

3. Muscle Biology and Biochemistry

Microsomal Lipid Peroxidation in Relation with Autoxidation of Bovine Oxymyoglobin

M. ANTON, P. GATELLIER and M. RENERRE.

Meat Research Station, INRA, 63122 Ceyrat, France.

Lipid peroxidation in microsomal fractions is a major factor responsible for quality deterioration in raw and cooked meat and myoglobin is implicated as playing a dominant catalytic role. On the other hand, free oxygen radicals formed by the decomposition of lipid peroxides can oxidatively modify proteins as myoglobin.

The aim of this experiment was to appreciate with two bovine muscles different from the viewpoint of colour stability, the relations between microsomal lipid peroxidation and oxymyoglobin (MbO₂) autoxidation.

From bovine Longissimus dorsi (LD) and Psoas major (PM) muscles, microsomes were prepared and oxymyoglobin was purified on a Mono Q column. MbO₂ autoxidation was appreciated by spectrophotometric measurements at 580 nm in the presence of microsomes and required cofactors. Enzymic lipid peroxidation was evaluated following 2-thiobarbituric acid reactive substances. Fluorescence decay (emission at 340 nm) was used as a test of tryptophan loss after exposition of the reaction medium to free radicals formed during the reaction.

Role of different radical scavengers and cellular antioxidants was precised. For nonenzymic catalysis, microsomal lipid peroxidation was obtained after interaction of hydrogen peroxide with metmyoglobin (MetMb) and studied by TBA test and fluorescence emission at 420 nm.

It was shown that enzymic microsomal peroxidation particularly in presence of cofactors generated oxygen radicals which increased MbO₂ autoxidation. Fluorescence decay was more pronounced with microsomes extracted from PM muscle than with those extracted from LD muscle. The inhibitory effect of superoxide dismutase and vitamin E on MbO₂ autoxidation and lipid peroxidation were confirmed and participation of superoxide anion underlined. For nonenzymic catalysis, it was observed that metmyoglobin activated by hydrogen peroxide enhanced lipid peroxidation at the same extent for the two muscles.

Myofibre composition and metabolic aspects in different strains of Belgian White-blue bulls and their relation to meat colour.

BATJOENS P., VAN VOOREN T., VAN HOOFF J. AND VEREECKE D.

State University of Ghent, Faculty of Veterinary Medicine, Laboratory of Hygiene and Technology of Food from Animal Origin, I.W.O.N.L., Wolterslaan 16, 9000 Ghent, Belgium.

Two groups of Belgian White-Blue bulls (BWB) are investigated on their myofibre composition and metabolic capacity in relation to the meat colour: one group of 5 normal bulls (N) and another group consisting of 8 double-muscