}	0.40 ^{B}	0.98 ^{B}	0.97 ^{b}	0.98 ^{b}	1.89 ^{Aa}	*	**	0.530

$^{A, B, C, D}$: P<0.01; $^{a, b, c, d}$: P<0.05

Table 4. Fatty acids of meat fat (%)

	Diet x Genotype								
	Test			Grape skin					
N (samples)	4	4	4	4	4	4		P	SED
Variables	Ch x Pod	Mar x Pod	Pod	Ch x Pod	Mar x Pod	Pod	Diet	Genotype	FD=18
Saturated	49.80 ^{aB}	48.80 ^{Bb}	48.35 ^{C}	54.00 ^{A}	48.85 ^{Bb}	47.30 ^{D}	**	**	0.496
Unsatured	50.20 ^{bC}	51.20 ^{aC}	51.65 ^{B}	46.00 ^{D}	51.15 ^{aC}	52.70 ^{A}	**	**	0.496
Monounsaturated	45.00 ^{Bb}	47.20 ^{Ab}	45.75 ^{aB}	40.60 ^{C}	45.95 ^{aB}	48.00 ^{Aa}	**	**	0.502
Polyunsaturated	5.20 ^{Abc}	4.00 ^{Bd}	5.90 ^{Aa}	5.40 ^{Aab}	5.20 ^{Abc}	4.70 ^{Bc}	n.s.	*	0.365
ω6	4.20 ^{B}	3.10 ^{C}	4.95 ^{A}	4.60 ^{AaB}	4.05 ^{Bb}	3.45 ^{C}	n.s.	*	0.271
ω3	0.30 ^{Aa}	0.15 ^{B}	0.30 ^{Aa}	0.20 ^{b}	0.20 ^{b}	0.20 ^{b}	*	*	0.033
ω6/ω3	16.12 ^{b}	23.00 ^{a}	16.50	23.00 ^{a}	20.25	17.25	n.s.	n.s.	8.593
Unsatur./Satur.	1.01 ^{Cb}	1.05 ^{aBC}	1.07 ^{B}	0.85 ^{D}	1.05 ^{aBC}	1.12 ^{A}	**	**	0.021
Atherog. index	0.97 ^{aB}	0.95 ^{aB}	0.95 ^{aB}	1.06 ^{A}	0.87 ^{Bb}	0.97 ^{aB}	n.s.	**	0.047
Thrombog. Index	1.83 ^{B}	1.80 ^{Bca}	1.72 ^{CDb}	2.21 ^{A}	1.81 ^{BC}	1.68 ^{D}	**	**	0.042
PCL/PCE	0.83 ^{Bc}	0.85 ^{BD}	0.92 ^{Aa}	0.74 ^{C}	0.92 ^{Aa}	0.87 ^{Ab}	n.s.	**	0.030

$^{A, B, C, D}$: P<0.01; $^{a, b, c, d}$: P<0.05

DECOCTIONS OF NORWEGIAN HERBS STABILISE MARINATED TURKEY THIGHS

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Background

One of the major problems during the processing and storage of meat and meat products is lipid oxidation. This process initiates several changes, which adversely affects the products' colour, flavour, texture and nutritional value. Rancidity in pre-cooked products, especially made from turkey meat, is a great challenge for the food industry. Lipid stability in processed poultry meat may be improved by various components added or injected into the meat during a marination process (Mielnik *et al.,* 1996). The composition of the brine influences the course of chemical reactions, which in turn affect oxidative stability.

The major strategies for preventing lipid oxidation are the use of free radical scavengers such as phenolic antioxidants. In the past few years a variety of plant materials containing phenolic compounds has been proved to be effective antioxidants in model systems. Herbs and spices were traditionally added to food for improving sensory properties and as antioxidants and preservatives. Many herbs and spices rich in polyphenols exhibit antioxidative activities similar to those of synthetic phenolic antioxidants (Cuvelier *et al.,* 1996). Since ancient times, herbs and spices have been used to prolong shelf life and improve the taste of meat products. In various studies, herbs of the mint family (*Labiatae*) have exhibited powerful antioxidant activity (Madsen and Bertelsen, 1995; Herrmann, 1981; Chipault *et al.,* 1956). Marination, originally used for the preservation and tenderisation of meat, is nowadays applied by industry and restaurants to give meats new and exciting tastes and appearances. Marketing of marinated poultry parts is one of the fastest growing segments of the food industry around the world.

Objectives

The presented study was carried out to examine the effect of marinades made with decoction from rosemary, sage and thyme on the oxidative stability of turkey thighs, pre-cooked and chill-stored for one week.

Materials and methods

Marinating of turkey thighs

The turkey thighs were obtained from a local processing plant the day after slaughter. Cuts of thigh meat without skin were soaked in one of five different marinades. Decoctions of rosemary, sage and thyme obtained from Norske Eteriske Oljer A/S were used to prepare three of them. These marinades consisted of decoction with 12% salt. The fourth marinade consisted of 12% salt and 160 mg/L butylated hydroxyanisole (BHA) in water. Water with salt (12 %) and no antioxidant was used as a control. The turkey meat cuts were wiped with paper towel after overnight marination and were vacuum packed in plastic bags (ca 450 g). The samples were cooked in a water bath at 85 °C for 30 min and cooled for 45 min in running water. The meat cuts were divided into smaller portions, transferred to plastic boxes covered with aluminium foil to get air-exposure, and stored for 7 days in the dark at 4 °C.

Chemical methods

Antiradical power (ARP)

The antioxidant activity of the different marinades were determined by using the 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) method according to the procedure described by Brand-Williams *et al.* (1995) with some modifications (Mielnik *et al.*, 2003). Antioxidant activity was expressed as antiradical power (ARP) in units of mg $DPPH^{\cdot}$/ml marinade.

Thiobarbituric acid reactive substances (TBARS)
The TBARS values were determined by the extraction method according to Sørensen and Jørgensen (1996). Results expressed as mg malondialdehyde (MDA)/kg meat, were calculated from a standard curve of TEP (1,1,3,3-tetraethyoxypropane). TBARS values were examined before and after marinating, directly after cooking and after 1, 3, 5 and 7 days of chill-storage at 4 °C in the dark.

Hexanal
Analysis of hexanal formed during 5 days of storage at 4 °C in marinated cooked turkey thighs was performed by a dynamic headspace technique as described earlier (Mielnik *et al*., 2002). Semi-quantitative amounts of the volatile compound were obtained by relating peak intensities to that of the internal standard ethyl heptanoate (ng), weight of meat sample (g) and total volume of purging gas (L), giving the units ng/g x L.

Sensory analysis
The marinated cooked turkey thighs were evaluated after 5 days of storage at 4 °C. Prior to serving, meat samples sealed in vacuum pouches were heated in a water bath at 70 °C for 30 min. Sensory analysis was performed according to international standard methods (ISO 1985). The panel, consisting of eleven trained assessors, carried out a descriptive test (ISO 1985). Prior to the analysis the panel was trained in the definition and intensities of odours (turkey, acidulous, spicy, rancid), flavours (turkey, acidulous, salt, spicy, bitter, metallic, rancid), colour (whiteness, hue, intensity) and texture attributes (hardness and juiciness). Samples from each marinade were served twice in a warm steel cup with a lid. Serving order was randomised according to sample, replicate and assessor. Each panellist evaluated the samples at individual speed on a computerised system for direct recording of data (CSA, Compusense, Version 4.2, Guelph, ON, Canada) using value 1.0 for the lowest intensity and value 9.0 for the highest intensity of each attribute.

Data analysis
Two replications were carried out with all samples. Effects of marinade and storage time on TBARS values were analysed by ANOVA. The main trends of variation among the samples were studied by principal component analysis (PCA) using the Unscrambler version 8.05 software program (CAMO, Oslo, Norway). The variables were mean-centred prior to PCA. All variables were standardised by 1/standard deviation and full cross-validation was used for the model validation (Martens and Næs, 1989).

Results and discussion

Antiradical Power (ARP) of marinades
The $DPPH^{\cdot}$ radical scavenging activities of the marinades applied in the present study are shown as antiradical power (ARP) in Table 1. The rosemary marinade showed the highest ARP values which means that this marinade had the highest antioxidative activity. Thyme and sage marinades showed considerably lower antioxidative activity compared with the rosemary marinade; 36.9 % and 10.7 % lower, respectively. The BHA marinade had the lowest antiradical power.

Table 1. ARP (mg DPPH/ml marinade) for tested marinades

Marinade	Rosemary	Sage	Thyme	BHA
ARP	85.7	9.2	31.6	1.2

TBARS values
TBARS values measured in the turkey thigh meat showed that both the marinating process and the heat treatment affected the oxidation rate (Table 2). The control samples soaked in 12% salt solution had significantly higher TBARS values than samples soaked in 12% salt solution with decoction of herbs or BHA. TBARS values in control samples were 13 times higher than in the other samples. The differences between turkey thighs marinated with various antioxidants were not significant.

Table 2. Evolution of TBARS values (mg malondialdehyd/kg meat) in turkey thighs during marination, cooking and dark storage at 4 °C in air

Marinade	Raw meat	Marinated meat	Cooked and stored meat (days)				
			0	1	3	5	7
Rosemary	0.15	0.14 ^{b}	0,12 $^{c}{}_{v}$	0,39 $^{e}{}_{w}$	0,82 $^{e}{}_{x}$	1,45 $^{e}{}_{y}$	2,33 $^{e}{}_{z}$
Sage	0.15	0.12 ^{b}	0,63 $^{b}{}_{v}$	0,75 $^{c}{}_{w}$	2,40 $^{b}{}_{x}$	2,55 $^{c}{}_{y}$	3,87 $^{c}{}_{z}$
Thyme	0.15	0.14 ^{b}	0,09 $^{c}{}_{v}$	0,66 $^{d}{}_{w}$	1,50 $^{d}{}_{x}$	1,77 $^{d}{}_{y}$	3,35 $^{d}{}_{z}$
BHA	0.15	0.12 ^{b}	0,18 $^{c}{}_{v}$	1,09 $^{b}{}_{w}$	1,88 $^{c}{}_{x}$	3,21 $^{b}{}_{y}$	4,15 $^{b}{}_{z}$
Control	0.15	1.91 ^{a}	7,38 $^{a}{}_{x}$	7,32 $^{a}{}_{x}$	7,69 $^{a}{}_{yx}$	8,28 $^{a}{}_{zy}$	8,45 $^{a}{}_{z}$

$^{a\text{-}e}$ Means with different letters within a column are significantly different at $p < 0.05$.
v-z Means with different letters within a row are significantly different at $p < 0.05$.

The highest TBARS values, measured directly after cooking, were obtained in the control samples without antioxidant. The increase was about four fold compared to raw meat soaked in salt solution, while turkey thigh marinated with rosemary and thyme showed no increase in TBARS values from raw state through marinating and cooking. Meat marinated in rosemary or thyme marinades had the lowest TBARS values; 1.6% and 1.2% of the TBARS value noticed in control samples, respectively. The results demonstrated that marinating with herbs reduced oxidation both during marinating and cooking. However, cooked meat marinated in sage showed significantly higher TBARS values compared with raw and marinated samples and with meat treated with the other herb marinades.

Oxidative rancidity measured as TBARS values increased during storage in all samples. The highest rises in TBARS over time were seen in the control samples without added antioxidant. Meat from the rosemary marinade had the lowest TBARS values throughout the storage period although the development in oxidation increased continually. TBARS values in meat marinated with thyme, sage and BHA also increase during storage, but the TBARS values were significantly higher than those with rosemary. BHA had the lowest stabilising effect among the antioxidants.

Hexanal, a typical off-flavour volatile is often used as an indicator for lipid degradation and rancidity. In raw and marinated turkey thigh meat the amount of hexanal was negligible until cooking (results not shown). After cooking and later during chill storage, the amount of hexanal increased considerably especially in the control samples. The rosemary decoction was most efficient in retarding the formation of hexanal.

The PCA showed that the two first principal components described 81% of the variation between samples treated with different marinades. The loading plot (Fig.1) illustrates contributions of the scaled variables to the PCs. The first principal component appears to be composed of spicy and acidulous flavour together with juiciness on one side. These variables were highly correlated to each other and gave high negative loadings. On the opposite side, rancidity attributes like rancid and metallic flavour, TBARS and hexanal as well as hardness contributed to the positive loadings. The second principal component was mainly related to bitter flavour. The pattern in the score plot showed that control samples were strongly related to the variables describing development in oxidation while the meat marinated with decoction of herbs, especially rosemary, had high scores for spicy and acidulous flavour. However, the turkey thigh marinated with rosemary was assessed as the most bitter among the samples.

Conclusions

Utilisation of herb decoctions may have a great potential in the catering sector in order to prevent the development of rancidity in stored heat-treated meat products. This study showed that decoctions, as by-products from distillation of essential oils, could be used as a source of antioxidants in marinades. However, procedures need to be standardised, and the decoction should undergo further processing before being used commercially.

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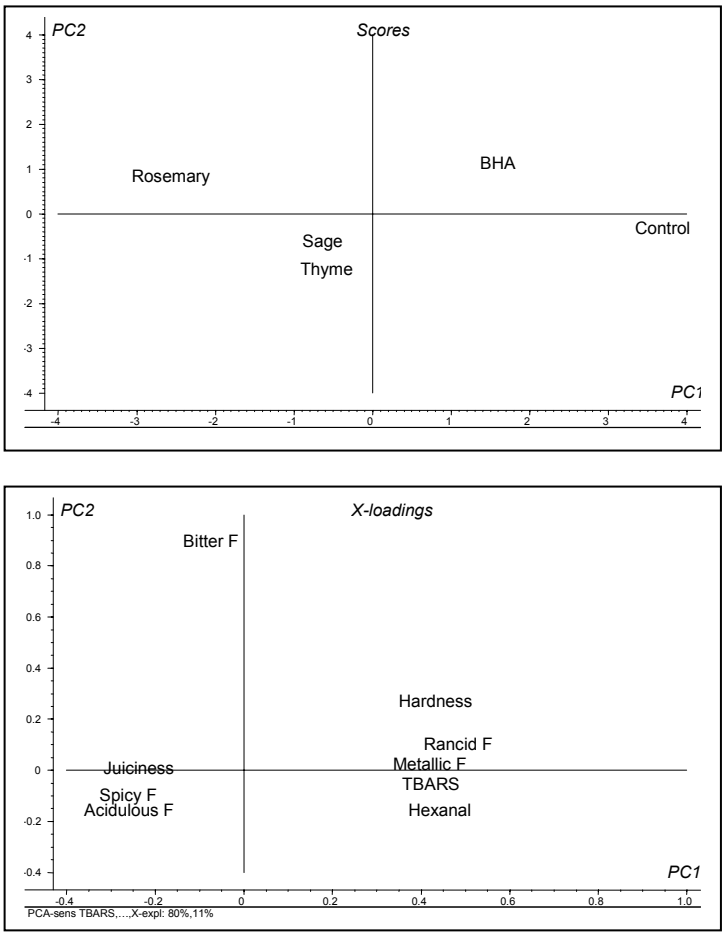

Figure 1. Score and loading plot for PC1 and PC2 obtained from PCA of sensory attributes analysed together with TBARS and§ hexanal measured in marinated meat from turkey thigh, cooked and stored for 5 days at 4 °C. (F = flavour)

EFFECT OF FORAGE LEGUME, STOCKING RATE AND CONDENSED TANNINS ON CORRIEDALE LAMB MEAT QUALITY IN URUGUAY

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Background

Meat color, visible fat content and odour are variables that the consumers judge at the time of purchase. However, when meat is being consumed, tenderness, juiciness and flavour became the main characteristics. Meat quality, as a general concept, is difficult to define, because it can have different meanings for different consumers. Given this consumer behaviour variability, it is not common to study this concept based on just one characteristic of meat quality. Instead, it should be considered on a broader manner, taking into account a number of characteristics measured objectively. Despite of this approach of scientific studies, one must consider that for consumers, the general concept or impression is what really matters (Purchas, 1994). In the actual export markets under high competitive pressure and particularly in the food industry around the world, marketing strategies have to be based on solid scientific means to certify and assure food safety and product quality. It is also known, that other attributes associated with the environment sustainability, animal welfare and social aspects, where animal are produced, constitute other important aspects of the promotion strategies and development of market niches for each country (Montossi *et al.*, 2003).

Objectives

The purpose of this study was to evaluate the effects of forage legume (*Lotus pedunculatus* cv. Maku, *Lotus subbliflorus* cv. El Rincón, *Lotus corniculatus* cv. Draco and *Trifolium repens* cv. LE Zapicán), condensed tannins (CT) and stocking rates (8 and 12 lambs/ha) on meat quality in Uruguayan Corriedale lambs.

Materials and methods

Description of the experiment

One hundred and twenty eight castrated male Corriedale lambs (9 months old), born in August-September 2000, were used for this study. At the beginning of the experiment, the average liveweight (LW) and condition score (CS) were 23,8 ± 2,1 kg (fasted) and 2,2 ± 0,3 units, respectively. The selected lambs were divided into balanced groups according to LW and CS. Half of the lambs received two oral administrations (0730 and 1730 hours) of polyethylene glycol (PEG; MW 6000) to inactivate CT (2 g of PEG per 1 g of CT), whilst the remaining lambs received oral administration of water. PEG supplementation was used to study the interaction between condensed tannins (CT) and proteins (Jones and Mangan, 1977; Barry and Manley, 1986; cited by Barry *et al*., 2001), because of its capacity to make strong binds with CT.

Animal measurements

Objective parameters of meat and carcass quality were measured on half of the animals (n=64): meat temperature and pH at 1, 3 and 24 hours *pos mortem*, between 12th and 13th rib (*Longissimus lumborum* muscle; LL). The muscle pH was measured using a hand-held pH meter (Orion A 230) with a probe type electrode (BC 200, Hanna Instruments), standardized against two pH buffers (4 and 7). The probe was cleaned with alcohol and rinsed with water between uses. The temperature was determined by a thermometer (Barnant 115) with stainless steel thermocouple (type E). Muscle and fat color measurements were made using a Minolta Colorimeter (model C-10). They were recorded in triplicate from the approximate geometric center of the exposed LL muscle at the 13th rib, after 24 hours *pos mortem*, taking the readings of L*, a* and b* parameters, according to the Hunter system.

A portion of the LL was removed from the left side of carcasses, labeled, vacuum-packaged, and measured for shear force after 10 days of aging at 2 - 4 °C. The samples were cooked by immersion within a plastic bag in a water bath at an internal temperature of 70°C for 75 min. The internal temperature was monitored using type E thermocouples placed in the approximate geometric center of the sample. Six cores (2,54 cm in

diameter) parallel to the muscle fiber orientation were removed from each sample. A single peak WBSF measurement was obtained for each core using a WBSF machine (G-R Electric Manufacturing Co, Manhattan, KS). Individual-core peak shear force values were averaged to assign a mean peak WBSF value to each sample. Further procedures concerning measurements have been described in Montossi *et al.* (2003).

Statistics

The animal information was analyzed using the statistical package SAS (SAS, 2000), based on Split-Split-Plot design using 2 blocks, with the 4 forage species (*Lotus corniculatus* cv. INIA Draco, *Lotus pedunculatus* cv. Maku, *Lotus subbiflorus* cv. El Rincón and *Trifolium repens* cv. LE Zapicán), being the main plot arranged in a 2 x 2 factorial structure. Stocking rate (SR; 8 or 12 lambs/ha) was treated as the split-plot factor, while PEG (CT inactivated or activated) was used as the split-split-plot factor. All data were initially tested for normality and homogeneity of variance. Before the statistical analysis was performed, some variables (tenderness, temperature, pH, meat and fat color) were normalized by 3√, Ln or 1/Ln correction factors. Liveweight gain (LWG) was adjusted by covariance for initial live weight. Carcass fatness (estimated by GR point) was evaluated using hot carcass weight (HCW) as a co-variate.

Results and Discussion

Animal performance and carcass quality

Animal performance and carcass quality traits are presented in Table 1. Lambs grazing *Trifolium repens* cv. LE Zapicán (white clover; WC) reached the highest liveweight gain (LWG; g/d), intermediate values were found for *Lotus corniculatus* cv. INIA Draco (D) and *Lotus pedunculatus* cv. Maku (M) lambs, and the lowest value was for lambs grazing on *Lotus subbiflorus* cv. El Rincón (R) swards. These results of LWG between animals grazing different species caused differences in final liveweight (FLW), hot carcass weight (HCW), cold carcass weight (CCW) and fat cover (GR). Stocking rate (SR) affected animal performance and carcass traits, where animals of the lower SR obtained higher values than animals of higher SR. PEG supplementation did not affect these variables, probably caused by the high quantity and quality of the forage offered (Barry *et al.*, 2001).

Meat quality

It is desirable to decrease muscle temperature after slaughter in order to reduce the losses of proteins and to inhibit bacterial growth, but this reduction has to be slow to prevent muscle fibre shortening (cold shortening)(Brito, 2002). Table 2 shows the results of muscle temperature and pH at 1, 3 and 24 hours *post mortem*. Spp affected significantly temperature at 1 and 3 hours. Despite the differences found between SR in fat cover (GR), there was no effect of this factor on the reduction of the temperature *post mortem*, probably caused by the small differences shown. PEG supplementation did not affect temperature measurements. Muscular pH of alive animals is between 7.1 and 7.3, reducing itself down to 5.5-5.7 at 48 hours *post mortem* (Garrido and Bañón, 2000). In this study, the differences recorded in temperature at 1 and 3 hours between forage species did not cause *Longissimus lumborum* pH differences (1, 3 and 24 hours *post mortem*). SR and PEG supplementation did not affect pH measurements.

Consumer preferences are greatly affected by meat tenderness, being considered as the most important characteristic of meat quality and determinant of the repetition of purchasing (Brito *et al*., 2002). Tenderness results, obtained by shear force measurements (SF; kgF) of *Longissimus lumborum* muscle, were not affected by the effect of any of the evaluated factors (Table 3). These results could be due to the similar pH and temperature reduction shown by the different treatments (Brito *et al*., 2002). Anyway, the small differences found between treatments applied on tenderness, are strongly influenced by the experiment protocol used to determine the value of shear force, which includes 10 days of meat ageing. This process allows the tenderization of the muscular fibers, reducing existing differences caused by initial meat tenderness or some processes applied pre, during or after slaughter (Brito *et al*., 2002). Camesasca *et al*. (2002) did not find differences in meat tenderness despite the differences in temperature and pH. This suggests that the 10 days of ageing at 2-4 °C would explain these results, given the importance that this process has in reducing differences in meat tenderness (Brito *et al*., 2002; Koohmaraie *et al*., 1995, cited by Camesasca *et al*., 2002).

The findings on temperature, pH and tenderness (Tables 2 and 3), are similar to those obtained by Brito *et al*. (2002) for lamb carcasses (male and females) of different genotypes and less than 12 months old, reared with different nutritional regimes under grazing conditions. In the case of pH, the values are also similar to those obtained by Wheeler and Koohmaraie (1994). Both factors, in addition to the ageing process, and the high liveweight gains during the experiment, could be explained by the low values of SF obtained, which were very similar to those reported by Camesasca *et al*. (2002). Bickerstaffe (1996), cited by Brito *et al*. (2002), suggested that the values of lamb meat tenderness standardized by the meat industry of the United States and New Zealand, to maintain or access to new markets, has to be less than 5 kgF in terms of shear force.

According to the results obtained in the present experiment, these animals would be eligible to those important markets. Adams and Huffman (1972), cited by Osório and Sañudo (1996), reported that the meat color is one of the most important consumer attributes, and is the most relevant quality factor that the consumer considers at the time of purchase. Color of meat is affected by pre-slaughter (animal species, age, sex and feeding system, etc, Albertí, 2000), as well as post-slaughter factors (cooling conditions, pH reduction, etc.; Brito *et al*., 2002). Table 4 shows the results of the effect of Spp, SR and PEG on muscle and fat color. Spp and PEG did not have a significant effect on the parameters of lightness (L*), relative redness (a*) or yellowness (b*) of muscle. However, SR significantly affected muscle lightness (L*m), being higher in the SR of 12 lambs/ha, but did not affect the parameters a* and b*.
Channon and Leury (1992), comparing different diets (*Trifolium yannicum vs. Lolium rigidum*), did not find differences on muscle pH (*L. lumborum*) and lightness, but the treatments affected parameters a* and b*. In another work made by the same authors, animals fed at maintenance level and well fed later, did not show differences in meat color in comparison with animals without restrictions. Due to the lack of information gather from R treatment in fat color, the comparison is restricted to the remaining treatments. The parameters of fat a* and b* did not show differences of statistical significance between the evaluated factors. On the other hand, L* was affected significantly by forage species, but not by the remaining evaluated factors. WC achieved a higher value than M, and D did not have differences with both species. The values of muscle and fat L*, a* and b* are similar to the results reported by Brito *et al*. (2002) in Uruguayan national evaluations on lambs with different nutritional regimes.

Conclusions

For the conditions imposed in this study, under grazing conditions with adequate feeding levels and meat aging period of 10 days, lambs meat quality attributes were not substantially affected by the type of legume, feeding regimes and PEG supplementation used. The values of temperature, pH and tenderness found in this study, and those provided by Montossi *et al*. (2003) in a national sheep meat quality audit, suggest that the meat produced by these Uruguayan Corriedale lambs would be eligible to the most important external markets.

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Table 1. Effect of forage species (Spp), stocking rate (SR) and PEG supplementation (PEG) on animal performance and carcass quality traits.

Variable	Species (Spp)					Stocking rate (SR)			PEG		
	D	M	R	WC	P	12	8	P	No	Yes	P
LWG (g/d)	176[b]	182[b]	150[c]	221[a]	**	171[b]	193[a]	**	181	183	ns
FLW (kg)	39.8[b]	40.5[b]	37.1[c]	44.7[a]	**	39.4[b]	41.7[a]	**	40.4	40.7	ns
HCW (kg)	19.4[b]	19.3[b]	16.3[c]	22.4[a]	**	18.8[b]	19.9[a]	**	19.3	19.4	ns
CCW (kg)	19.0[b]	18.8[b]	15.9[c]	22.0[a]	**	18.4[b]	19.4[a]	*	18.9	18.9	ns
GR (mm)	9.2[b]	8.4[c]	4.5[d]	12.6[a]	**	7.5[b]	9.8[a]	**	8.9	8.5	ns

ns: not significant (P>0.05), *: P<0.05 and **: P<0.01.
[a, b, c y d]: means with different letters within each variable are statistically different (P<0.05).
D: *L. corniculatus* cv. INIA Draco; M: *L. pedunculatus* cv. Maku; R: *L. subbiflorus* cv. El Rincón; WC: *T. repens* cv. LE Zapicán; LWG: liveweight gain; FLW: final liveweight; HCW: hot carcass weight; CCW: cold carcass weight; GR: fat cover.

Table 2. Effect of forage species (Spp), stocking rate (SR) and PEG supplementation (PEG) on meat temperature and pH *post mortem*.

Variable	C F	Species (Spp)					Stocking rate (SR)			PEG			Spp x SR	Spp x PEG	SR x PEG	Sppx SRx PEG
		D	M	R	WC	P	12	8	P	No	Yes	P				
Temp.1	=	23.6[b]	23.2[b]	20.7[c]	26.1[a]	**	23.3	23.5	ns	23.5	23.2	ns	ns	ns	ns	ns
Temp.3	R[3]	16.8[a]	17.0[a]	14.4[b]	17.7[a]	*	16.0	16.9	ns	16.5	16.4	ns	ns	ns	ns	ns
Temp.24	=	4.2	4.4	4.2	4.2	ns	4.2	4.4	ns	4.3	4.3	ns	ns	ns	ns	ns
pH 1	Nl	6.4	6.4	6.5	6.4	ns	6.4	6.4	ns	6.4	6.4	ns	ns	ns	ns	ns
pH 3	Nl	6.2	6.1	6.2	6.2	ns	6.2	6.2	ns	6.2	6.1	ns	ns	ns	ns	ns
pH 24	Nl	5.8	5.8	5.8	5.8	ns	5.8	5.8	ns	5.8	5.8	ns	ns	ns	ns	ns

ns: not significant (P>0.05), *: P<0.05 and **: P<0.01.
a, b, c y d: means with different letters within each variable are statistically different (P<0.05).

Table 3. Effect of forage species (Spp), stocking rate (SR) and PEG supplementation (PEG) on meat tenderness.

Variable	CF	Species (Spp)					Stocking rate (SR)			PEG			Spp x SR	Sppx PEG	SRx PEG	Sppx SRx PEG
		D	M	R	WC	P	12	8	P	No	Yes	P				
SF (kgF)	Nl	1.63	1.60	1.71	1.60	ns	1.65	1.61	ns	1.61	1.66	ns	ns	ns	ns	ns

ns: not significant (P>0.05), *: P<0.05 and **: P<0.01.

Table 4. Effect of forage species (Spp), stocking rate (SR) and PEG supplementation (PEG) on parameters of muscle and fat color.

	CF	Species (Spp)					Stocking rate (SR)			PEG			Spp x SR	Spp x PEG	SR x PEG	Spp x SR x PEG
		D	M	R	WC	P	12	8	P	No	Yes	P				
L*m	l/Nl	36.4	37.2	36.7	38.2	ns	37.7[b]	36.5[a]	*	36.6	37.6	ns	ns	-	-	-
a*m	Ln	19.8	20.1	19.6	19.8	ns	19.8	19.8	ns	19.8	19.8	ns	ns	-	-	-
b*m	R[3]	8.5	8.6	7.8	8.4	ns	8.3	8.3	ns	8.1	8.4	ns	ns	-	-	-
L*f	Nl	73.0[ab]	70.8[b]	-	75.0[a]	*	72.1	76.0	ns	73.2	72.6	ns	-	-	-	-
a*f	R[3]	4.5	3.9	-	3.6	ns	4.0	4.0	ns	3.9	4.1	ns	-	-	-	-
b*f	R[3]	10.6	11.1	-	10.4	ns	10.1	11.3	ns	10.7	10.7	ns	-	-	-	-

ns: not significant (P>0.05), *: P<0.05 and **: P<0.01.
a, b, c y d: means with different letters within each variable are statistically different (P<0.05).

EFFECT OF HIGH PRESSURE TREATMENT AND SUBSEQUENT STORAGE ON THE COLOUR OF A BEEF PUREE

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Background

The effects of high pressure on meat have been reviewed by Cheftel and Culioli (1997) and include changes in muscle ultra structure, denaturation of proteins, inactivation of microorganisms and extension of shelf-life. The colour of meat depends on the amount and type of heme pigment, and the scattering properties of the meat (MacDougall 1983). High-pressure treatment of myoglobin solutions has been shown to result in partial denaturation with later renaturation (Defaye *et al* 1995). The effect of high-pressure treatment on myoglobin solutions depends on the temperature at which pressure treatment occurs. Zipp and Kauzmann (1973) did not observe denaturation of myoglobin below 225 MPa at 20°C, and Ooi (1994) did not observe denaturation until 500 MPa at 10°C. Carlez *et al* (1995) found increases in L* values and decreases in a* values when minced beef was pressure treated above 350 MPa at 10°C. High-pressure treatment is undertaken on vacuum packed meat and it is well established that when vacuum packs are opened the myoglobin oxygenates to oxymyoglobin. Thus given the partial denaturation reported by Defaye *et al* (1995) it is important to standardise colour measurement at a set time after opening of the vacuum pack of pressure treated meat.

Objectives

The purpose of this study was to compare the effects of high-pressure treatment at different temperatures followed by storage on the CIELAB colour values of vacuum packaged beef puree samples.

Materials and methods

Fresh minced beef was purchased from a local supplier in Belfast. The beef was mixed thoroughly to give a homogenous sample. A total 180 beef puree samples, each weighing approximately 100 g and formed into 5 cm sided cubes, were prepared after which each sample was placed in a sterile polyethylene/polyamide vacuum pouch, vacuum packed and stored overnight at 1ºC prior to high pressure treatment.

Samples were pressure treated in a Stansted Foodlab 900 high pressure isostat capable of operating at 900 MPa (Stansted Fluid Power Ltd., Stansted, UK). The pressure transmission fluid was a 10% vegetable oil in water emulsion. A submerged thermocouple was used to monitor the temperature of the pressurisation fluid during treatment. The pressure come-up time using this system was approximately 300 MPa per min and the pressure release time was 4.5 s per 100 MPa. The temperature increase due to adiabatic heating was approximately 2.5°C per 100 MPa. The puree samples were given pressure treatments of 100, 300 and 600 MPa or left untreated to serve as controls. Pressure treatment was carried out at temperatures of 5, 20 or 40ºC. Samples were treated at each pressure/temperature combination for 15 min. For each pressure treatment, 15 samples were treated at each temperature to give a total of 45 samples. Following treatment, colour measurements were carried out on three samples from each pressure/temperature combination. The reflectance spectra of the vacuum packs were measured immediately after opening and again at 45 min post-opening using a Monolight spectrophotometer and CIELAB values calculated (Moss *et al* 2000).

Results and discussion

High-pressure treatment had a statistically significant effect on all CIELAB colour parameters both on initial opening and after 45 min post-opening. In general, changes in CIELAB values were not observed until pressure treatment was applied at 300 MPa and above (Tables 1 & 2). These results are similar to those of Carlez *et al* (1995) who observed increases in L* from above 200 MPa and decreases in a* particularly above 400 MPa when minced beef was treated. Carlez *et al* (1995) observed little change in b* values with increasing pressure treatment, whereas in the present experiment b* values on initial opening increased with

pressure treatment above 300 MPa, whereas after 45 min opening only the b* values at 300 MPa were significantly different. Carlez *et al* (1995) undertook colour measurements 10 min post-opening with pressure treatment carried out at 10°C which could explain some of the differences observed, although in the current studies the temperature at which the meat was pressure treated had little effect on CIELAB unless at 40°C (Fig 1). O'Connor (2001) studied the effects of combination pressure/temperature treatments on both colour and microbiological shelf-life and showed that, in general, combination of high pressure with higher temperature resulted in greater inactivation of microorganisms.

The main effect of storage was an increase in L* values and decrease in a* values with storage time on both the initial opening and 45 min later. The major effect was seen between 0 and 2 weeks storage. The statistically significant interaction between pressure treatment and storage (Tables 1 and 2) shows that for a* values, in particular, the decrease between 0 and 2 weeks storage was greater for the 300 and 600 MPa treatments than for the control and 100 MPa treatments (Fig 1).

The effect of storage of beef in vacuum packs on colour values has been studied extensively. In the current studies the potential for the meat puree to oxygenate ('bloom') is evident at all storage times as indicated by the higher a* values in the packs 45 min post-opening (Fig 1). Studies on myoglobin solutions have shown denaturation occurs at 500 MPa at 10°C (Ooi 1994) and 350 MPa at 30°C (Taniguchi *et al* 1994). It has been suggested that the effect of pressure on myoglobin was similar to denaturation by heat, with spectral changes indicating the 6th co-ordination position (water for myoglobin) was replaced by imidazole group of histidine (Zipp & Kauzmann 1973).

In intact meat systems the contribution of the meat matrix to colour must be considered, particularly denaturation of myofibrillar proteins, sarcoplasmic proteins and consequently influences on enzyme activity (Govindarajan *et al* 1977). Studies on the effect of high-pressure individual proteins have shown a number of changes including aggregation of myosin (O'Shea *et al* 1976) and disaggregation of actomyosin (Ikkai and Ooi 1969). Changes in a number sarcoplasmic proteins and enzyme activity have also been observed (Cheftel and Culioli 1997). The paler appearance and higher L* values of PSE pork are due to an increase in the scattering coefficient (McDougall 1983).

In these studies the $^{k}/_{s}$ ratio at 730 nm decreased due to high-pressure treatment from 0.41 and 0.48 at 0 and 100 MPa to 0.15 at both 300 and 600 MPa. This decrease indicates general denaturation of the meat protein matrix and consequent increase in scattering coefficient, since at 730 nm absorption due to myoglobin is low (Millar *et al* 1996). The $^{k}/_{s}$ ratio at 525 nm shows a similar trend (3.2, 3.7, 1.4 and 1.4 at 0, 100, 300 and 600 MPa, respectively). Since 525 nm is generally considered to be the isobestic form of the myoglobin, oxymyoglobin and metmyoglobin (Millar *et al* 1996), any denaturation of myoglobin should have little effect on the absorption coefficient. Thus, the increased $^{k}/_{s}$ ratio at 525 nm is also indicative of a general increase in scattering coefficient. The colour of meat depends on oxygen penetration into the meat and the formation of oxymyoglobin in bloomed meat. The darker appearance of DFD meat and greater contribution of myoglobin to the observed colour is due in part to the lower scattering coefficient of high pH meat. Thus conversely, an increased scattering coefficient due to high pressure would result in a greater contribution of the outer surface to the observed appearance/measured surface reflectance. It might be expected that high pressure treated spectra show a more oxymyoglobin type spectra. Further detailed evaluation of the reflectance spectra at the meat surface and at depths through the meat is required to evaluate this.

Conclusions

High-pressure treatment of beef puree results in changes in CIELAB colour values. Increased L*, decreased a* were found at 300 MPa and above, but were not evident at 100 MPa. The results indicate that the myoglobin is not completely denatured and the beef puree has the potential to 'bloom' when the vacuum packs are exposed to air. The temperature at which high pressure treatment is undertaken also has an effect on CIELAB colour parameters with higher L* and lower a* values when pressure treated at 40°C. Decreases in a* values with storage are dependent on the pressure treatment used.

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Table 1: Effect of high pressure treatment on the colour of vacuum packaged beef puree samples. Measurements taken upon initial opening of pack.

	L*	a*	b*	hue	Metric Chroma
Effect of pressure (MPa)					
0	45.32	10.74	14.66	53.82	18.22
100	44.59	10.16	14.49	55.02	17.75
300	56.28	4.47	15.96	74.63	16.68
600	58.14	4.24	16.64	75.82	17.27
Significance of effect	***	***	***	***	***
SEM (n = 45)	0.334	0.133	0.131	0.399	0.141
Effect of temperature (ºC)					
5	50.30	7.69	15.22	63.77	17.40
20	50.52	7.51	15.48	64.62	17.40
40	52.43	7.01	15.61	66.07	17.44
Significance of effect	***	***	*	***	NS
SEM (n = 60)	0.289	0.116	0.113	0.345	0.122
Effect of storage (weeks at <4ºC)					
0	49.75	9.77	15.46	57.88	18.32
2	50.93	7.32	14.91	64.59	16.98
4	51.03	6.59	15.23	66.69	16.95
6	51.39	6.59	15.40	67.00	17.14
8	52.31	6.73	16.21	67.95	17.88
Significance of effect	***	***	***	***	***
SEM (n = 36)	0.373	0.149	0.146	0.446	0.153
Significance of interaction					
P x T	NS	NS	**	*	**
P x S	*	***	***	***	***
T x S	NS	***	NS	*	NS
P x T x S	NS	NS	***	*	***

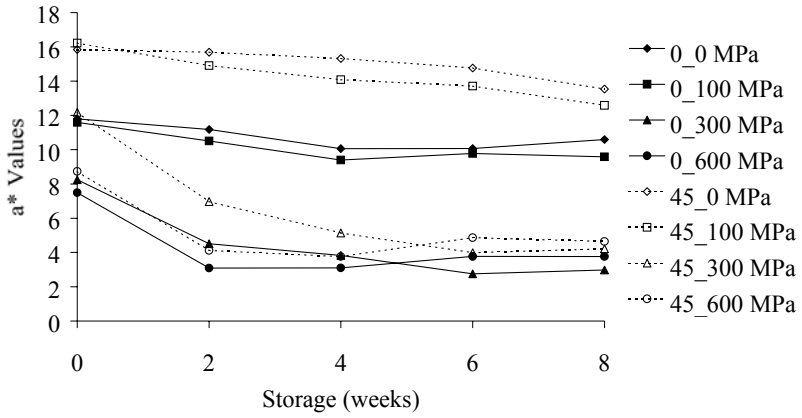
SEM = standard error of mean; *** = p<0.001; ** = p<0.01; * = p<0.05; NS (not significant) = p>0.05

Table 2: Effect of high pressure treatment on the colour of vacuum packaged beef puree samples. Measurements taken 45 min after initial opening of pack.

	L*	a*	b*	hue	Metric Chroma
Effect of pressure (MPa)					
0	44.28	15.03	17.08	48.78	22.78
100	44.67	14.30	17.04	50.25	22.30
300	55.37	6.49	16.05	68.86	17.49
600	56.58	5.22	17.03	73.10	17.92
Significance of effect	***	***	***	***	***
SEM (n = 45)	0.380	0.179	0.152	0.353	0.197
Effect of temperature (ºC)					
5	49.25	10.65	16.75	59.24	20.24
20	49.53	10.62	16.81	59.52	20.34
40	51.89	9.52	16.85	61.98	19.79
Significance of effect	***	***	NS	***	‡NS
SEM (n = 60)	0.329	0.155	0.132	0.306	0.171
Effect of storage (weeks at <4ºC)					
0	48.95	13.24	17.64	53.71	22.20
2	49.34	10.42	16.38	59.40	19.85
4	50.50	9.58	16.52	61.87	19.60
6	50.53	9.34	16.69	62.50	19.61
8	51.80	8.74	16.78	63.76	19.35
Significance of effect	***	***	***	***	***
SEM (n = 36)	0.425	0.200	0.170	0.395	0.220
Significance of interaction					
P x T	NS	*	*	**	**
P x S	*	***	***	***	***
T x S	NS	NS	NS	*	NS
P x T x S	NS	*	NS	***	NS

SEM = standard error of mean; *** = p<0.001; ** = p<0.01; * = p<0.05; NS (not significant) = p>0.05; ‡p = 0.056

Figure 1: Effect of high pressure processing and storage on the a* values of beef puree samples



CONSUMER PREFERENCES OF PORK CHOPS: RESULTS OF AN INTERNATIONAL CROSS-CULTURAL COMPARISON.

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Background

The pork industry is competitive at both international and national levels and is responding to consumers' demands and expectations for safe, nutritious products, which conform to their life-styles. With increasing international trade, the industry needs to be aware of different consumer preferences for meat according to their different cultures and traditions. This will be increasingly important for the pork market.

Consumer preferences of pork are based on expectations of enjoyment within a given context. Expectations are perhaps different when the pork is for oneself, others or for a particular meal or event. These contextual differences play a role in the selection of pork and the type of pork to be bought. At the point of purchase, the choice is based on an expectation of good eating quality, an expectation that the pork will be tender and juicy. These expectations are based on information and previous experiences. Choice is therefore based on expectations, price and the appeal of the pork, where appeal is strongly expressed as a preference for the appearance characteristics.

Appearance characteristics of the pork are thought to be the main factors governing choice and comprise the main characteristics: colour, amount of fat cover, marbling and drip. Preferences for pork characteristics have been determined separately in different countries in several local studies but the practical limitations, imposed by the short display-life of meats, make it inevitable that the people in different localities have assessed different meats. The meats also will have differed simultaneously in several of those characteristics and the relative importance of those characteristics is uncertain. The conclusions from such studies are therefore limited when considering the cultural and international dimensions.

These limitations, particularly so when surveying large numbers of people in different countries, have been overcome by using photographs varying systematically in four appearance characteristics. This is the first time a large-scale systematic study has been conducted on meat appearance and consumer preference.

Objectives

- To identify the most important characteristics of fresh pork which determine preference
- To show any variations in preferences among people from different countries

Materials and methods

The methodology and chop characteristics are described in detail in Ngapo *et al.* (2004). Briefly, photographs of 16 pork chops were computer-modified to give two levels of each of the characteristics: fat cover, colour, marbling and drip. The pork chops were purchased at local supermarkets and butcher shops. The resulting 256 (2x2x2x2x16) images have been published as a book (Dransfield *et al.*, 2001), which can be used as a tool for analysing the importance of those factors in consumer choice. The book is comprised of 6 series of which series 1+2, 3+4, and 5+6 each contain all 256 images. A series constitutes 16 (A4) pages or 8 double-pages. Every double-page contains the 16 different chop shapes and each chop represents one of the combinations of the four characteristics studied. Therefore every double-page contains a complete set of all 16 combinations of the 2 levels of each of the four characteristics. Both the order of representation of the characteristics with respect to the chop shape and the position of the chops in a double-page are randomised. It is important to note that the chop shape was not a factor studied, but can be considered a distraction and a means to realistically present a range of characteristics to the consumer.

Consumers, older than fifteen years of age and who eat pork, were chosen at random and asked to select their preferred chop from each double-page. The selection was repeated 8 times completing one series. The consumers then completed a short questionnaire (translated by the research group undertaking the survey into the language of that country) asking basic socio-demographic and purchase- and eating-behaviour information (Table 1). Each new consumer was given a series in the order 1 to 6 so that all series were used approximately equally throughout a survey period. Consumers were surveyed at a range of sites, including agricultural shows, supermarkets and at their workplaces. The surveys were undertaken by 28 research groups in 26 countries and coordinated by the French group. These countries and the number of consumers surveyed in each were Argentina (505), Australia (498), Belgium (353), Brazil (710), Canada (Alberta and Quebec; 1053), China (544), Denmark (200), Estonia (248), Finland (305), France (573), Germany (143), Greece (412), Ireland (300), Japan (645), Korea (1014), Mexico (751), New Zealand (327), Poland (480), South Africa (562), Spain (358), Sweden (200), Taiwan (716), The Netherlands (873), United Kingdom (290), USA (Iowa and Texas; 732) and Yugoslavia (488).

Detailed analyses of the French results have been reported earlier (Ngapo *et al.*, 2002; Ngapo *et al.*, 2004) and, more briefly, the results of the Korean (Cho, *et al.*, 2003) and Brazilian (Cipolli *et al.*, 2003) surveys.

Results

The characteristics of the images of the pork chops used in these surveys are given in Table 1. Visual differences in colour were mainly due to average differences of 8.6 units in lightness (L*) and 5.4 units in redness (a*). Subcutaneous fat cover of the fat chops was, on average, twice that of the lean chops. Drip was either absent or represented almost 6% of the surface area in the samples modified to show drip. Bone and loin muscle area were similar across appearance variables.

Table 1. Mean composition of the 256 pork chop images.

	Light	Dark
Colour L*	64.3	55.7
Colour a*	18.3	23.7
Colour b*	20.8	18.8
(% chop surface area)	Fat	Lean
Cover fat	16.9	7.9
Drip	5.4	5.7
Bone	15.7	16.1
Loin muscle	39.2	43.9

From the results of the 8 replicates given by each person, the frequency of choice for the 4 main characteristics was calculated. For each of the 2 levels of each characteristic, the choice was classed as consistent when the same level was chosen at least 6 times from the 8 replicates, otherwise the choice was deemed inconsistent. Three classes were then produced for each characteristic: with the percentage of people choosing level 1 (for example for colour, light red), level 2 (dark red) and inconsistent (with <6 of the replicates the same choice) for each of the 26 countries.

This frequency of choice was then subjected to a correspondence analysis (SAS, 1999) to determine the relationships among countries of choice for each of the 12 classes (3 frequency classes for 4 appearance characteristics). The first 2 dimensions of the correspondence analysis accounted for 80% of the total variation and are shown graphically in Figure 1. The positions of the countries are given relative to 8 of the choice options (the 4 inconsistent options are not given for clarity, but are included in the analyses). Countries shown close to a given choice have a greater percentage of their people who chose consistently that characteristic as its preferred appearance. Conversely, large distances from a choice characteristic usually denote preference for the other option of the characteristic. Inconsistent choices tend to be found in the central region of Figure 1.

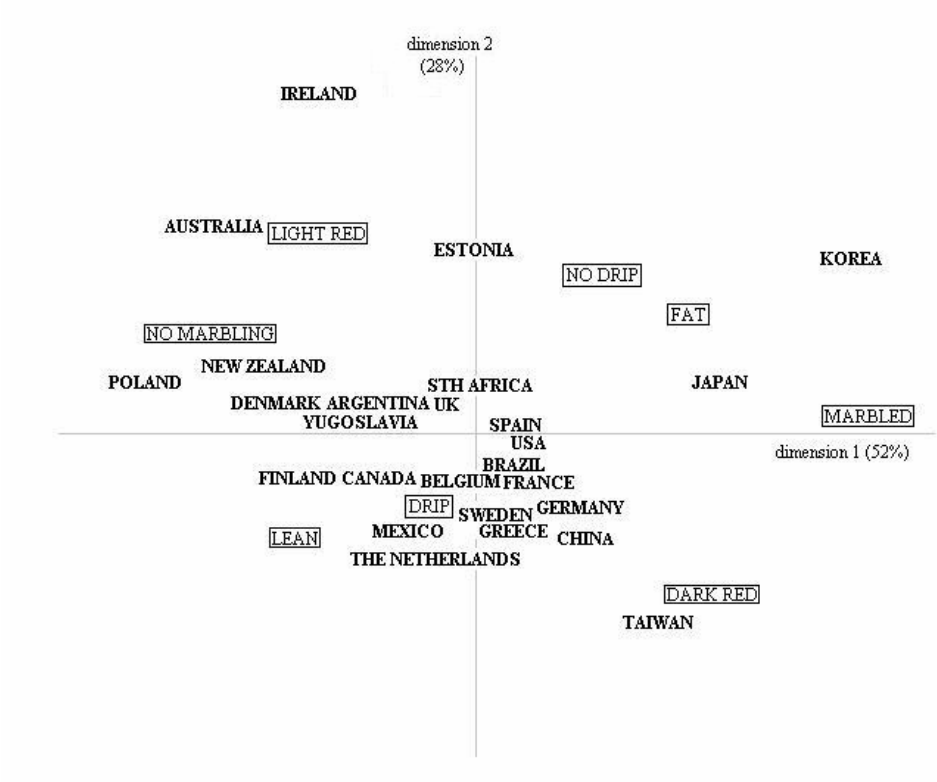

Figure 1. Preferences for 4 pork characteristics from surveys conducted in 26 countries.

Large differences in preferences were found between individuals, groups of people and between countries. Compared to the other consumers, more of the Polish, Australian and Irish consumers preferred non-marbled, light red pork, that is, they are positioned in the upper left quadrant. Those in Estonia preferred light red pork without drip. On the contrary, more people from Korea and Japan tended to prefer the fat and marbled options and are positioned in the upper right quadrant of Figure 1. More people in Taiwan preferred the dark red pork whilst the Dutch and Finnish preferred lean meat with little overall preference for colour. Most countries tended to group close to the 'centre' of Figure 1 showing that preferences for colour, marbling and drip were not strongly in favour of any one option but they were more consistent in their choice of the leaner option.

France, USA and Canada conducted surveys in different regions, and estimates can be made of the within and between country variation. In France, 3 regions were studied (Ngapo *et al.*, 2004), which showed differences in preferences between the regions composed of about 200 consumers each.

Discussion

This unique study of preferences for appearance characteristics, in which consumers in 26 countries viewed exactly the same appearance characteristics, has shown that choice of pork is influenced by its colour, fatness, marbling and drip and that preferences differed considerably between countries. The range of characteristics chosen for the study was not exceptional and can be found in the market within Europe.

Using replicate choices, the study was able to show those characteristics which were consistently chosen and those which were not. This is a unique consumer study conducted with replication, but without the consumers knowing it. Consistency is interpreted as a measure of importance to the individual, who presumably paid more attention to those characteristics to be able to give a consistent choice. So it was shown that, overall, colour and fatness were the most important appearance characteristics and marbling and drip were less important.

Using groups of people from different regions within the same country has also enabled the variation within country to be established which was found to be much less than that between countries.

Conclusions

- Significant differences were found for consumer choice of pork chops based on 4 appearance characteristics. The greatest differences were for external fat cover with preferences for both fat and lean chops differing among countries. The second criterion for selection was for colour where both dark and light red chops were preferred by different people.
- Differences in preference between regions within country were generally smaller than those between many of the countries surveyed.
- Significant market segmentation exists in preferences on a global scale.
- Pork producers should be aware and prepared to respond to such market opportunities.

Acknowledgements

For organising the surveys in different countries, thanks are expressed to: Dr F. Carduza, ITA-INTA Castelar, Buenos Aires, Argentina; Dr C. Russell, University of Ballarat, Ballarat, Victoria, Australia; Dr W. Verbeke, Ghent University, Gent, Belgium; Dr E.T.F. Silveira, ITA, Campinas, Sao Paulo, Brazil; J. Fortin, Agriculture and Agri-Food Canada, St-Hyacinthe, Quebec, Canada; Dr J. Aalhus, Agriculture and Agri-Food Canada, Lacombe, Alberta, Canada; Dr G.H. Zhou, Nanjing Agricultural University, Nanjing, China; L. Bredahl, MAPP, Aarhus, Denmark; Prof M. Rei, Estonian Agricultural University, Tartu, Estonia; Prof E. Puolanne, University of Helsinki, Helsinki, Finland; Dr M. Bruhn, University of Kiel, Kiel, Germany; Dr P. Fortomaris, Aristotle University of Thessaloniki, Thessaloniki, Greece; Dr S. O'Reilly, University College Cork, Cork City, Ireland; Prof K. Samejima, Rakuno Gakeun University, Hokkaido, Japan; Dr S. Cho, National Livestock Research Institute, Suwon, Korea; Dr A. Alarcon-Rojo, Universidad Autónoma de Chihuahua, Chihuahua, Mexico; P. Fenwick, New Zealand Pork Industry Board, Wellington, New Zealand; Prof N. Barylko-Pikielna, Szkoła Główna Gospodarstwa Wiejskiego, Warsaw, Poland; Dr H. de Kock, University of Pretoria, Pretoria, South Africa; Dr C. Sanudo, University of Zaragoza, Zaragoza, Spain; Dr M. Magnusson, Uppsala University, Uppsala, Sweden Dr M.-T. Chen, Da-Yeh University, Chung Hwa, Taiwan; Dr S. Hillebrand, ID-Lelystad, Lelystad, The Netherlands; G. Nute, Bristol University, Bristol, United Kingdom; Dr S. Lonergan, Iowa State University, Ames, Iowa, USA; Dr R. Miller, Texas A & M University, College Station, Texas, USA; Dr L. Turubatović, Laboratorija Instituta za Higijenu i Tehnologiju Mesa, Belgrade, Yugoslavia

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FRENCH CONSUMER APPRECIATION OF A NOVEL POLISH-STYLE SAUSAGE

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Background

In Poland, product development of new meat products which are both healthy alternatives as well as novel competitors to the wide range of pork-based products already on the market is being undertaken. Poultry meat is being considered as an alternative using a new source derived from the soft separation of meat and fat from sinew, cartilage and bones, an application employed in the German poultry industry.
After removal of trade restrictions upon entry into an expanded EU, Ploand will likely look to increasing exportation of meat products into European markets. To achieve this successfully, any new meat products must meet the demands and tastes, not only of the consumers in Poland, but also of consumers in these potential new markets.
One such new product, that is a development of a typical Polish pork-based smoked sausage, is a poultry-based cheese-filled smoked sausage. Consumer acceptability of this product is currently being studied in Poland. Its acceptability in other markets which are unfamiliar to similar pork-based products is unknown.

Objectives

The objective of this study was to determine consumer acceptability of a novel Polish cheese-filled, smoked poultry sausage in France.

Materials and methods

Sausages were made in Olsztyn, Poland from a combination of turkey, goose and ostrich meats and transported to France in a chilled state. The turkey meat had been obtained using a Baader soft separator machine (Baader 1200-ST, Lubeck, Germany). The sausages that did not have any added fat, were formed with cubes of a low-fat cheddar-type cheese and smoked. For comparison, to be used for French consumers, 3 other types of sausages, of similar diameter, were bought from a French supermarket: a pork chipolata, a pork frankfurter and a poultry frankfurter with a cheese centre (containing a cheese was similar to sour cream).
A total of 72 consumers from the Clermont Ferrand region in Central France tasted the sausages (in April and May, 2003) after completing a questionnaire asking basic socio-demographic information. The consumers were told that some new sausage products were to be tasted and that two contained cheese. No information of the origin of the products, or their contents was given. The sausages were cooked on a plate grill with frequent turning and presented monadically to the consumer. The order of presentation was determined using Latin-square. Consumers were asked to score the acceptability of each product on a 12.5 cm scale from “I don’t like at all” to “I like a lot”, with a given midpoint. The consumers were encouraged to write comments.
The acceptability results were analysed by ANOVA and differences between sausage types compared using students t-test. A hierarchical cluster analysis was undertaken using the SAS CLUSTER procedure (SAS, 1996). Three clusters were evident considering the ‘distance’ between clusters and the profile of the resulting graph. A disjoint cluster analysis was carried out using the SAS FASTCLUS procedure (SAS, 1996) forcing the consumers into the 3 different clusters.

Results and discussion

Acceptability differed significantly (P<0.0001) among the products for the entire panel (Table 1). The cheese-filled smoked sausage was the least acceptable overall with an average score of 3.9 compared to the most acceptable product, the chipolata with an average score of 5.8.

Table 1. Acceptability scores of the sausages.

	Mean (maximum 10)	Standard deviation
Chipolata	5.83	2.38
Frankfurter	4.73	2.72
Cheese-filled frankfurter	4.58	2.67
Cheese-filled smoked sausage	3.90	2.92

Paired comparisons (Table 2) showed that the cheese-filled smoked sausage was less acceptable than the chipolata and the frankfurter (P<0.05), but equally as acceptable as the cheese-filled frankfurter.

Table 2. Differences in acceptability scores of the sausages.

	Pr > |t|
Chipolata vs frankfurter	0.0062
Chipolata vs cheese-filled frankfurter	0.0018
Chipolata vs cheese-filled smoked sausage	<0.0001
Frankfurter vs cheese-filled frankfurter	0.6939
Frankfurter vs cheese-filled smoked sausage	0.0360
Cheese-filled frankfurter vs cheese-filled smoked sausage	0.0876

Of the 72 consumers in total, 42 of them made 149 written comments which are summarised as negative comments, such as “horrible smoky flavour” or “too fatty”, or positive, including “interesting new idea” or “good level of fat” (Table 3). Although the sample size was relatively small and therefore extension of this work to the entire French population can only be undertaken with extreme caution, it was clear that most of the comments were negative (62%) with 34 consumers commenting negatively, and in particular, about the smoked nature (14) or the fatness (15) of the cheese-filled smoked sausages. No equivalent smoked sausage could be found in France and these comments may derive from its unfamiliarity. The sausage was made without added fat, but using a low-fat cheese which exuded a lot of oil upon cooking. The presence of the melted cheese could have contributed to the fatty perception of the sausage. Negative comments related to fatness also for the frankfurter (8) and the chipolata (10) and to a lack of taste for the cheese-filled frankfurter.

Table 3. Number of consumers giving comments about the sausages.

	Cheese-filled Smoked		Frankfurter		Chipolata		Cheese-filled Frankfurter	
Nature of comment	negative	positive	negative	positive	negative	positive	negative	positive
Consumers commenting	34	13	19	11	20	17	23	12
Fat	15	1	8	1	10	6	3	2
Taste	1	4	4	10	1	13	5	4
Texture	9		7	2	1	5	3	4
Colour	3		6	1	6	3	6	
Smoky	14		2				2	
No taste	1		2		4		9	
Smell	3		3	2		3	2	3
Salt	5		2	1	3	1	1	
Artificial	1		4		3		3	
Novel		8						3
Appearance	1			1	1	3	2	2
After-taste/acid	5							
Traditional/rustic		3				1		

The next step was to try to determine whether or not differences between the acceptability of products could be related to different consumers. For this, a cluster analysis was undertaken on the acceptability scores and three approximately equal sized clusters were retained (Table 4).

Table 4. Mean acceptability scores of the sausages (maximum 10) for each cluster with the significant differences within a cluster represented by different letters (P<0.05).

	Cheese-filled Smoked	Frankfurter	Chipolata	Cheese-filled Frankfurter
Cluster 1	5.8 [b]	5.8 [b]	7.4 [a]	6.1 [b]
Cluster 2	2.2 [b]	2.0 [b]	5.7 [a]	2.0 [b]
Cluster 3	2.9 [b]	5.6 [a]	3.5 [b]	4.8 [a]

Comparing acceptability scores within each cluster of consumers (Table 4), it can be concluded that:

- Cluster 1: 26 consumers (36%) who prefer chipolata, but like all four products
- Cluster 2: 23 consumers (32%) who prefer only the chipolata, (they do not like the other products)
- Cluster 3: 23 consumers (32%) who prefer the frankfurter products (both with and without cheese)

The socio-demographic questionnaire and responses are given in Table 5. The ages of the consumers were well distributed among the age group and 44% of the consumers were men. A large proportion of the consumers were in the lower income groups (<2300 Euros/month) and 63% were married.
To identify characteristics from the socio-demographic profiles relating to consumer acceptability, a chi-square test was applied to each response within each cluster of consumers. The results (degrees of freedom and probability) are given in Table 5 and show that the only socio-demographic characteristic significantly (P<0.025) linked with the consumer clusters was that of 'shopping for meat in the supermarket'. Cluster 1 consumers, who liked all the products, and in particular, the chipolata, did not shop for meat in the supermarket whereas a quarter of the consumers in the other two clusters did shop in supermarkets (Table 6). This result is in contrast to the traditional view that the novelty seekers buy their "trendy" foods at specialist stores or large supermarkets. Instead, the traditionalists who buy their meat at butchers are those that most liked the new smoked cheesy sausages.

Table 6. Relationship between shopping for meat in the supermarket and the clusters of sausage acceptability. Significant differences compared to the entire sample are shown in bold (P<0.05).

	Shop for meat in the supermarket	
	Yes	No
Cluster 1	**0**	100
Cluster 2	26	74
Cluster 3	23	77
Total	16	84

Conclusions

While the sample size was relatively small and therefore extension of this work to the entire French population can only be undertaken with extreme caution, this project has shown that the French consumers studied did not find the cheese–filled smoked sausage acceptable, in particular the smoky and fatty nature of the sausage were disliked. The poultry-based smoked sausage is similar to pork-based smoked sausages familiar to the Polish consumer. However, the added cheese is a novel concept in the Polish market and therefore the acceptability of these products is also being investigated in Poland.

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Table 5. Some socio-demographic information obtained from the consumers

Question	Response options	Consumers number	Consumers %	Degrees of freedom	P
What is your age (years)?	16-24	11	15	6	0.562
	25-34	15	21		
	35-44	18	25		
	45-54	17	24		
	>54	11	15		
Gender?	female	39	54	2	0.747
	male	32	44		
Marital status?	Single/widowed	27	38	2	0.214
	married/cohabitating	45	63		
How many people live in your household?	1	15	21	10	0.478
	2	21	29		
	3	11	15		
	4	16	22		
	5+	5	7		
What is the total monthly income of your household? (Euros)	< 1500	17	37	10	0.363
	1500-2299	21	22		
	2300-2999	12	18		
	3000-4499	16	8		
	>4500	2	9		
Are you the member of you household who normally shops for meat?	no	13	18	2	0.215
	yes	56	78		
Where do you normally purchase your meat?	butcher	18	25	2	0.324
	supermarket	59	82	2	**0.025**
	farmer	9	13	2	0.582
How often do you eat meat?	every meal, everyday	7	10	6	0.209
	once a day, everyday	25	35		
	several x/week	38	53		
	1x/week	0	0		
	<1x/week	1	1		
	never	0	0		
How often do you eat small-goods products?	every meal, everyday	0	0	8	0.247
	once a day, everyday	2	3		
	several x/week	38	53		
	1x/week	21	29		
	<1x/week	9	13		
	never	2	3		
List some new foods you have recently eaten. (number of products given)	0	8	11	12	0.131
	1	15	21		
	2	18	25		
	3	11	15		
	4	11	15		
	5	8	11		
	6	1	1		
List some foreign foods you have recently eaten. (number of foods listed)	0	9	13	14	0.380
	1	7	10		
	2	13	18		
	3	20	28		
	4	14	19		
	5	7	10		
	6	1	1		
	7	1	1		

MONITORING ACCELERATION (G-FORCE) DURING ANIMAL TRANSPORTS

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Background

Transport to the abattoir may stress animals (Bradshaw et al. 1996) and affect meat quality (Lehenska et al. 2002). One potential stressor is the motion of the truck. Vibration leads to increased heart rate (Perremans et al. 1998), increased plasma levels of cortisol (Perremans et al. 1995; Perremans et al. 2001), beta-endorphin (Perremans et al. 1995), and adrenocorticotropic hormone (Perremans et al. 2001). Vibration is also believed to be the reason why pigs develop motion sickness with vomiting (Randall and Bradshaw, 1998), whereas acceleration can cause animals to loose balance and receive injuries. The physical and physiological reactions of the animals during transport have a strong impact on the energy consumption within individual muscles, which in turn has strong implications for the quality of the final meat product (Rosenvold & Andersen, 2003).

Objectives

It is the objective to develop a monitoring system of G-force / vibration on animal transports fully integrating data on vehicle speed, road type, and balance of the animals on the truck. The system should be able to save data for evaluation and documentation. Also the system should be prepared for future modifications allowing the system to provide on-line alarms to the driver when certain pre-set G-force values are exceeded.

Materials and methods

Based on LabView software from National Instruments Corporation a programme ('G-force Viewer') was developed. 'G-force Viewer' integrates the information into a screen display that simultaneously shows data for G-force ('G-logger'), vehicle speed (GPS), animal balance (video camera), road type (video camera) and altitude ('G-logger').

A tri-axis G-force logger ('G-logger') was developed. The 'G-logger' constantly measures G-forces in all three axis and calculates the resultant G-force. It also measures barometric pressure that can be converted into altitude. The unit can store data up to 200 times per second.

Two units of the 'G-logger' were tested on a real pig transport on a fully air suspended three-axel truck (photo). The 'G-loggers' were set to log 100 times per second (100Hz). Each logger was placed on the rubber-coated floor and a 15 mm thick polyethylene foam was placed on top of the logger before a metal protective casing was firmly pressed down over it. The casing was fastened by four screws through the rubber layer and into the underlying aluminium floor. In this way it was believed that the 'G-logger' would fairly realistically record the vibrations / G-forces that the pigs are subjected to through their contact with the rubber floor. One 'G-logger' was mounted in the rear pen. This logger was placed 94 cm from the rear end; 151 cm aft of the rearmost axel and 274 cm aft of the central pivotal point of vertical movements. The central pivotal point was estimated to be half way from the fore axle to the centre point between the two rear axles (see photo). Another 'G-logger' was mounted in the pen located directly over the central pivotal point.

A video camera (Sony DCR-TRV10E) equipped with a wide-angle lens was mounted over the pigs in the rear pen in which the floor area per 100kg pig was 0.42 m^2. Another camera was mounted in the driver's cabin filming out the windscreen. A Global Positioning System receiver (Garmin eTrex Summit GPS) was used to record vehicle speed and location.

The test drive was intentionally made on secondary roads to get a picture of the worst scenario for transport conditions in Denmark. With very few exceptions the roads were paved.

Results and discussion

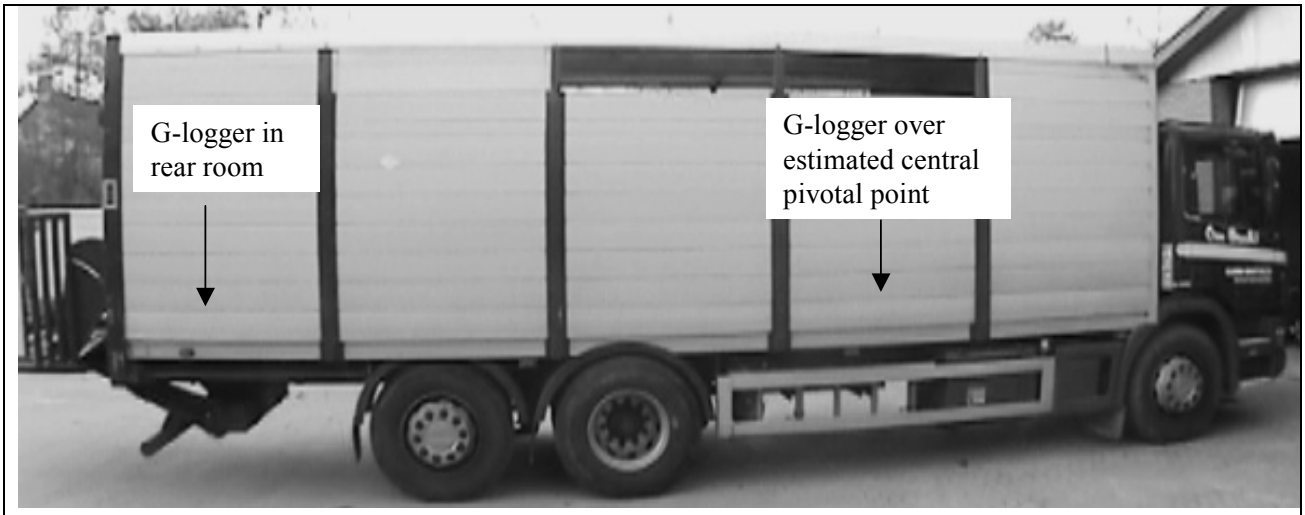

Photo. Truck used for the test of 'G-loggers'. Scania P94 6x2, OptiCruise automatic gear. Fully air suspended. The truck was loaded with approximately 5 tons of pigs.

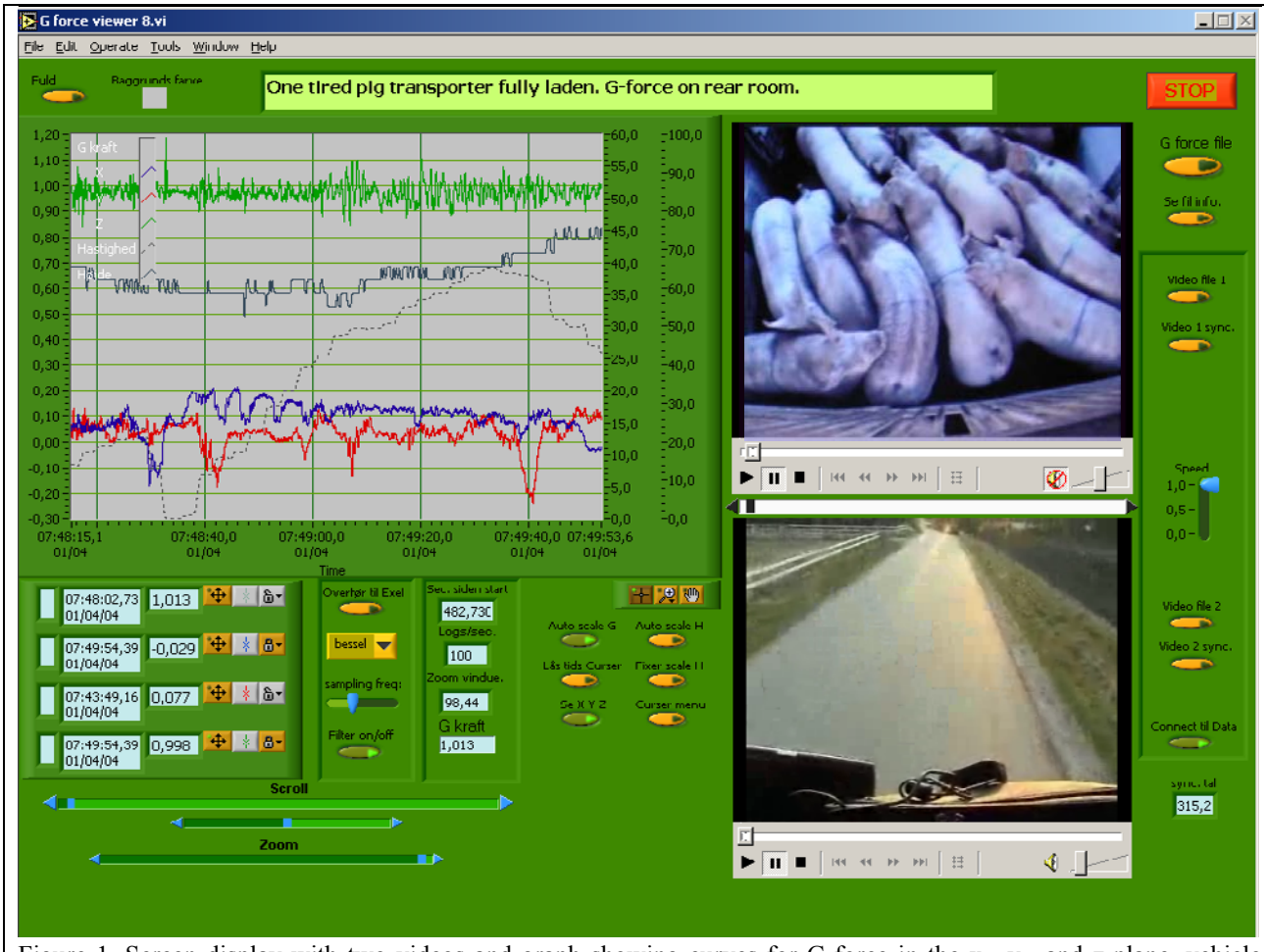

Figure 1. Screen display with two videos and graph showing curves for G-force in the x-, y-, and z-plane, vehicle speed, and altitude. The graph for the resultant force has been omitted for clarity. Data from rear pen. 1st order Bessel low pass filtered.

G-force measurements. Figure one is a screen display from the 'G-force Viewer' showing 1 minute:38 seconds of the test drive. Figure two shows another screen display from the same part of the test drive. Most imbalances of pigs seem to be caused by roundabouts as these caused repetitive shifts in direction of G-forces in two planes i.e. 0.4G laterally due to turns, and 0.3G for-aft due to acceleration and de-acceleration. When travelling at 70 km/h and maximum brake was applied - triggering the ABS anti-blockage system - the

G-force in the fore-aft direction (**X**) was 0.6g. With a loading density of 100 kg per 0.42m^2 this braking forced pigs forward in the pen and the ones not leaning against anything lost their balance - this happened again when the brake was released or when the truck came to a complete halt. Other loading densities would obviously have given different results. G-force in the vertical plane had negligible effects on balance.

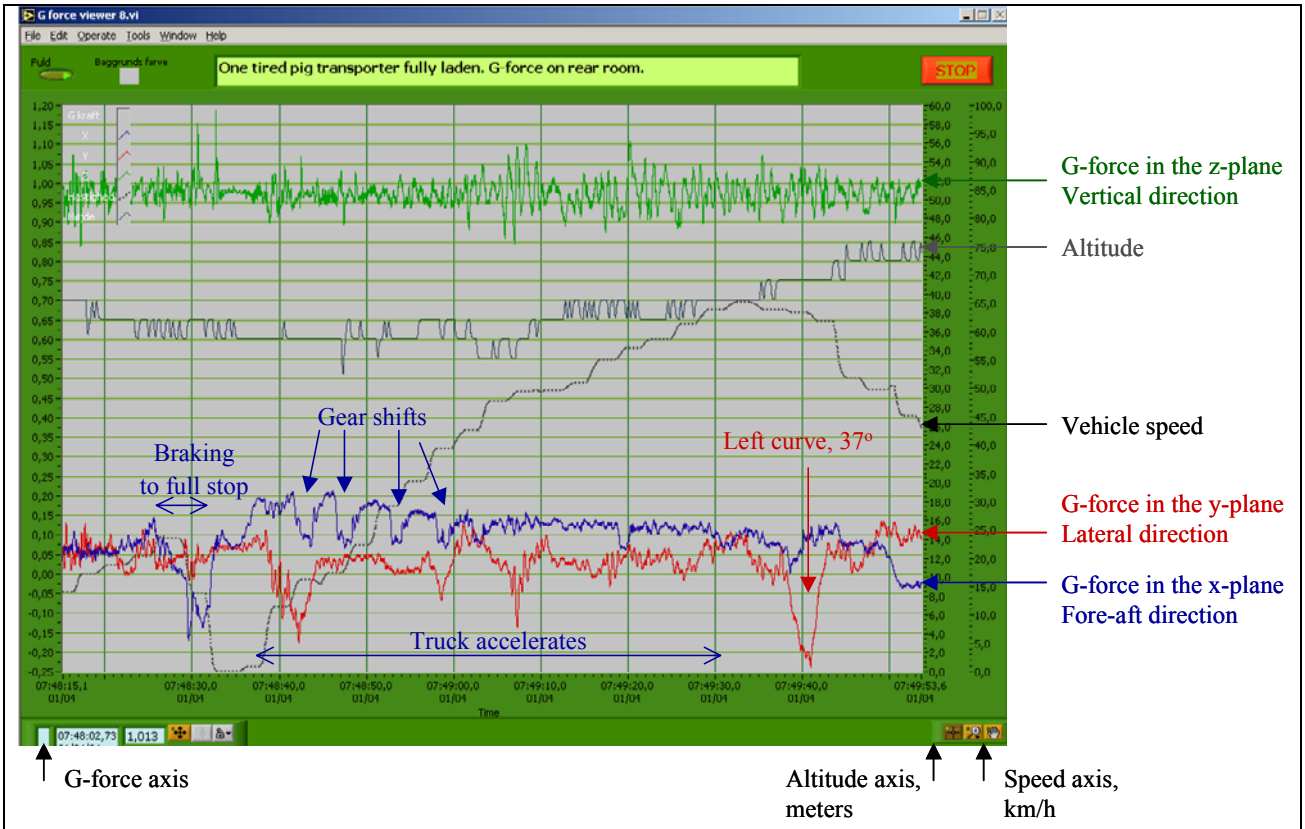

Figure 2. Screen display with only graphs. Data from rear pen. 1st order Bessel low pass filtered. The graph for the resultant force has been omitted for clarity.

Vibration measurements. The raw unfiltered G-force data (figure 3) reveals that in the vertical plane G-forces of ultra short duration (<3/100 second) of up to 3G occasionally occurred. However, this also happened occasionally when the fully laden truck was stationary leading to the conclusion that it was caused by the movements of the pigs. Probably when a pig stepped on the 'G-logger'. Ultra short peaks will probably have only minor effects on the pigs.

At a vehicle speed of 60 km/h the prevailing vibration frequency was 25-33 Hz with an amplitude of 0.05G in the lateral direction (**Y**); 25-33 Hz with an amplitude of 0.1G in the fore-aft direction (**X**); and 20-25 Hz with an amplitude of 0.2-0.4G in the vertical direction (**Z**). With reference to human vibration studies (Griffin, 1990; BSI, 1987), Randall et al (1995) speculated that pigs would feel less discomfort at these frequencies than at lower frequencies. With piglets Perremans et al (2001) found that - with regard to short durations (less than one hour) of vertical vibration - higher frequencies (18Hz) are less stressful than lower frequencies (2-8Hz). Since the prevailing vibration recorded in the current test was predominately of sinusoidal nature, the vertical amplitude of 0.2-0.4G can be estimated to correspond to 1.4-2.8 m/s^2 RMS (Griffin, 1990 p 468) and thus well below the maximum RMS value of 3 m/s^2 recommended by Perremans et al (1998). The effect on pigs from lateral vibration is unknown. With exception of the fore-aft direction (X) the vibration during driving conditions on the fully laden truck resembled the vibrations recorded on the stationary empty truck with the engine running. Thus the vibrations are caused by the engine. In the X-direction very little vibration occurred on the stationary empty truck with the engine running.

Conclusions

The 'G-logger' and the 'G-force viewer' provide easy to use tools for measurements of G-force and vibration on pig transporters. With minor modifications it can be used as an on-line alarm system educating the driver.

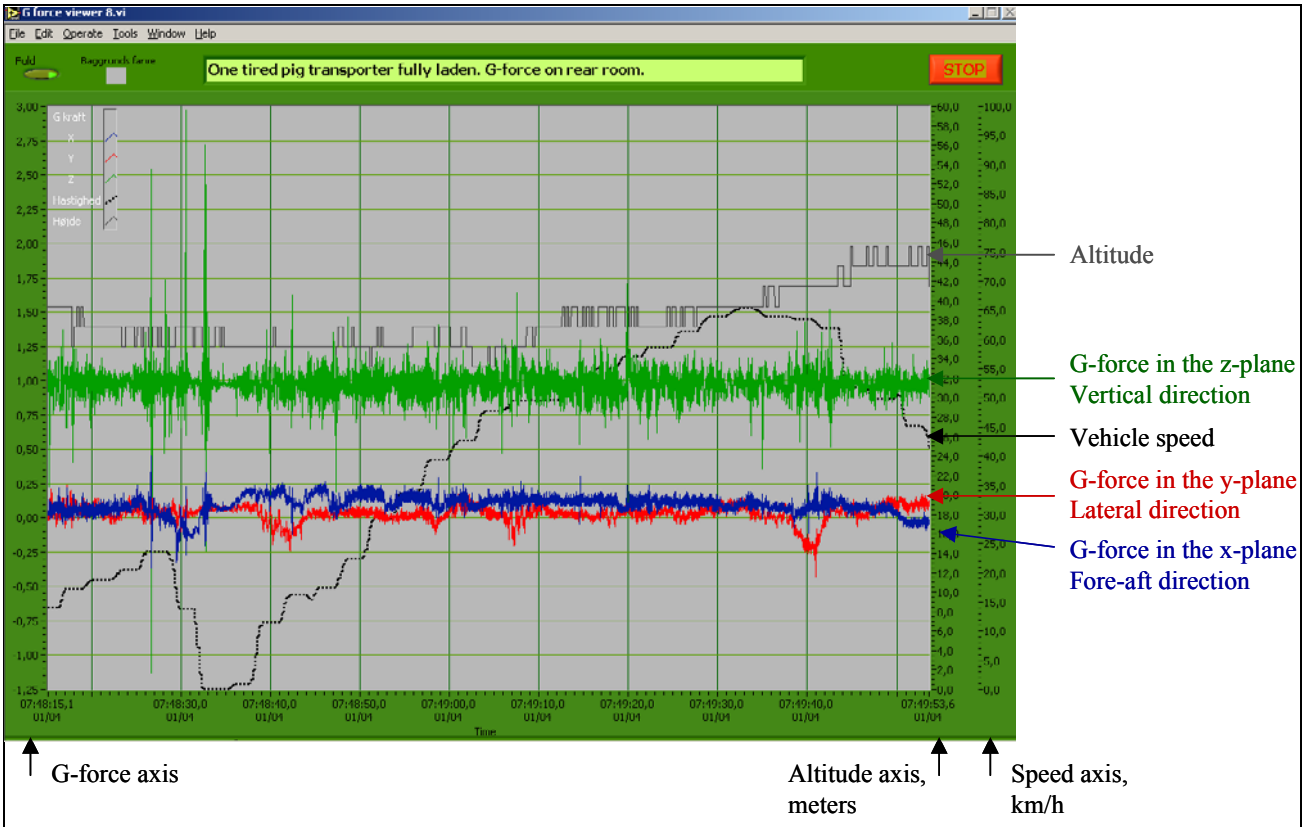

Figure 3. Screen display with only graphs. Data from rear pen. Raw data without filter. The graph for the resultant force has been omitted for clarity.

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EFFECT OF LINSEED AND GRASS FEEDING ON THE FLAVOUR PROFILE AND TASTE-PANEL EVALUATIONS OF BEEF FROM BELGIAN BLUE DOUBLE-MUSCLED BULLS

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Background

Nowadays, a lot of research is performed aiming at increasing the n-3 polyunsaturated fatty acid content (PUFA) of meat to better meet human nutritional guidelines with respect to the P/S and n-6/n-3 ratios. Increasing the n-3 PUFA content of beef can be achieved by feeding fish oil/algae, linseed (oil) and/or grass or grass silage to cattle (see review Raes et al., 2004). However, it has been demonstrated that increasing the n-3 PUFA content may affect meat flavour. This effect seems to depend on the n-3 PUFA level in the meat and the feed source. Beef from grass-fed animals may elicit a typical grass-like flavour (Melton, 1990). Both C20:5n-3 and C22:6n-3 may cause an undesirable fishy flavour above certain levels, which is observed by consumers after heating the meat (Mottram, 1998). Also the heating method affects flavour profiles, however, most research on beef flavour has been done after moist cooking, and few references are available on flavour profiles of roasted or grilled beef (Elmore et al., 2004; Raes et al., 2003a).

Objectives

The aim of this study was to evaluate the flavour profile of meat from bulls fed a C18:3n-3 rich diet using linseed and/or grass or grass silage. The flavour profile as chemical components was measured by gas chromatography – mass spectrometry. Flavour was also evaluated by a semi-trained taste-panel.

Materials and methods

Experimental design

Thirty-one Belgian Blue double-muscled young bulls (mean (sd) live weight at start 339 (44.4) kg) were fed different feeds aiming at increasing the n-3 intramuscular fatty acid content (as fully described by Raes et al., 2003b), and studying the effects on oxidative stability and flavour of the meat. The animals were divided in 4 groups receiving different diets during three subsequent phases (last growing phase, prefattening phase and fattening phase). The diets differed according to the main fat source in the concentrate as well as in the type of roughage, as shown in Table 1. To increase the n-3 fatty acid content in the muscle tissue, linseed was added as the main C18:3n-3 source in the concentrates, and grass or grass silage as a C18:3n-3 roughage source. Animals were slaughtered at a mean (sd) live weight of 681 (26.3) kg. After cooling the carcasses for 24 h, the *longissimus thoracis* (LT) was sampled for flavour profile analysis, while the LT, *semitendinosus* (ST) and *triceps brachii* (TB) were sampled for taste-panel evaluations. The meat samples were vacuum packed and aged for 14 days at 2°C. After ageing, the samples were stored at –18°C until analysis.

Analyses

Taste-panel evaluations

Sensory characteristics ie., tenderness, juiciness, flavour intensity and flavour preference, were evaluated by a trained taste panel of ten members (22-55 years of age). In the first series of sessions, tenderness and juiciness were evaluated, while in the subsequent series of sessions flavour intensity and flavour preference were scored. All evaluations were performed for all three muscles with the exception that flavour preference of LT muscle was not ranked. Two servings were provided at a session. Each serving included one sample (from the same muscle) of each of the 4 feeding groups. As in Belgium, grilling is a very common heating method for beef steaks**,** samples (3x3x2 cm) were grilled for 2 minutes on a double-contact grill and served on pre-heated plates for sensory evaluation. The panellists were asked to assess tenderness and juiciness on

an 8-point scale. The values of 1 and 8 correspond with extremely tender or juicy and extremely tough or dry, respectively. Flavour intensity and flavour preference were evaluated using a ranking test. The values of 1 and 4 correspond to the lowest and the highest flavour intensity or flavour preference, respectively.

Flavour analysis

For each group, 3 samples of the LT were grilled and extracted using the Likens-Nickerson extraction. The aroma compounds were collected and analysed by gas chromatography-mass spectrometry (GC-MS) as described by Raes et al. (2003a). Semi-quantitative data of the flavour compounds were obtained by relating the peak intensities to the intensity of nonane, added to the dichloromethane phase as an internal standard.

Table 1. Composition of the diets for the different feeding phases depending on the feeding group

	Group C_1 (n = 7)	Group GC_2 (n = 8)	Group GC_3 (n = 8)	Group MC_4 (n = 8)
Last growing phase (70 d)				
Concentrate	----------	+ linseed	----------	– linseed
Roughage		Fresh grass		Maize silage
Prefattening phase (56-98 d)				
Concentrate	-------	+ linseed	-------	+ linseed	– linseed
Roughage		Whole triticale silage		Grass silage	Maize silage
Fattening phase (42-190 d)				
Concentrate	– linseed	+ linseed	+ linseed	+ linseed
Roughage	–	Grass silage	Grass silage	Maize silage
Concentrate/roughage ratio (on DM basis)	100/0	80/20	70/30	80/20

Statistical analysis

Data were subjected to analysis of variance (ANOVA), using Duncan as post-hoc test (SPSS 9.0). Principal component analysis (PCA) was carried out using data of the flavour profile analysis.

Results and discussion

In Figure 1, a PCA bi-plot of the flavour volatiles of the LT muscle and the average feeding group scores are shown. Group MC_4 is situated in an area where little volatiles were found, while group GC_2 show a strong association with derivatives of pyrrol and furan. Group C_1 and group GC_3 are situated in the areas characterised by pyrazines, branched and aromatic aldehydes, saturated aldehydes as well as sulphur containing compounds. In Table 2, the semi-quantitative analysis of the volatile categories is given per feeding treatment. Significant differences between feeding groups were only observed in pyrrol and furane derivatives, while no differences were found for the other volatiles. In grilled products, the pyrazines are important flavour compounds formed by Maillard reaction and Strecker degradation. No differences were observed between the feeding groups in the amount of these volatiles, which is in agreement with Elmore et al. (1999) who reported that the dietary fat source had no effect on the production of pyrazines. Overall, the amount of volatiles formed during heating of Belgian Blue beef is small, and lower compared to that originating from cattle with a higher degree of fat and/or different feeding regime (Raes et al., 2003a).

As it is known that flavour evaluations by a taste-panel can be influenced by differences in tenderness and juiciness, both these parameters were evaluated by the taste-panel. No differences in tenderness could be observed between the feeding groups (Table 3). For juiciness, however, the taste-panel evaluated the LT and TB samples from the groups GC_2 and GC_3 significantly juicier than those from the groups C_1 and MC_4. The analytical flavour profile corresponds to the results observed by the taste-panel (Table 3). Indeed, the taste-panel appointed the lowest flavour intensity to meat from animals of group MC_4, which is in agreement with the flavour profiling (Figure 1), the differences between the feeding groups being small, however.

A non-significantly higher amount of saturated aldehydes was found in the group MC_4 compared to the other groups, due to higher amounts of hexanal ($P = 0.076$) and 2,4-decadienal ($P = 0.128$). Both these volatiles originate from oxidation of n-6 PUFA. A significantly higher amount of 1-octen-3-ol ($P = 0.010$), formed during oxidation of C18:2n-6, was found in group MC_4 compared to the other groups. This corresponds with

the fatty acid analysis of the beef, where MC_4 showed a higher n-6 PUFA content than GC_2 and GC_3 (Raes et al., 2003b). Similarly, Elmore et al. (2004) found higher concentrations of hexanal and 1-octen-3-ol for concentrate fed animals compared to silage fed animals.

Concerning flavour preference, the taste-panel had the lowest appreciation for the steaks from group C_1 (Table 3). Thus, the flavour may have been positively affected by the linseed in the finishing diet.

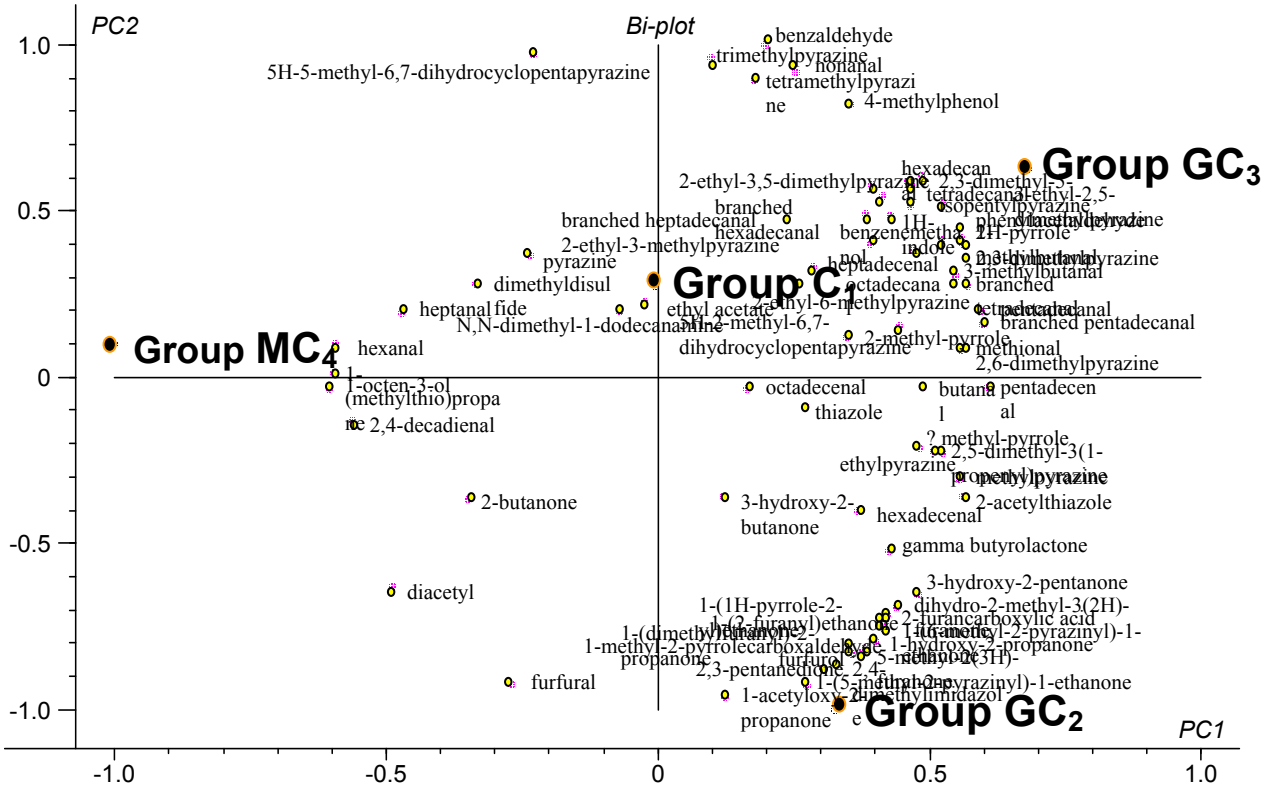

Figure 1. PCA bi-plot of the GC-MS flavour profile analysis of *longissimus thoracis* samples of the different feeding groups

Table 2. Semi-quantitative analysis of the volatile classes (µg/kg meat) for the different feeding groups[1]

	Group C_1	Group GC_2	Group GC_3	Group MC_4	P
saturated aldehydes	46	42	45	55	0.303
branched and aromatic aldehydes	420	375	443	322	0.069
higher saturated aldehydes	14241	11956	14705	9832	0.146
higher unsaturated aldehydes	4431	4269	3953	3283	0.164
alcohols	13	13	16	23	0.199
ketons	1136	1109	885	868	0.579
pyrrol derivatives	89^{ab}	122^{a}	90^{ab}	56^{b}	0.018
pyrazines	418	422	485	403	0.273
furan derivatives	150^{a}	184^{b}	153^{a}	150^{a}	0.002
thiazol derivatives	19	21	21	18	0.598
other nitrogenous compounds	54	66	72	66	0.090
sulphur containing compounds	70	67	70	55	0.385
esters	27	31	25	25	0.171

[1] Mean values of three observations
[a,b] Means with a different superscript are significantly different ($P < 0.05$)

Table 3. Mean values (n = 7) for tenderness[1], juiciness[1], flavour intensity[2] and flavour preference[2] scored by a semi-trained taste-panel from samples of the different feeding groups

	Group C1	Group GC2	Group GC3	Group MC4	P
Tenderness					
LT	3.2	2.9	2.8	3.2	0.338
ST	4.6	4.1	4.4	4.2	0.490
TB	3.9	3.6	3.6	3.7	0.625
Juiciness					
LT	4.0^{ab}	3.6^{ab}	3.5^{a}	4.2^{b}	0.025
ST	4.2	4.2	4.1	4.2	0.970
TB	4.0^{a}	3.2^{b}	3.6^{ab}	3.9^{a}	0.011
Flavour intensity					
LT	2.4	2.5	2.8	2.2	0.121
ST	2.7^{a}	2.8^{a}	2.5^{a}	2.0^{b}	0.002
TB	2.5	2.7	2.6	2.2	0.236
Flavour preference					
ST	2.2	2.7	2.5	2.6	0.365
TB	2.0^{a}	2.8^{b}	2.5^{b}	2.7^{a}	0.003

[a,b] Means with a different superscript are significantly different ($P < 0.05$)
[1] Evaluation on a 1 to 8 scale where 1 = extremely tender or juicy, and 8 = extremely tough or dry
[2] Evaluation using a 1 to 4 ranking test where 1 = lowest intensity or preference, and 4 = highest intensity or preference

Conclusions

Relatively small differences in the flavour profile were detected in grilled meat from Belgian Blue double-muscled young bulls fed rations differing in the presence of linseed in the concentrate and the use of grass or grass silage as roughage. However, the semi-trained taste-panel was able to observe flavour differences between the feeding groups.

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Acknowledgements

This research was financially supported by the Ministry of Small Enterprises, Traders and Agriculture and the EU-community (project QLRT-2000-31423, 'Healthy Beef'). INVE Technologies (Baasrode, Belgium) are thanked for their advice in feed formulations and for supplying the n-3 premix.

EFFECT OF LAMB FEEDING WITH LINSEED MEAL ON SOME MEAT QUALITY TRAITS

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Background

The EC Regulation n. 1829/2003 sets at 0.9% the limit of genetically modified organisms (GMO) allowed in feeds for animals without any mention in the label. Such a limit dramatically stresses the need to identify raw materials free from genetically manipulated substances. Nowadays, about 63% of the soy cultivated all over the world is genetically modified, thus representing a serious problem for animal feeding since soybean is still considered as the main protein source. The possibility of using some traditional oilseeds can be a valid alternative to soy in order to obtain quality performances. Contrary of other seeds, normally rich in linoleic acid (LA), linseed is particularly rich in α-linolenic acid (approximately 350 g fat/kg fresh material, of which LNA accounts for about 50% of the total fatty acids). Omega-3 fatty acids, that may be only partially transferred to ruminant fat (Dufrasne *et al.*, 1991; Enser *et al.*, 1999; Choi *et al.*, 2000; Scollan *et al.*, 2001; Raes *et al.*, 2002; Raes *et al.*, 2004), are very important in the human diet, since they reduce the risk of cardiovascular diseases with their anti-thombogenic and anti-atherogenic effect. They inhibit tromboxane A2 synthesis, starting from the arachidonic acid in platelets, and the migration of monocytes in the atherosclerotic plaque (Condor, 1997). Several authors have used linseed meal in ruminant nutrition, especially for cattle, with positive results on digestibility (Khorasani *et al.*, 1994; Dufrasne *et al.*, 1991, Dixon *et al.*, 2003a; Dixon *et al.*, 2003b), productive performances (Dufrasne *et al.*, 1991; Berge *et al.*, 1993; Dumont *et al.*, 1997; Dixon *et al.*, 2003a; Dixon *et al.*, 2003b; Raes *et al.*, 2004) and meat quality traits (Dufrasne *et al.*, 1991; Berge *et al.*, 1993; Enser *et al.*, 1999; Choi *et al.*, 2000; Raes *et al.*, 2002; Scollan *et al.*, 2001; Raes *et al.*, 2004). There are, however, few reports in literature concerning the use of linseed meal in lamb production.

Objectives

The present study aimed to investigate some of the most important meat quality traits in lambs fed a linseed meal in comparison with a traditional diet based on soybean meal.

Materials and methods

The experiment was carried out on samples of *Longissimus lumborum* (Ll) and *Semimembranosus* (Sm) muscles isolated from Comisana male lambs (n. = 14). Lambs were fed *ad libitum* for 6 weeks from the age of 50 days on either a concentrate pelleted diet (Control group) or on a diet containing 20% linseed meal (LM). Diets were planned to contain approximately the same amount on dry matter of protein (16.5%), fat (5.0%) and crude fibre (10.5%). Following 24 hours of refrigeration at 4 °C, the lumbar region and the pelvic limb were excised from the right half carcass and dissected into their tissue components, i.e. lean, fat and bone. Ll and Sm muscles were assessed for pH using a glass electrode at slaughter (pH_1) and after 24 hours refrigeration at 4 °C (pH_2). Samples of both muscles were evaluated for colour and tenderness, while chemical analysis (ASPA, 1996) and the fatty acid profile were performed only on Ll samples. Lipids were extracted according to the 2:1 chloroform-methanol method described by Folch *et al.* (1957), whereas the acidic profile was assessed using a Chromopack CP 9000 gas chromatograph. Meat colour was estimated by the Hunter Lab system using a colorimeter (illuminant D 65), which measures the values of Lightness (L), Redness (a) and Yellowness (b) by making 5 readings for each meat sample, approximately 2.5 cm thick. Tenderness was measured using a Warner Bratzler shear device applied to an Instron 5544 and expressed as the shear force (kg/cm^2) required to cut perpendicularly to the direction of the fibres half an inch diameter cylinders of raw meat, taking three measurements for each muscle per subject. The atherogenicity and thrombogenicity indexes were calculated accordingly to Ulbricht and Southgate (1991). The PCL/PCE (plasma cholesterol lowering/plasma cholesterol elevating) ratio was also determined (Reiser and Shorland, 1990). Data were analysed for variance using the GLM procedure of SAS (1999/2000).

Results and discussion

By the end of the trial, the lambs had achieved similar live weights in both groups (23.67 *vs* 23.59 kg, respectively for the LM and control group), thus proving the productive efficiency of the linseed meal diet. As for pH values, no significant differences between groups were detected for Ll and Sm muscles at slaughter, nor after refrigeration (Figure 1). Dissecting data of the lumbar region and pelvic limb were not markedly different, although a higher incidence of lean was observed in the experimental group (Figure 2). The Sm meat colour parameters were quite similar between the two groups, while the yellowness of the Ll muscle was significantly lower ($P<0.01$) in the linseed meal diet (Figure 3). In both muscles, tenderness was slightly improved by LM feeding (Table 1). With regard to the chemical composition of meat, no statistical differences were assessed (Table 2). However, a slight increase of the protein content was found in the experimental group, corresponding to the greater amount of lean found at the dissection in the same group, in accordance with the results of Berge *et al.* (1993). Meat from lambs fed on the LM diet showed better dietetic properties, as evidenced by a markedly higher amount of unsaturated fatty acids and a lower one of saturated (Table 3), along with a lower percentage of C12:0 and C14:0 ($P<0.05$). As a consequence, lower indexes of atherogenicity and thrombogenicity and a higher PCL/PCE ratio were found in meat of the LM group ($P<0.05$). No significant differences were found in meat PUFA content of either group concerning the ω6 or the ω3 fractions. However, the LM diet significantly improved the ω6/ω3 ratio, as well as the UFA/SFA ($P<0.05$).

Conclusions

The results obtained in this research suggest that without altering the productive efficiency of lambs, the use of a linseed meal positively affected meat healthiness and dietetic properties, since a lower concentration of C12:0 and C14:0 fatty acids was found, corresponding to an increase in the total unsaturated fatty acids as well as the ratio of unsaturated to saturated fatty acids. Furthermore, this diet improved the atherogenicity and thrombogenicity indexes as well as the PCL/PCE ratio, with positive effects for human health. Finally, meat lean content and its tenderness were also better following linseed meal feeding.

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Acknowledgements

The research was supported by Project ALBIO (D.M. MiPAF n° 441/7303/01 - 28/11/2001). The Authors are grateful to Mr. Massimo Lacitignola for his technical collaboration.

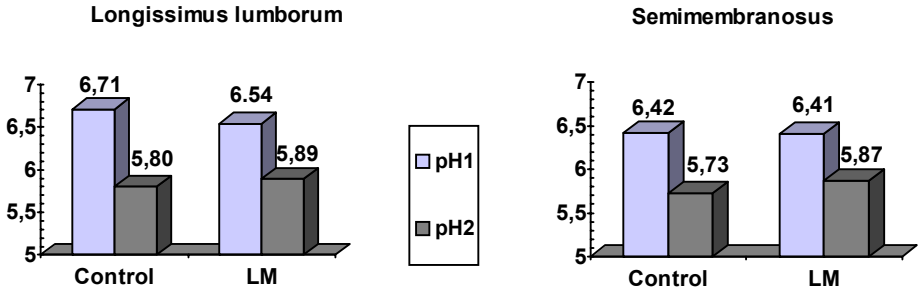

Figure 1. pH measurement in *Longissimus lumborum* and *Semimembranosus* muscles

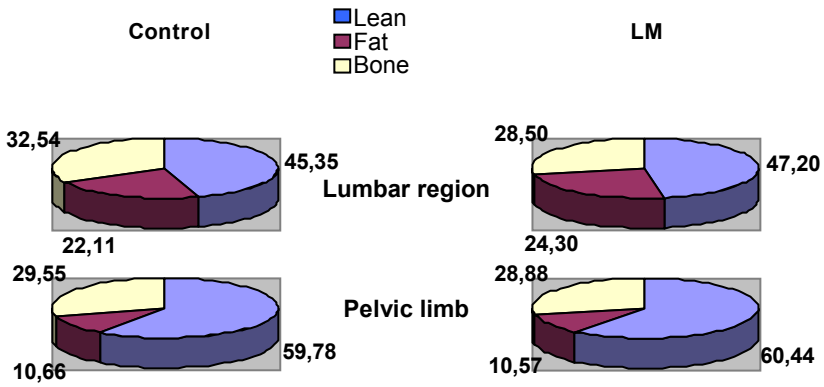

Figure 2. Dissecting data (%)

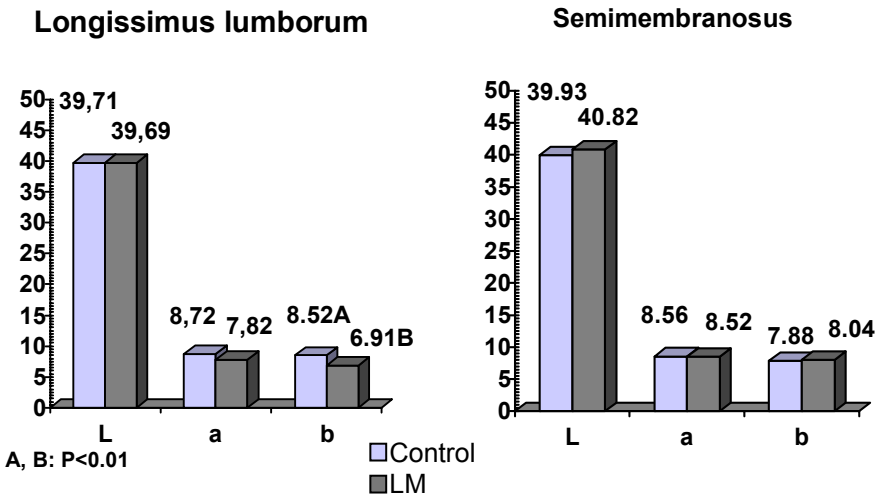

Figure 3. Meat colour of Longissimus lumborum and Semimembranosus muscles

Table 1. Meat tenderness of *Longissimus lumborum* and *Semimembranosus* muscles

	Control	LM	SED
Samples (n.)	7	7	(DF = 12)
WBS Ll (kg/cm^2)	5.38	4.14	1.896
WBS Sm (kg/cm^2)	5.18	4.74	2.192

Table 2. Chemical composition of Longissimus lumborum muscle (%)

	Control	LM	SED
Samples (n.)	7	7	(DF = 12)
Moisture	75.50	75.68	0.719
Protein	18.60	18.83	0.398
Fat	3.93	3.73	0.694
Ash	1.31	1.18	0.222
N-free extract	0.45	0.60	0.282

Table 3. Fatty acid composition of Longissimus lumborum muscle (%)

	Control	LM	SED
Samples (n.)	7	7	(DF = 12)
C12:0	0.63^a	0.40^b	0.199
C14:0	5.58^a	4.10^b	1.111
C16:0	24.13	22.33	1.937
C18:0	15.41	17.38	2.368
Total Saturated	48.91^a	46.90^b	1.658
Total Monounsaturated	42.60	44.60	2.069
Total Polyunsaturated	8.46	8.54	1.788
Total Unsaturated	51.06^b	53.14^a	1.647
ω6	7.67	7.51	1.649
ω3	0.78	1.03	0.329
ω6/ω3	10.08	8.49	3.354
Unsaturated/Saturated	1.05^b	1.13^a	0.072
Atherogenicity index	0.92^a	0.74^b	0.125
Thrombogenicity index	1.64^a	1.50^b	0.113
PCL/PCE	0.99^b	1.16^a	0.145
Polyunsaturated/Saturated	0.17	0.18	0.039

a, b: P<0.05

QUALITY OF MEAT FROM STEERS OF TWO DIFFERENT FRAME SIZES GRAZING HIGH QUALITY PASTURES SUPPLEMENTED WITH HIGH MOISTURE MAIZE GRAIN OR WHOLE PLANT MAIZE SILAGE

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Background

Beef produced from grazing cattle results in higher levels of unsaturated fatty acids (Miller, *et al.,* 1987 and García and Casal, 1992), lower n-6:n-3 fatty acids (Enser, *et al.* 2001) and higher levels of conjugated linoleic acid (CLA) in body fat (French, *et al.* 2000) as opposed to meat from animals fattened in high concentrate diets. However, short term supplementation with maize grain or soybean harvest by-products in the finishing stage did not affect fatty acid profile (Grigera Naón, *et al.*, 2000 and Grigera Naón, *et al.*, 2003).; longer supplementation periods may be necessary to overcome seasonal grass production , thus the widespread use of other supplements such as high moisture maize grain and maize silage (Abdelhadi, 2000), which in turn may affect meat quality. Frame size of animals can have decisive bearings on the length of the fattening stage (Di Marco, 1998) and on some aspects of quality such as tenderness and cooking loss (Muir *et al.*, 1998 and Camfield *et al.* 1999).

Objectives

The objective was to evaluate the effect of type of energy fall-winter supplementation with high moisture maize grain or whole plant maize silage on meat qualitiy of Aberdeen Angus steers with contrasting mature body weight.

Materials and methods

Over 177 days, covering autumn and winter, 32 male calves of six months of age, grazing pastures were assigned to the following treatments: LM, animals of low (L) mature body weight supplemented with high moisture maize grain (M); LS, animals of low (L) mature body weight supplemented with whole plant maize silage (S); HM , high (H) mature body weight animals supplemented with high moisture maize grain (M) and HS, high (H) mature body weight animals supplemented with whole plant maize silage (S). Individuals were weighed every 21 days and fat depth between ribs 12th and 13th was measured by ultrasound using a 3.5 MHz trasductor. At the end of the experiment, eight animals from each treatment were slaughtered. Colour was assessed on the *Longissimus* muscle exposed between the 12th and 13th rib, blooming time was 60 minutes (Wulf and Wise, 1999), readings were taken in L* a* b* colour space, using a Minolta CR-300 (Minolta Co. Ltd., Japan) colorimeter. A Testo 230 pH-meter with a puncture type combination electrode (Testo GmbH & Co., Germany) was used to measure muscle ultimate pH (pHu). Fatty acids were extracted according to Folch *et al.* (1957) and analyzed as methyl esters by gas cromatography. Tenderness was measured with an Instron 4442 Universal Testing Machine (Canton, MA, USA) with a Warner-Bratzler shearing attachment on samples cooked in a water bath at 70 °C for 50 minutes. Data were analyzed using GLM procedure SAS (SAS Inst. Inc., Cary N.C.).

Results and discussion

Animal performance data is shown in Table 1, H calves had higher ($P < 0.01$) initial liveweight as expected. Initial fat depth was the same accross treatments. At the end of the trial L steers had a deeper ($P< 0.01$)

subcutaneous fat layer, lower liveweight gain ($P < 0.01$) and were lighter ($P < 0.01$) than H reflecting that smaller animals were more mature. Steers on M gained more ($P < 0.01$) in autumn than those supplemented with S.

Among treatments , the proportion of ether extract (EE), saturated fatty acids (SFA), polyunsaturated fatty acids (PUFA), n-6 fatty acids, n-3 fatty acids and the ratio between the latter two (Table 2) in meat was similar ($P > 0.1$). The n-6: n:3 ratio was below 4:1, therefore considered healthy for human beings (Holman, 1995). Concentration of monounsaturated fatty acids (MUFA) was higher in L steers, in accordance to their higher degree of fatness. Animal size influenced the proportion of conjugated linoleic acid (CLA) for LS and LM was 1.08 g/100 g and 0.82 g/100 g respectively ($P< 0.05$), whereas for HS was 0.93 g/100 g and 1.06 g/100g for HM ($P > 0.10$). In smaller animals the effect of the different feeding regime became apparent, the rich forage diet enhanced CLA body fat levels as reported elesewhere (French *et al.,* 2000 and Grigera Naón *et al,*., 2003). In case of larger steers this difference was not detected, which can be associated with the fact that they were thinner animals. Ultimate pH (Table 3) was higher for H ($P < 0.05$) than for L and similar for both supplements. There was a significant ($P< 0.05$) interaction between animal size and diet for a* and b*, LM showed higher values in comparison to LS, both parameters were similar for large frame steers. Grigera Naón *et al*. (2001), reported that a* and b* were affected when maize grain was fed to grazing steers. Tenderness was not affected neither by type of animal nor by the supplement fed. However, there was a trend for those animals on S to produce somewhat more tender meat. Cooking loss was higher in H as compared to L ($P< 0.05$), Camfield *et al.* (1999), reported comparable results from three muscles of steers differing in frame size. Such higher cooking loss was recorded in spite of higher pHu in H ($P< 0.05$), but such value (pHu = 5.8) can seldom affect juiciness (Lawrie, 1998). Cooking losses were similar ($P> 0.1$), when comparisons were made between supplements, Geay *et al.* (2000) consider that juiciness cannot be modified by feeding regimes.

Conclusions

During the supplementation period, meat from large animals showed lower MUFA and higher pHu than meat from smaller animals; supplements had some effect on CLA concentration and colour parameters a* and b* in small frame steers.

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Table 1. Animal performance

	Factors					Contrasts, *P* <		
Variable	H	L	M	S	se	H vs.L	M vs. S	B*D
Initial liveweight, kg	185	153	171	166	6.4	<0.01	0.47	0.21
Final liveweight, kg	296.1	260.1	283.9	272.3	7.8	<0.01	0.15	0.23
Daily gain , g/d								
-Autumn	356	426	457	325	43	0.10	<0.01	0.64
-Winter	1103	924	1016	1011	36	<0.01	0.86	0.88
Initial fat depth, mm	2,2	2,2	2,2	2,2	0,11	0,71	0,97	0,21
Final fat depth, mm	2.88	4.46	4.01	3.34	0.47	<0.01	0.03	0.34

Table 2. Ether extract and fatty acid composition (%) of muscle lipids

	Factors					Contrasts, *P* <		
Variable	H	L	M	S	se	H vs.L	M vs. S	B*D
CLA	0.99	0.95	0.94	1.00	0.08	0.55	0.38	0.02
EE, %*	8.12	8.70	8.02	8.79	1.08	0.58	0.46	0.25
SFA	50.0	45.9	48.0	47.9	2.41	0.07	0.98	0.98
MUFA	41.6	47.9	43.7	44.7	1.59	<0.01	0.56	0..30
PUFA	8..34	7..34	8.27	7.41	1.18	0.34	0.41	0.13
n-3	1.96	1.62	2.03	1.55	0.40	0.34	0.18	0.06
n-6	5.31	4.63	5.19	4.76	0.36	0.52	0.58	0.12
Ratio n-6:n-3	3.36	3.27	2.99	3.64	0.52	0.85	0.21	0.39

* on a dry matter basis

Table 3. Cooking loss, pH, colour and tenderness

	Factors					Contrasts, *P* <		
Variable	H	L	M	S	se	H vs.L	M vs. S	B*D
Cooking loss, g/g	0.17	0.14	0.16	0.14	0.01	0.04	0.12	0.43
pH	5.80	5.64	5.66	5.77	0.08	0.04	0.14	0.15
a*	18.6	20.2	20.3	18.5	1.55	0.27	0.22	0.04
b*	6.65	7.00	7.27	6.39	0.55	0.53	0.13	0.03
L	36.0	36.6	36.8	35.8	1.1	0.55	0.36	0.20
Shear force, kg	6.79	6.65	7.30	6.14	0.59	0.82	0.07	0.58

CARBON MONOXIDE AS A SUBSTITUTE FOR NITRITE IN MEAT BATTER SYSTEMS

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Background

Nitrite ($NaNO_2$) and nitrate ($NaNO_3$), a precursor to nitrite, are widely used as additives in processed meat for enhancing colour and other properties of the products. Due to the risk of formation of carcinogenic compounds, finding alternative additives that can replace or reduce the addition of nitrite are highly desirable (Pegg and Shahidi, 2000). Carbon monoxide (CO) binds strongly to the muscle pigment myoglobin creating stable, bright red carboxymyoglobin. CO can be safely used in concentrations below 1 % in fresh meat packages, or in similar or higher concentrations for pretreatment of fresh meat (Sørheim *et al*., 1997). Carboxymyoglobin has a higher denaturation temperature than other forms of myoglobin, e.g. deoxymyoglobin (Hunt *et al*., 1999; Sørheim *et al*., 2001), thus having a potential use as a colourant in typical cooked meat products.

Objective

The main objective was to study the effect of replacing nitrite with CO on the colour of cooked meat batter systems.

Materials and methods

The study was a multifactoral design with a total of 44 batches including:
- colour processes (4): CO gas flushing directly to batter (CO-D), CO pretreatment of raw materials (CO-R), addition of nitrite to batter (N), no CO/nitrite as control (C)
- meat sources (2): semimembranosus muscles of pork or beef
- antioxidants for CO-D and CO-R (4): ascorbic acid, phosphate, ascorbic acid + phosphate, none
- sodium chloride level for CO-D and CO-R (3): 0, 1.4 and 2.8 %

The design was unbalanced with some factors considered more important than others. Significance testing was performed by univariate and multivariate (Langsrud, 2002) analysis of variance. Effects were illustrated by least square means adjusted for unbalance.

The basic batter receipe consisted of:
- 70.0 % meat
- 24.4 % water
- 1.4 % NaCl
- 3.5 % native potato starch (Hoff, Gjøvik, Norway)
- 0.7 % Na-caseinate (Tine, Oslo, Norway).

The following ingredients were added to some batches:
- 85 ppm $NaNO_2$
- 500 ppm ascorbic acid
- 0.3 % sodiumtripolyphosphate (A.B. Corneliussen, Oslo, Norway)

The raw batters contained on average 16.4 % protein, 1.4 % fat, 76.9 % water and 2.8 % carbohydrates. Total batter weight was 1.430 kg.

Fresh meat was ground twice through a 4 mm plate. Meat for the CO-R treatment was placed in polyamide pouches, compressed to < 5 mm, packaged in 1 % CO/ 99 % N_2 with < 0.2 % residual O_2 and stored at 3 °C

for 4 and 5 days for pork and beef, respectively. The meat pouch was turned at 2 days of storage to access gas from both sides. Batters were prepared in a Stefan UM5 chopper (A. Stephan u. Söhne, Hameln, Germany) with a lid and double bladed knives with a chopping time of 3 min and 20 sec. For CO-R, pretreated meat with CO was used without supply of CO in the chopper. Meat for CO-D was not pretreated with CO, but the batters were flushed with 1 % CO/ 99 % N_2 at 2 bars for the last 2 min of the chopping. All batters were filled in 50 ml polyethylene tube casings of 28 mm in diameter, centrifuged at 2000 rpm for 5 min, stored overnight at 3 °C, heated for 30 and 40 minutes in circulating water baths at 80 and 100 °C, respectively (the latter 8 batches only), chilled in ice water, and stored at 3 °C for 3 hours. Core temperature after cooking was measured with a 1 mm needle thermometer (Teck Instrument AS, Tranby, Norway). Casing was peeled off the cooked products, which were then sliced vertically in duplicate halves of 15 mm height for instrumental and visual colour analyses taking place at 0, 15 and 60 min of air exposure at 20 °C.

In a small additional experiment, two fermented batters of 1 kg each were prepared of beef *semimembranosus* muscle (94.5 %), dextrose (0.7 %), starter culture, NaCl (4.5 %), and ascorbic acid (500 ppm). In one batter, 180 ppm $NaNO_2$ was added, while the other batter was flushed with 1 % CO/ 99 % N_2 (see above for details). The batters were stored in 50 ml tubes for 6 days at 20 °C.

L*a*b* values (lightness, redness, yellowness) were measured with a Minolta Chroma Meter CR-300 (Minolta Camera Co., Osaka, Japan) with an 8 mm viewing port and a D_{65}-illuminator. Visual colour evaluation was performed by two trained assessors using a 5 point scale where 1 = very red/pink and 5 = extremely gray/brown. Denaturation of myoglobin was analysed by the method of Krzywicki (1979) (8 batches, each raw and at 80 °C). pH of the raw as well as the fermented batters was analysed with an Ingold Xerolyt gel electrode (Mettler-Toledo, Greifensee, Switzerland).

Results and discussion

Figs. 1 and 2 demonstrate a* values of pork and beef batters, respectively, heated into 80 °C, (actual core temperature was 79.5 °C), and exposed to air for 0, 15 or 60 min. Fig 3. shows the b* values for both pork and beef batters cooked to 80 °C. Fig. 4 demonstrates visual colour score of the batters at 80 °C. Immediately after slicing, the CO-D samples with CO gas flushing were more red with higher a* values than N samples on beef ($p<0.05$), but similar to the a* values on pork. However, after 15 and 60 min CO-D samples lost more redness than N in both species. The results of the visual colour evaluation corresponded to the a* values. Initial yellowness values (b*) were lower for CO-D than N beef samples ($p<0.05$), but were not different anymore at 15 and 60 minutes of O_2 exposure. CO-D samples were slightly lighter, expressing L* values of about a unit higher than the N samples at all measuring points (results not shown).

The CO-D gas flushing seemed to effectively replace O_2 with CO and bind to myoglobin in the final batters. The CO-R pretreatment was less efficient than direct flushing in producing a red colour of the samples, as shown in Figs. 1 - 4. Based on visual examination of the CO-R pretreated raw materials, 100 % of the pork and 80 – 90 % of the beef pigments were in the state of carboxymyoglobin. Despite the almost complete saturation of the meat pigment with CO, exchange of CO with O_2 during chopping was the likely cause for the less intense redness after cooking of CO-R samples. Although the red colour of CO-treated samples faded during air exposure, the colour could perhaps be maintained by keeping the samples in an anaerobic environment. The level of NaCl or the use of additives, either as ascorbic acid, phosphate or ascorbic acid + phosphate, did not much affect the colour of the final cooked batters of CO-D and CO-R (results not shown). The pH of the raw batters without additives was 5.51. Addition of phosphate increased the pH of the batter by 0.10 unit ($p<0.05$). Myoglobin denaturation at 80 °C was in the range 73 – 81 % for pork and 91 – 95 % for beef batters, respectively, with no apparent differences between CO-D, N and C samples. These results suggest that carboxymyoglobin denaturates to about the same extent as nitrosomyoglobin at 80 °C.

At 100 °C (actual core temperature was 98 °C), initial a* values of CO-D beef samples were slightly lower (about a unit) than at 80 °C, yet expressing a bright red colour (results not shown). However, pork CO-D samples gave approximately 2.5 units lower a* readings, and were clearly paler at 100 °C than the same samples at 80 °C.

In the fermented beef batters, pH was reduced from appr. 5.4 to 4.5 during fermentation. CO flushing produced a brighter red colour into the fermented samples than nitrite, but the colour of the CO sample faded away along with exposure to air (results not shown).

Conclusions

Direct flushing of pork and beef batters with 1 % CO produced an initial bright red colour into samples cooked into 80 °C. The internal colour of these samples immediately after slicing was equally or more intensely red than samples treated with nitrite. Pretreatment with 1% CO to raw materials that were later used for batter production, resulted in less efficient colour formation than the flushing of gas during chopping, probably due to exchange with O_2 during batter blending. Beef seemed to be more responsive than pork to colour improvement by CO flushing and pretreatment. The lack of colour stability of CO samples in O_2 exposure needs to be addressed further, perhaps with new processes or additives to counteract pigment oxidation.

Acknowledgements

The Research Council of Norway is thanked for financial support of the study. The authors are grateful to Anne Kari Arnesen, Grethe Enersen, Håvard Johansen, Kjell Merok, Karin Solgaard and Hans Sundell at Matforsk for skilful technical assistance.

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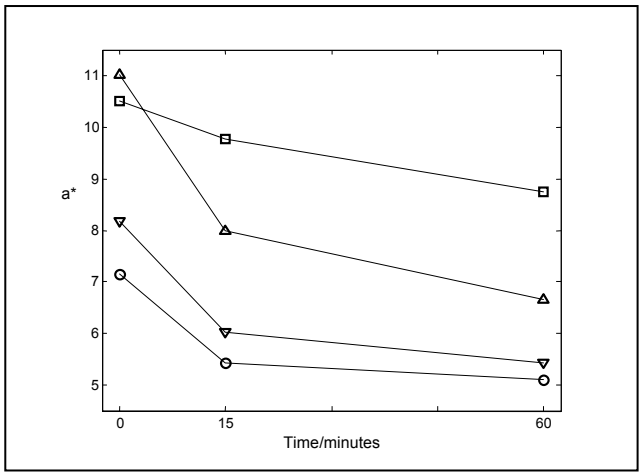

Figure 1. a* redness values of pork batters cooked to 80 °C and exposed to air for up to 60 minutes. Symbols: Δ = CO flushing of batters (CO-D), ∇ = CO pretreatment of raw materials (CO-R), ڤ = nitrite (N), o = no CO/nitrite as control (C).

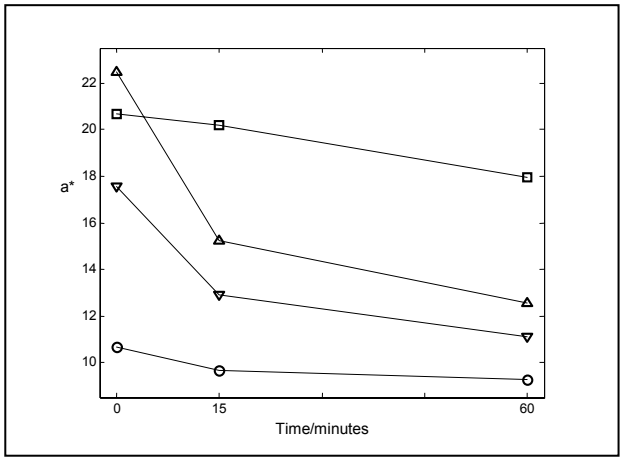

Figure 2. Redness values (a*) of beef batters cooked into 80 °C and exposed to air for up to 60 minutes. For symbols, see Fig. 1.

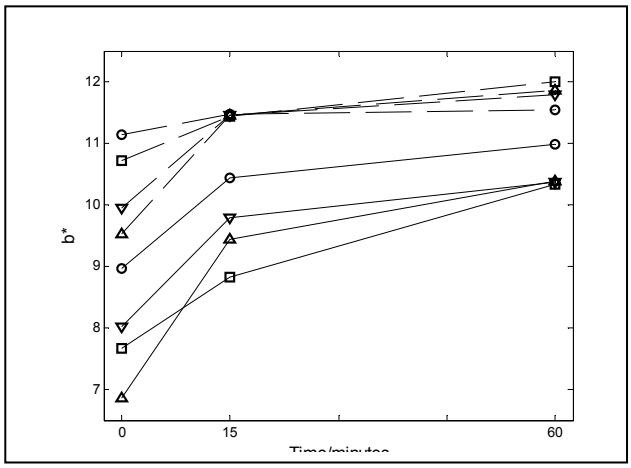

Figure 3. Yellowness values (b*) of pork and beef batters cooked into 80 °C and exposed to air for up to 60 minutes. Pork = solid lines, beef = dashed lines. For symbols, see Fig. 1.

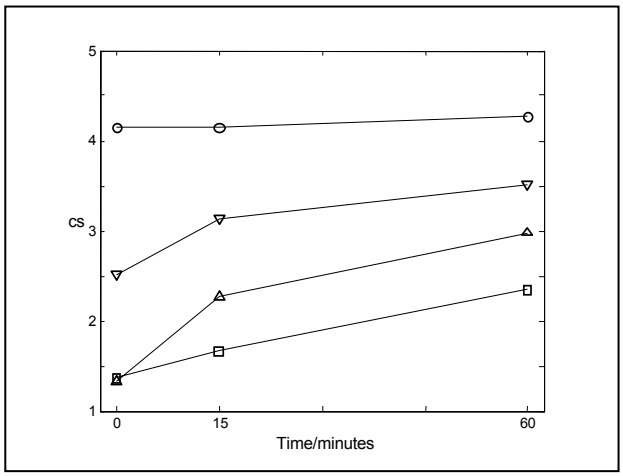

Figure 4. Visual colour evalution of batters cooked to 80 °C and exposed to air for up to 60 minutes. Colour score (cs): 1 = very red/pink, 2 = some red/pink, 3 = slightly red/pink, 4 = some gray/brown, 5 = extremely gray/brown. For symbols, see Fig. 1.

EFFECT OF DIETARY OIL AND PROTEIN LEVEL ON CARCASS AND FAT QUALITIES AND PROCESSING CHARACTERISTICS IN PIGS

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Background

The use of dietary fats and oils to alter the fatty acid profile in meat animals to produce healthier and higher quality meat suitable for processing as well as consumption has been well documented, for example by Enser *et al*. (2000). In Ghana and other developing countries, local vegetable oils and agro-industrial by-products (AIBP) such as palm oil, palm kernel oil, palm kernel meal, coconut meal and cotton seed meal, are being used in animal feed, and recommended inclusion levels have been established (Okai, 1998). The economic incentive of using palm products is their relatively favourable cost compared to cereals and other oilseeds due to increasing global production (Shahidi, 1990). Furthermore, palm oil is cholesterol free, trans fatty acid free and fortified with carotenoids, tocopherols and tocotrienols (Sulaiman *et al*., 2001). There is, however, no information on the effect of Ghanaian AIBPs on the fatty acid profile of pork and pork fat. The processing characteristics of pork fat are influenced by its fatty acid composition. Poor slicing quality of floppy bacon is caused largely by soft fat (Morgan *et al*., 1994). High concentrations of saturated fatty acids (SFA) particularly stearic acid (18:0) as well as low concentrations of unsaturated fatty acids, mainly linoleic (18: 2), improve firmness/hardness of fatty tissues (Wood *et al*., 1985). The physical properties of fat also influence fat loss during cooking of comminuted meat products (Evans and Ranken, 1975; Teye *et al*., 2004).

Objectives

The objective of this study was to evaluate the effects of palm and palm kernel oils, and lysine level on the fatty acid composition of pork fat, fat firmness and the quality of belly bacon and frankfurter sausage.

Materials and methods

Sixty crossbred pigs (0.5 Duroc, 0.25 Large White, 0.25 Landrace) were used, with equal numbers of males and females. Each group of 10 were fed one of six diets in a 3x2 factorial design with 3 oil types (40 g/kg) and 2 lysine levels (HL-11 and LL-7 g/kg). Palm oil (PO) and palm kernel oil (PKO) were compared with soya bean oil (SBO). Pigs were housed in straw-based pens and fed *ad libitum*. Animals were reared from an initial mean live weight of 40 kg and slaughtered at an average live weight of 100 ± 10 kg. Measurements taken were cold carcass weight (CCW), pH_{45} min, pH_u (24h), drip loss, texture in *M. longissimus*, P2 backfat thickness and fat firmness at two positions (shoulder fat firmness SFF, loin fat firmness, LFF) at 2ºC with a custom-made digital penetrometer. Analysis of backfat fatty acid composition was by gas-liquid chromatography following extraction into chloroform. Slip point was determined by the open capillary method (BSI, 1985). Ten 4 mm thick slices from each cured belly were subjectively evaluated for slice integrity and classified as grade A, B or C. Bacon cohesion (BC) was assessed by a tensile test on cylindrical samples of 25 mm diameter. Pork frankfurter sausages, formulated with 5 kg lean and 2 kg fat from the shoulder and neck, were cooked individually in sealed vacuum-packed type polyethylene bags at 80ºC for 1h. Fat loss in the exudates was determined gravimetrically. Data were analysed by general linear model (Minitab 13). Correlations between fatty acids and fat firmness, bacon quality and fat loss from sausages were determined.

Results and discussion

Means for cold carcass weight, P2, pH45 and pHu, drip loss, texture of m. longissimus, backfat firmness and slip point are given in Table 1. Table 2 shows the means for fatty acid composition. The relationships between fatty acid concentration and fat firmness slip point, bacon and sausage qualities are given in Table 3. Oil type did not have a significant effect ($P > 0.05$) on carcass quality (Table 1), as also found by Okai (1998) and Rentfrow *et al*. (2003). PKO and PO significantly ($P \leq 0.001$) increased fat firmness and slip point,

relative to SBO (Table 1). The LL resulted in a significantly higher ($P<0.01$) shoulder fat firmness. PKO increased the concentrations of lauric (12:0), myristic (14:0), palmitic (16:0) and stearic (18:0) acids and decreased linoleic acid (18: 2). Valencia *et al*., (1993) found a similar result in poultry, consequently making the fat firmer (Wood *et al*., 2003). The LL diet increased the concentrations of 16:0 and 18:0 and decreased 18:2 and 18:3 concentrations (Table 2). PKO as well as the LL diet significantly ($P\leq 0.001$) reduced the P/S ratio (Table 2), apparently lowering the nutritional value of the fat. Fat firmness and slip point had moderate to strong positive relationships with SFA and 18:0/18:2 ratio, and strong negative relationships with PUFA (Table 3). Sausage fat loss was positively related to SFA and C18:0/C18:2, and negatively related to PUFA, as observed previously (Teye *et al*., 2004). There was no significant correlation between the grade A bacon slices and fatty acid concentrations (Table 3). Rentfrow *et al*. (2003) reported a similar finding, which is, however, contrary to what was observed by Teye *et al*. (2004) who found a positive correlation ($r=0.5^{**}$) existed between 18:0 and grade A bacon slices.

Conclusions

Fatty acid composition and backfat firmness were affected by dietary oil, with PKO and PO increasing fat firmness. PKO produced the most saturated fat and the lowest P/S ratio, which is considered having adverse implications to human health. The work suggests that PO could be used to improve pork fat firmness in lean pigs without adversely affecting its healthiness. The LL diet increased the concentration of saturated fatty acids and fat firmness. High concentrations of medium chain saturated fatty acids (12:0, 14:0 and 16:0) may not be suitable for frankfurter type sausage production due to high fat losses. Optimum inclusion levels of palm products for quality meat production need to be determined in all countries where these products are being used, such as in Ghana.

Acknowledgements

This study was funded by the Association of Commonwealth Universities and supported by DEFRA.

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Table 1. Effect of dietary oil and lysine level on pork carcass and fat qualities

	PKO	PO	SBO	sed	sig.	HL	LL	sed	sig.
CCW (kg)	73.6	73.7	73.7	0.11	ns	73.6	73.8	0.10	ns
P2 (mm)	12.7	13.2	13.6	0.98	ns	12.8	13.6	0.80	ns
pH_{45}	6.3	6.3	6.4	0.06	ns	6.3	6.3	0.06	ns
pH_u	5.4	5.4	5.5	0.03	ns	5.4	5.5	0.03	ns
Drip loss (g/100 g)	4.3	4.3	4.9	0.63	ns	5.0	4.2	0.52	ns
LD texture (kg)	6.0	6.3	6.0	0.44	ns	6.2	6.1	0.36	ns
SFF (Pe)	952^{b}	810^{a}	733^{a}	28.2	***	814^{a}	877^{b}	23.0	**
LFF (Pe)	781^{b}	672^{a}	588^{a}	60.8	**	651	711	49.5	ns
Slip point (ºC)	32.8^{b}	28.1^{a}	27.8^{a}	1.15	***	29.0	30.1	0.94	ns

Pe: Penetrometer units; Means in the same row prior to each 'sig.'-column with different superscripts differ significantly. ns- not significant; ** $P \leq 0.01$; *** $P \leq 0.001$

Table 2. Effect of dietary oil and lysine level on backfat fatty acid composition (g/100g fatty acid)

	PKO	PO	SBO	sed	sig.	HL	LL	sed	Sig.
C12:0	1.14^{b}	0.06^{a}	0.06^{a}	0.06	***	0.40	0.44	0.04	ns
C14:0	3.86^{b}	1.14^{a}	1.14^{a}	0.11	***	2.01	2.09	0.09	ns
C16:0	24.3^{c}	22.8^{b}	21.4^{a}	0.39	***	22.5	23.2	0.32	*
C18:0	13.0^{b}	11.7^{a}	11.6^{a}	0.40	**	11.7^{a}	12.5^{b}	0.32	*
C18:1n-9	34.0^{a}	39.1^{b}	33.0^{a}	0.68	***	34.8	35.9	0.55	ns
C18:2n-6	12.6^{a}	14.9^{b}	21.9^{c}	0.66	***	17.6^{b}	15.4^{a}	0.05	***
C18:3n-3	1.31^{a}	1.45^{ab}	1.53^{b}	0.09	***	1.91^{b}	1.53^{a}	0.06	***
C18:0 / C18:2	1.1^{c}	0.8^{c}	0.5^{a}	0.05	***	0.7^{a}	0.9^{b}	0.04	***
P/S	0.3^{a}	0.5^{b}	0.7^{c}	0.03	***	0.6^{b}	0.5^{a}	0.02	***
Total lipids (g/100g)	81.0	79.5	81.0	1.95	ns	78.8^{a}	82.2^{b}	1.59	*

Means in the same row prior to each 'sig.'-column with different superscripts differ significantly. ns: not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

Table 3. Correlations between fatty acid concentration and qualities of backfat and pork products

	SFF	LFF	Slip point	BC	% Grade A slices	Total sausage fat loss
C12:0	0.6***	0.3*	0.6***	0.4**	-0.04^{ns}	0.6**
C14:0	0.6***	0.3*	0.6***	0.4**	-0.02^{ns}	0.6**
C16:0	0.6**	0.3*	0.7***	0.02^{ns}	0.03^{ns}	0.6**
C18:0	0.5***	0.3*	0.8***	0.02^{ns}	-0.06^{ns}	0.4^{ns}
C18:2	-0.6***	-0.5***	-0.5***	-0.02^{ns}	-0.19^{ns}	-0.7***
C18:3	-0.6***	-0.5***	-0.5***	-0.01^{ns}	-0.14^{ns}	-0.6**
C18:0 / C18:2	0.8**	0.5***	0.7***	0.3^{ns}	0.10^{ns}	0.7**

ns: not significant; * $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$

THE FAITH OF THE UMAMI COMPOUND AND FLAVOUR PRECURSOR INOSINE MONOPHOSPHATE DURING AGING AND COOKING OF PORK

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Background

The flavour of meat develops largely through the cooking process, however, raw meat contains several constituents that are non-volatile and contribute to its taste (MacLeod, 1986). Consequently, many of the constituents in raw meat undergo substantial changes during the cooking process and subsequently provide roasted, boiled, fatty and species-related flavours, as well as the characteristic meaty aromas associated with all cooked meats. Model experiments have suggested that the Maillard reaction between amino acids and reducing sugars is one of the main pathways for formation of many of the aroma compounds, which have been identified in cooked meat. The dominating reducing sugar in meat is the pentose, ribose, which originates from the degradation of ribonucleotides (Mottram, 1998). Moreover, nucleotides and related compounds, e.g. the 5´-ribonucleotides, adenosine monophosphate (AMP), inosine monophosphate (IMP) and guanosine monophosphate (GMP), are also important in relation to meat flavour, as they contribute with umami taste (Durnford, Shahidi, 1998; Spurvey et al, 1998). Umami taste has a characteristic savoury quality, first characterized for glutamate. Beside the characteristic umami taste, umami compounds have flavour enhancing properties, and are reported to enhance meaty, brothy, mouth filling, dry and astringent qualities and suppress sulphurous notes (Kuninaka, 1981). Finally, bitterness in meat may be derived from hypoxanthine, which is a degradation product of IMP, together with anserine, carnosine, other dipeptides and several free amino acids.

Considering that IMP, ribose and hypoxanthine all are considered important constituents in meat flavour formation and development, an understanding of the post mortem metabolism in muscle and the subsequent degradation of the adenosine triphosphate (ATP) metabolite, IMP, during aging and cooking, as schematically outlined below (Scheme 1), becomes crucial in the further exploitation of flavour development in meat.

Scheme 1

ATP ⇒ ADP ⇒ AMP ⇒ IMP ⇒ Inosine ⇒ Hypoxanthine + Ribose

ATP ≡ adenosine 5´-triphosphate
ADP ≡ adenosine 5´-diphosphate
AMP ≡ adenosine 5´-monophosphate
IMP ≡ inosine 5´-monophosphate

IMP has been found to be a desirable flavour enhancer and umami compound in meat and fish (Maga, 1987; Madruga M.S., 1997; Murata, Sakaguchi, 1989). As the dephosphorylation of IMP to inosine is a slow process, IMP has been reported to accumulate in beef and fresh fish muscle (Dannert and Pearson, 1967; Konosu, Yamaguchi, 1989). The IMP concentration has in fish been found to be highest within one to two days post mortem with a subsequent decrease resulting in a less acceptable flavour development (Fletcher and Statham, 1988). Formation of bitter taste through degradation of IMP to hypoxanthine might be an element in flavour deterioration of fish during prolonged storage, as nucleotide degradation is found to contribute to bitter taste in muscle foods (Bremner et al., 1988). Likewise, Cambero et al. (2000a) reported sour and astringent flavour of beef broth at higher cooking temperatures, which also may reflect severe nucleotide degradation.

Several studies have reported a heat-induced increase in ATP metabolites during cooking of different muscle foods, and a significant increase in inosine and hypoxanthine during cooking has been demonstrated in goat and sheep meat (Arya and Parihar, 1979). Moreover, increasing cooking temperatures have been found to

result in a significant rise in the concentration of creatinine, IMP, AMP in beef broth, with IMP showing the highest correlation to the sensory data of the broth (Cambero et al., 2000b). The stability of IMP is reported to be both temperature- and pH-dependent due to the presence of weak chemical bonds, e.g. glucoside and ester bonds (Matoba et al. 1988). Consequently, pH in the fresh meat must be expected to influence IMP degradation through aging and cooking.
Interestingly, the contaminant formation of ribose upon hydrolysis of inosine is not considered in the literature even though ribose, as mentioned above, is known as a major reactant in the Maillard reactions, which take place in meat.
Most flavour studies related to meat have been carried out in model systems or in spiking studies where potential flavour pre-cursors have been added to meat or meat broth with subsequent chemical or sensory analysis. The present study is a preliminary study to exploit the faith of inherent inosine monophosphate during aging and cooking of pork, which subsequently will be investigated in relation to the potential contribution of IMP, hypoxanthine and ribose to the formation of volatile flavour compounds and sensory characteristics, as a consequence of aging and cooking temperature.

Objectives

The objective of this study was to investigate the faith of IMP during aging and cooking of pork, by measuring IMP and its degradation products inosine and hypoxanthine.

Materials and methods

The *M. longissimus dorsi* from four pigs (cross-breeds of Duroc boar and Danish Landrace x Yorkshire dams) reared and slaughtered at The Danish Institute of Agricultural Sciences (DIAS), Foulum, Denmark was used in the experiment. Rectangular meat samples (3x3x2 cm), from which all visible fat and connective tissue were removed, were cut, vacuum-packed in pairs and stored at 4°C for 24, 72, 120 and 216 h after slaughter. From all meat samples, 400 mg sub samples were taken at different time of aging.
The meat with different time of aging was cooked in an oven at 150°C to an inner temperature of 70 and 90°C and then cooled to room temperature. From each cooked piece two samples from 1 mm outer layer and two samples from the inner part of the meat were cut, and extractions according to the procedure described below were carried out to determine inosine monophosphate, inosine and hypoxanthine.
Pork samples of 400 mg were mixed for 5 seconds in 24 ml of ice-cold 0.6 M perchloric acid (PCA) containing a pH indicator (bromthymolblue and phenolphthalein 0.004% of each) using Sarstedt 50 ml conical vials. The samples were left on ice-bath for 15 minutes before neutralization with 21.6 ml of ice-cold 0.8 M KOH and addition of 1 ml ice-cold KH_2PO_4 buffer. Subsequently the mixtures were mixed for 10 seconds using an IKA MS 2 Minishaker, and the pH was adjusted to 7-8 using either KOH or PCA. Finally, the mixtures were centrifuged using an Eppendorf Centrifuge 5417R (4000 rpm for 10 min at 4°C), and 1 ml supernatant (in triplicate) was transferred to an Eppendorf vial and frozen at -80°C until further analysis.
Analysis of inosine 5´-monophosphate, inosine and hypoxanthine was carried out by high-performance liquid chromatography (HPLC) on a Hewlett-Packard HPLC system series 1100 using UV detection (210 nm). The samples were thawed and centrifuged, and the supernatants were transferred to cold HPLC vials and placed in a thermostatted auto sampler (1-2°C). A 10 µl sample was injected on a Lichrospher 250 x 4 mm RP18 column from which the three compounds were separated by isocratic elution using a solvent based on a buffer containing 10 mM tetrabutylammonium hydrogensulfate and 215 mM KH_2PO_4, to which 7.5 ml methanol/l was added. The following flow gradient was used to obtain optimal separation: 0.5 ml/min for 5 min, increasing to 1.5 ml/min in 1 min and keeping this flow for 9 min before a final decrease to 0.5 ml/min in 0.5 min. Quantification was based on standard curves using external standards and calculations carried out in the included software (HP Chemstation).

Results and discussion

Figure 1 shows the concentrations of IMP, inosine and hypoxanthine in fresh pork during aging. The concentrations of IMP and hypoxanthine were constant throughout the first 72 h, while the concentration of inosine increased significantly from 24 to 72 h. From 72 h to 216 h the concentration of IMP decreased with a simultaneous increase in the concentrations of inosine and hypoxanthine hereby resembling the data reported previously by Kato and Nishimura (1987).

Significant correlations were found between the concentrations of IMP and inosine (R=-0.55, P=0.0012) and between [inosine] and [hypoxanthine] (R=0.46, P=0.0077) during aging, while no significant correlation was found between [IMP] and [hypoxanthine] during aging (R=-0.26, P=0.1522). This is in agreement with the expected difference in the rate constants of the dephosphorylation of IMP and the hydrolysis of inosine, as described by Dunford and Shahidi (1998).
The observed maximum in the concentration of IMP and its degradation products inosine and hypoxanthine at 72 h after slaughter is in agreement with the data by Lindahl et al. (2003), who found that the post mortem metabolism proceeded after 2 days post slaughter.
Figure 2 shows the concentrations of IMP, inosine and hypoxanthine at the surface (o) and the centre (i) of pork samples aged for different time intervals and heated to a centre temperature of 90°C. In general, cooking resulted in a decrease in [IMP] with a concomitant increase in [inosine] and [hypoxantine] independent of time of aging. The concentrations of IMP and inosine in the centre of the cooked samples decreased independently of time of aging while [IMP] and [inosine] at the surface increased independently of aging compared with the concentrations in the fresh meat. Even though not as drastically, the same pattern was seen when samples were heated to a centre temperature of 70°C (data not shown). The highly significant difference between concentrations in the centre and at the surface of the pork samples is most probably due to dehydration of the outer layer during cooking even though a thermally induced dephosphorylation of residual ATP/ADP and AMP might also be a possibility. This aspect needs to be investigated further.

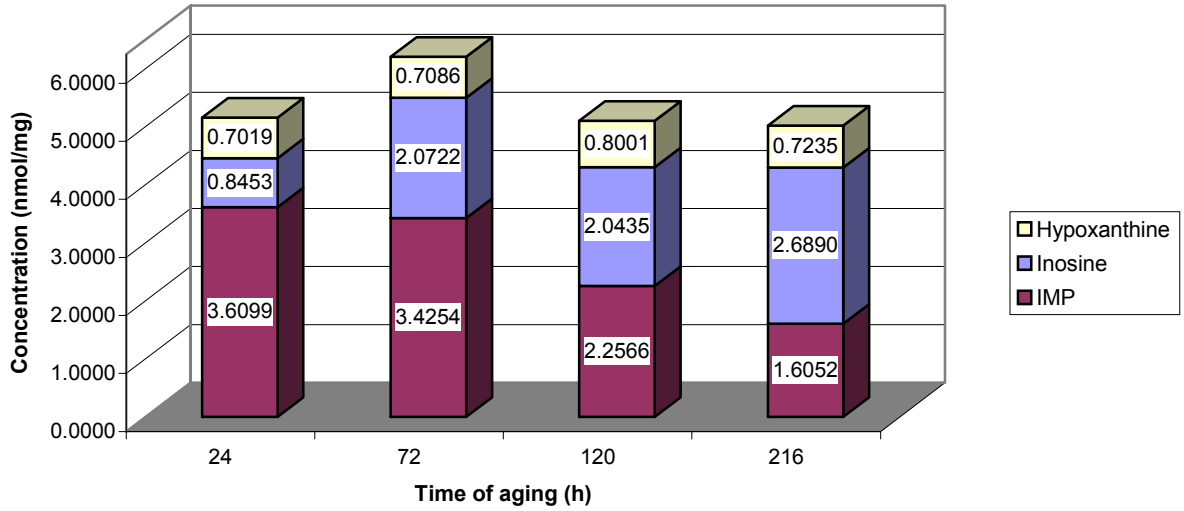

Figure 1. The concentration of IMP, inosine and hypoxanthine during aging of raw pork

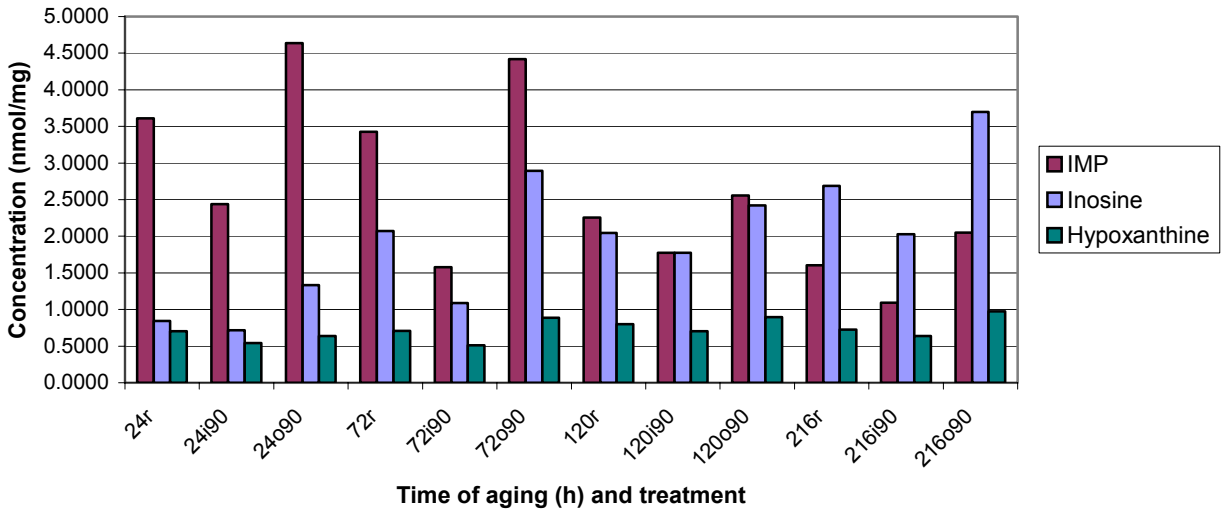

Figure 2. Influence of aging and heat treatment on the concentration of IMP, inosine and hypoxanthine. 24i90 means aging 24h, inside the meat at 90 ºC; r= fresh meat; o= outside layer; I= inside layer

Conclusions

This paper clearly demonstrates that aging and cooking are important for the development of essential meat aroma precursors. However, further studies including sensory analysis are needed to exploit the importance in relation to influence on sensory characteristics.

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EFECT OF SLAUGHTER WEIGHT AND DIET COMPOSITION OF CALVES ON MEAT AND FAT QUALITY

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Background

Many consumers in Spain and in other European countries prefer to consume meat from animals slaughtered at an early age, associating it with a healthy product, low amount of visible fat, high juiciness, high tenderness and a good smooth flavour. In fact, in the past, such meat was considered as the highest quality meat. In this sense, the use of local breeds, together with local production systems, could be a basis for establishing standards in order to improve local production and consumer confidence (Shackeldford *et al*., 2001; Dransfield *et al*., 2003).

However, in order to respond to consumer demands, it is essential to guarantee good quality meats, from a fundamental understanding of links between production factors and meat quality. Several factors, such as age, sex, breed and slaughter conditions, affect carcass and meat characteristics, but also diet is of importance. Most studies published about the effect of diet on calf performance, have been carried out using milk replacers (Beauchemin *et al*., 1990; Xiccato *et al*., 2002) and slaughtered at an early age or based on a relatively early weaning age (100-150 days), following an *ad libitum* diet of concentrate until slaughter (Hinks *et al*., 1999; Myers *et al*., 1999a, 1999b; Fluharty *et al*., 2000). However, the raising of calves on the basis of traditional systems, using whole milk, as unique feed or supplementing a concentrate diet may offer an alternative to the current systems as long as the quality of the final product could be guaranteed. On the other hand, this type of rearing system could also be used for other purpose, for example, in farms in which milk production is largely over the milk quota (Hornick *et al*., 1996). Owing to the peculiar digestive and metabolic characteristics of young calves, in which the effect of diet composition on body composition is of special importance, it may be assumed that the meat and fat from calves receiving a daily amount of milk until slaughter could show differential characteristics with respect to the meat from calves reared under intensive production systems. So, the aim of the present work was to study the effect of diet composition of calves might have on meat and fat quality.

Materials and methods

Animal Experiment

The animal experiment was conducted at the Agricultural Experimental Station of the Spanish Council for Scientific Research (CSIC) in León (Spain) while the analysis of meat quality characteristics were performed at the Meat Technological Station of Agronomic Institute (ITA) in Salamanca (Spain). Male Spanish Brown Swiss calves were used. Upon arrival, the calves were weighed and assigned to one of three groups, homogeneous in weight and age, according three experimental diets. Milk-fed (MF) calves were exclusively fed with whole milk, containing 13,8% powder milk served warm (39°C) twice daily (at 9 h and 19 h) in teat-feeder. These calves were slaughtered at 5 months of age. In the concentrate-fed (CF) treatment, after weaning at 100-kg body weight the calves received concentrate and barley straw, both available *ad libitum* until slaughter. The remaining calves, milk-concentrate group (MC), received 5 kg/d of whole milk made up the same way as for the MF group served individually once daily in buckets, and they were also fed with concentrate and barley straw, both available *ad libitum* until slaughter. The animals from last two groups were slaughtered at 7 months age. Individual weight was recorded before moving the calves to the slaughterhouse. Average slaughter weight for MF, MC and CF groups were 231.9, 346.0 and 345.5 kg respectively.

Analysis

pH was measured at 24 h post slaughter on *longissimus thoracis* muscle at 6th rib level by a penetration electrode. Dry matter, ether extract, crude protein and total collagen were determined according to official procedures (MAPA, 1979). Gross energy (GE) was determined by combustion in adiabatic calorimetric bomb. Concentration of haem pigments (mg mioglobin/g muscle) was measured by Hornsey method (1956). Labile collagen was analysed by Hill (1966) and collagen solubility was calculated as a percentage to total collagen. The fatty acid profile of subcutaneous, intermuscular and intramuscular fat from the 6th rib was obtained by gas chromatography (Perkin-Elmer Auto syst-X.L). Colorimetric parameters (L* a* b*) were obtained on *longissimus thoracis* muscle of the 6th rib, after the newly cut surface had been exposed to artificial light for 90 minutes at 10°C. Water holding capacity (WHC) was measured by four methods: drip loss (70 g aprox.) according to Honikel (1998), press loss by filter paper press method; thawing loss by thawing the sample during 4 hours in water at 18ºC (Ham, 1960) and cooking loss after cooking the sample in open polyethylene bags in water at 75°C, until the samples achieve 70ºC, measured with a thermocouple in the centre of the sample (Honikel, 1998). Water losses were expressed as a percentage of initial weight. Instrumental textural and sensory analyses were performed with the section between the 8th and 11th ribs aged through 7 days. Warner-Braztler test (Honikel, 1998) in heated meat was assessed using texture analyser TA-XT2. An eight-member trained sensory panel assessed the sensorial attributes, beef odour intensity, tenderness, juiciness; beef flavour intensity and general palatability, using a ten-point descriptive scale. Statistical analysis was performed using one-way analysis of variance (type of feeding) GLM procedure. However, in the case of fatty acid composition, in which the measurements were taken from several locations, two-way analysis of variance was used, considering the effect of diet, fat depot and the corresponding interaction. The statistical package used was SPSS 11.0.

Results and discussion

All calves shown pH values in normal range, and no differences between groups were found (5.5, 5.5 and 5.6 for MF, MC and CF respectively). The meat of calves whose diet was supplemented with milk until slaughter showed higher ether extract, lower crude protein and higher gross energy concentrations than exclusively milk-fed calves and concentrate-fed calves. Due the iron concentrate in milk and concentrate, myoglobin content was lower in exclusively milk-fed calves than in concentrate-fed calves, while calves received milk-concentrate diet provided an intermediate value (Table 1).

Table 1: Composition of *longissimus thoracis* muscle.

	Milk	Milk-Concentrate	Concentrate	P
Dry matter (%)	24.3 ± 0.78^{a}	25.7 ± 0.75^{b}	25.5 ± 0.75^{b}	*
Ether extract (% DM)	5.4 ± 2.23^{a}	8.2 ± 0.49^{c}	6.5 ± 1.53^{b}	*
Crude protein (%DM)	90.6 ± 1.33^{a}	88.0 ± 1.60^{b}	88.9 ± 2.46^{b}	***
Ash (%DM)	4.6 ± 0.25	4.3 ± 0.11	4.3 ± 0.18^{b}	ns
Gross energy (kcal/g)	5.4 ± 0.25^{a}	5.5 ± 0.07^{b}	5.5 ± 0.18^{b}	*
Myoglobin (mg/g)	2.1 ± 0.95^{a}	2.8 ± 1.04^{ab}	3.6 ± 0.62^{b}	*

a, b, c: values with different superscripts indicate significant differences between fat locations.
*** = p<0,001; ** = p<0,01; * = p<0,05; + = p<0,1; ns: p>0.1

The values of the colorimetric parameters obtained in *longissimus thoracis* muscle of the 6th rib are shown in table 2. As may be seen, statistically significant differences ($p<0.05$) were observed between the feeding strategies considered. Exclusively milk-fed calves provided the highest values of lightness and yellowness and the lowest value of redness. These results are probably related to the myoglobin content measured in *longissimus thoracis* muscle.

Whereas in the data concerning drip and thawing losses no statistically significant differences were observed between groups ($p>0.05$), solid feed supplemented with whole milk until slaughter led to a significant decrease ($p<0.05$) in press and cooking losses (table 2). This result is consistent with the findings of other authors who have observed a significant decrease in the percentage of liquid lost during cooking, parallel to the fat deposition (Gariépy *et al*., 1998; Scheeder *et al*., 1999).

Table 2: Colour, water holding capacity and Warner-Braztler for experimental groups.

	Milk	Milk-Concentrate	Concentrate	
Lightness (L*)	45.4 ±3.81 [a]	41.0 ± 1.84 [b]	40.9 ± 1.65 [b]	**
Redness (a*)	8.2 ± 2.78 [a]	12.6 ± 1.42 [b]	13.2 ± 0.64 [b]	***
Yellowness (b*)	10.6 ± 3.63 [a]	6.3 ± 0.71 [b]	7.3 ± 0.94 [c]	***
Drip loss (%)	3.0 ± 1.09	2.2 ± 0.52	2.5 ± 0.46	ns
Press loss (%)	17.4 ± 2.43 [a]	22.0 ± 2.86 [b]	24.7 ± 0.79 [b]	***
Thawing loss (%)	2.6 ± 1.13	3.1 ± 1.41	3.3 ± 1.45	ns
Cooking loss (%)	20.7 ± 3.71 [a]	22.3 ± 2.48 [ab]	26.3 ± 3.49 [b]	*
W-B shear force (kg)	6.4 ± 1.34 [a]	5.4 ± 0.19 [b]	6.5 ± 0.75 [a]	*

a, b, c: values with different superscripts indicate significant differences between fat locations.
*** = p<0,001; ** = p<0,01; * = p<0,05; + = p<0,1; ns: p>0.1

The lower value found in Warner-Bratzler test in calves receiving concentrate and milk until slaughter could be partially explained by the greater intramuscular fat content. Nishimura *et al*. (1998) have reported the important role of the fat content in shear force of cooked meat, indicating that the development of adipose tissue weakens the perimysium and hence increases the tenderness.

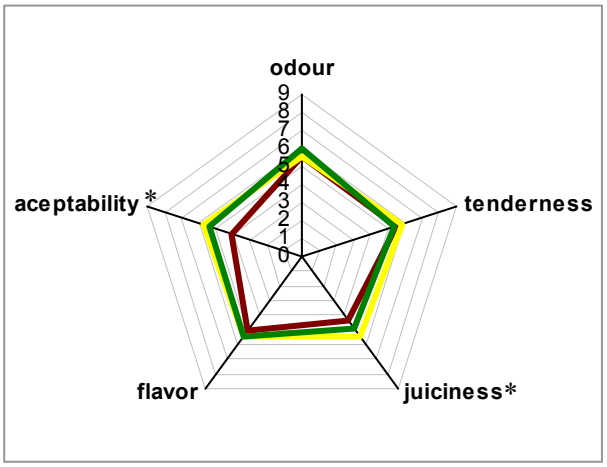

Figure 1: sensorial analysis for experimental groups

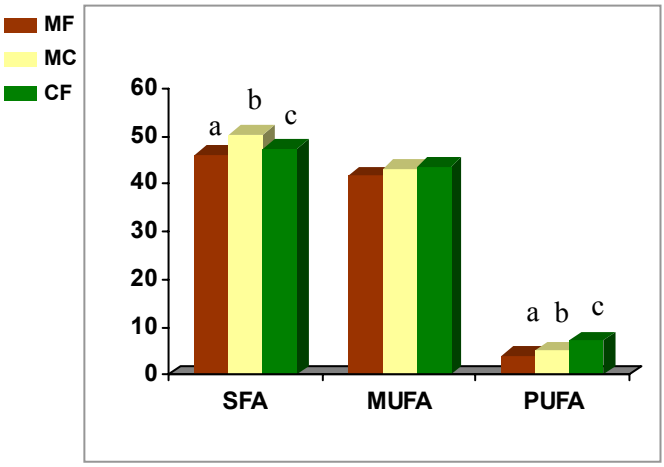

Figure 2: fatty acid composition as a function of type of feeding

The sensory analysis (figure 1) revealed statistically significant differences ($p<0.05$) for juiciness and acceptability, the lowest values corresponding to the calves received milk exclusively. These results are consistent with results obtained from water holding capacity and instrumental texture measurements.

Regarding fatty acid composition, due the absence of significant interaction between the location of fat depot and the type of feeding, both effects are described separately. The diet offered to both experimental groups provided a statistically significant effect on a large part of the fatty acids identified. Overall, the animals receiving a daily amount of whole milk until slaughter (MF and MC groups) showed a significantly higher percentage ($p<0.05$) of saturated fatty acids, which was particularly noteworthy in the case of short-chain fatty acids (figure 2).

Moreover, the calves from the CF group had a higher percentage of polyunsaturated fatty acids ($p<0.05$) and no significant differences ($p<0.05$) were found as regards the percentage of monounsaturated fatty acids. In relation with the saturation of the different fat depots, highly significant differences were found among them (date not shown). The highest proportion of polyunsaturated fatty acids corresponded to the intramuscular fat

and the highest percentage of monounsaturated fatty acids to the subcutaneous depot, being the intermuscular the most saturated fraction ($p<0.01$).

Conclusions

To sum up the results of the present work, we conclude that supplementing the concentrate diet with whole milk until slaughter may be an advantageous alternative to the traditional systems based in an early weaned and after fed only with concentrate and straw or based in exclusively milk fed. However, diets including whole milk changed the fatty acid profile in the carcasses by increasing the proportion of short- and medium-chain fatty acids and reducing their degree of unsaturation.

In either case, more studies are necessary to assess the economic costs involved in this process to achieve a compromise between production expenses and carcass, meat and fat quality.

Acknowledgements

This work was supported by project INIA SC 99-013-C2 (1/2). C. Vieira gratefully acknowledges receipt of a grant from the National Institute of Agrarian Research and Food Technology (Ministry of Science and Technology, Spain).

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PASTORAL-FLAVOUR DETECTION IN BEEF FAT USING SPME-GCMS

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Background

Some less desirable flavours have been reported in beef from forage-feeding, described as 'grassy' or 'gamey' (Larick et al., 1987). Maruri and Larick (1992) found six diterpenoids compounds in fat volatiles, phyt-1-ene, phytane, neophytadiene, phyt-2-ene, phytadiene and dihydrophytol, and showed these comprised 55.7% of the total volatiles from fat of pasture-fed steers. Bendall (2001) studied aroma compounds of milk, and showed phytol, phyt-1-ene and phyt-2-ene were more easily detected by panellists than neophytadiene and -diene compounds. The aroma of phyt-1-ene and phyt-2-ene were described as 'grassy', 'cardboard', or 'hay'. These volatiles as a component of pastoral flavour are important for consumer acceptance. However, their analysis requires a large amount of sample, and a costly module like purge-and-trap systems for concentrating volatiles.

Objectives

The solid phase micro extraction (SPME) fibre has been widely used because it is simple to use.. The aim of the present study was to establish the method for the sampling of head-space by SPME fibre and the analysis of pastoral-flavour by GCMS, and to clarify the kinetics of change in diterpenoids during a dry-lot period after grazing.

Materials and methods

Animals and sample preparation

Fourteen Japanese Shorthorn steers (14 months of age) were fed on pasture between May and September. After these five months, the steers were finished in a dry lot (concentrate diet 1.6 % of body weight/day, *ad libitum* access to rice straw), and serially slaughtered between 0 day to 154 days. Three steers which were fed on dry lot only were designated controls. Back fat samples were excised from carcasses two days after slaughter. The fat was minced and heated with an equal volume of distilled water at 100 ºC for 15min. This mixture was centrifuged at 3000 rpm for 5 minutes. An aliquot of rendered fat was removed into a glass vial and BHT was added as an internal standard and ant-oxidant (final concentration 0.5 mg/g of fat). Vials were purged with N_2 and stored with oxygen absorber in an aluminium bag at -80 ºC until analysed.

Flavour sampling and GCMS analysis

Fat samples were melted at 60 ºC, and 0.25g was placed in a glass crimp vial (10mL) with a glass fibre filter at the bottom. The vial was brought to 60 ºC for 5 min, after which the SPME fibre were inserted into the headspace and exposed for 30 minutes (static sampling). For selecting the right fibre, four types were purchased from Supelco (Bellefonte, PA), CAR/PDMS, DVB/CAR/PDMS, PDMS/DVB and CW/PDMS and were screened using the day 0 fat sample. The GC oven was held at –10 ºC for one minute after injection and rapidly increased to 40 ºC, held for one minute, and then increased to 290 ºC at 5 ºC /minute. Volatiles were analysed by MS at 70ev and a scanning range of m/z 29 to m/z 350.

Sensory evaluation

A trained sensory panel of 14 members was used. A triangle test was employed for the detection of the flavour difference between the fat from the pasture-fed animals at different dry lot periods, and the fat from the animals reared at the dry lot only.

Results and discussion

Optimization of the extraction by SPME

The day 0 fat sample was used to optimize the extraction of volatiles. The results of analyses are shown in figure 1 as chromatograms. Peaks were identified by mass spectrometry according to Maruri and Larick (1992). Phyt-1-ene, phytane, phytol, and phyt-2-ene were determined in all SPME-fibre extractions. The

CW/PDMS fibre showed the highst sensitivity for these volatile compounds, but lowest for the lower-boiling compounds. The CAR/PDMS fibre showed the higher sensitivity for lower and higher boiling compounds, but phytane was not clearly separated because of interfering peaks. The PDMS/DVB and the DVB/CAR/PDMS fibre were moderately useful for low and high boiling compounds. Although these data indicated that any SPME fibre could be used to study changes in phyt-1-ene, phytane, phytol, and phyt-2-ene, the DVB/CAR/PDMS fibre was selected for the present study.

Figure 2-a, for a DVB/CAR/PDMS fibre, shows the relationship between sampling temperature and extracted diterpenoids during the 20-minute exposure. Higher temperatures were more favourable. Figure 2-b shows the effect of holding time on diterpenoids extraction at 60 ºC, which indicates that the holding time of 40 minutes or more began to exceed the capacity of the absorption. Therefore, the sampling conditions were set to 30 minutes at 60 ºC with a DVB/CAR/PDMS fibre.

Changes of pastoral flavour and sensory analysis

Figure 3 shows the changes of pastoral flavour (phyt-1-ene, phytane, phytol and phyt-2-ene) during dry lot feeding after grazing. The headspace concentration of these compounds decreased with time of dry lot feeding. Among these compounds, phyt-1-ene was the most dominant in GC traces, and showed the highest negative correlation with time in the dry lot period, expressed by the equation of $Y= 12.455e^{-0.0135x}$ ($R^2= 0.90$). The concentration of phyt-1ene, relative value to BHT as internal standard, decreased from about 12 ppm to about one ppm during five months of dry lot feeding. The dry lot-only sample showed 0.10 ppm of phyt-1-ene.

Sensory evaluation revealed that the dry lot feeding over five months (154 days) completely eliminated the flavour. At that point, the concentration of phty-1-ene was below 0.98 ppm (Table 1). Young et al. (1999) reported that 4-methylphenol and indoles were among the compounds causing of pastoral odour and flavour in ruminant fat. These compounds were not detected with the present SPME method (Braggins et al. found already in 1999 that branch-chain fatty acids require dynamic SPME for detection). Phyt-1-ene, however, could be analysed quantitatively, and had a high negative correlation with the length of the dry lot period. These results indicate that phyt-1-ene may be used as a marker of pastoral flavour.

Conclusions

These results showed that SPME-GCMS technique is useful for rapid detection of pastoral flavour, for which phyt-1-ene could be used as a marker. The recognition of this kind of flavour is difficult if the concentration of phyt-1-ene is below one ppm (relative concentration to BHT in fat). Five months of dry lot feeding after grazing eliminated the flavour.

The authors wish to express their sincere appreciation to Dr. T. Braggins (Hill Laboratories, New Zealand) and Dr. O.Young (Auckland University of Technology, New Zealand) for their helpful comments.

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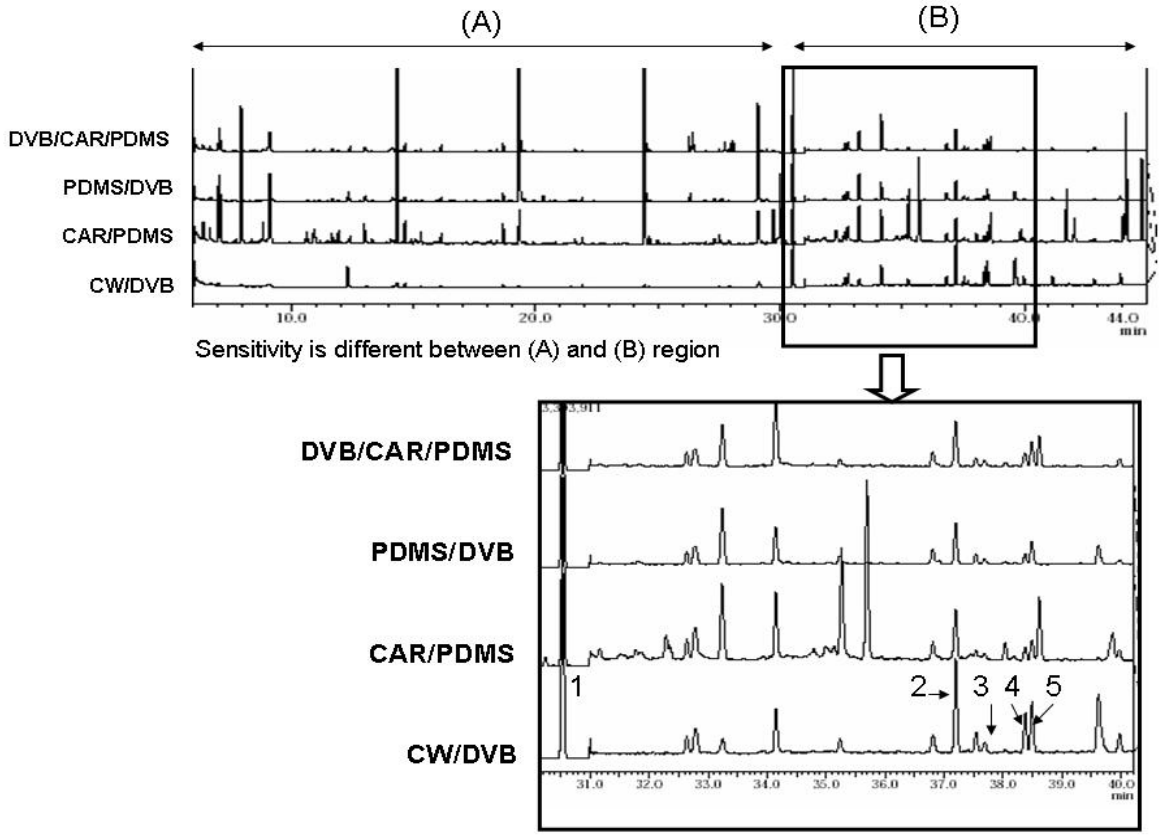
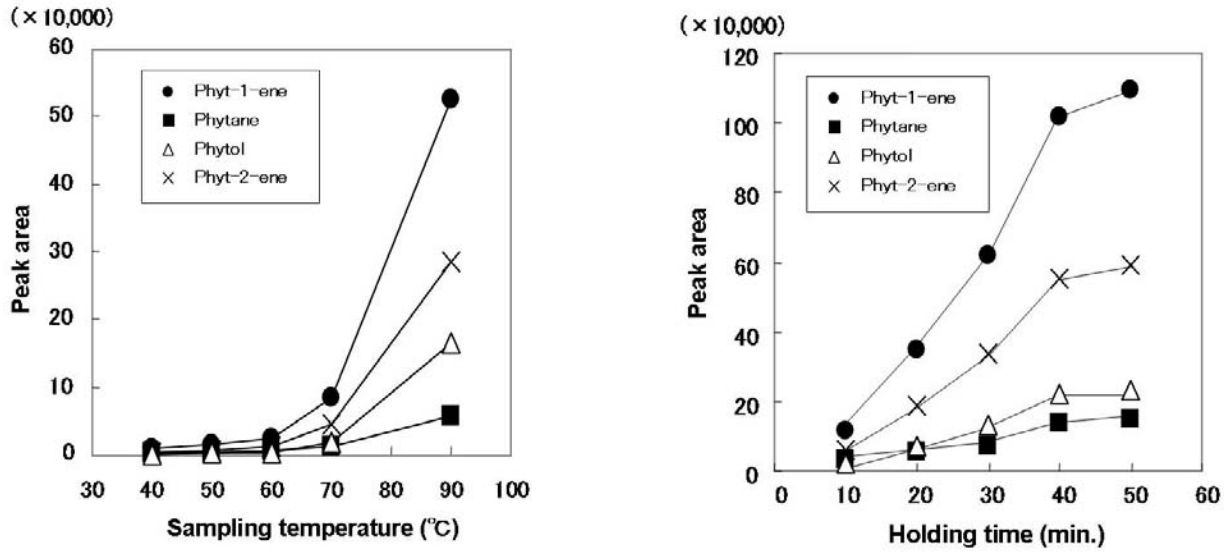


Figure 1. Chromatograms from different SPME fibers.
Four kinds of SPME fiber were tested for detection of components responsible for pastoral flavor, ie., phyt-1-ene (2), phytane (3), phytol (4) and phyt-2-ene (5) were determined. Peak No.1 is BHT used as an internal STD.



(2-a) **(2-b)**

Figure 2. Effects of sampling temperature (2-a), and holding time (2-b) on extraction volume.

Sampling temperature was varied while the holding time remained 20 minutes (2-a), then the holding time was varied while the temperature remained at 60 °C.

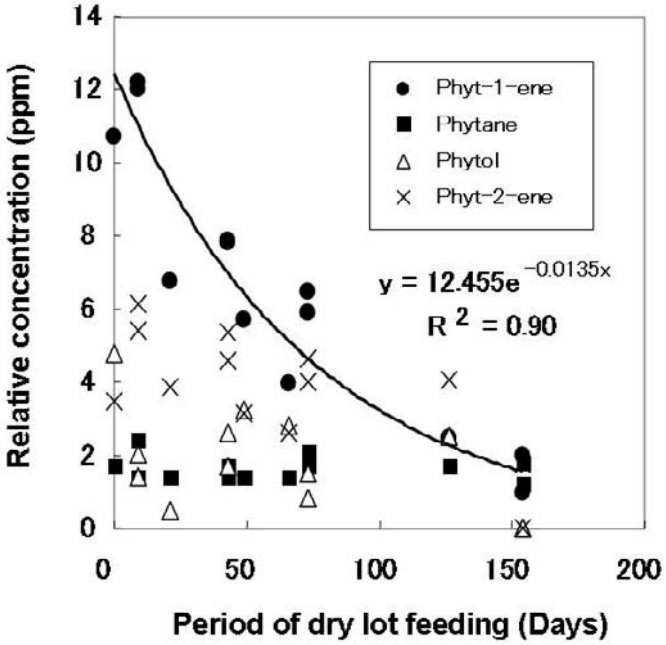


Figure 3. Effects of the dry lot feeding after grazing on flavour compounds

Phyt-1-ene showed the highest correlation to the period of dry lot feeding after grazing.

Table 1. Result of sensory evaluation (triangle test)

Dry lot period [a)]	Phyt-1-ene [b)]	Correct Answer	Significance [C)]
9 days	12.19 ppm	71 %	**
43 days	7.89 ppm	79 %	***
73 days	5.87 ppm	71 %	**
154 days	0.98 ppm	36 %	N.S.

Triangular test was performed between samples from dry lot after grazing and dry lot only (0.10 ppm of phyt-1-ene). a) Steers were fed concentrate (1.6 % of body weight/day) and given *ad libitum* access to rice straw during dry lot period after grazing. b) Phyt-1-ene concentration was defined as a relative value to BHT in fat. c) ***; $p<0.001$. **; $p<0.01$. N.S; not significant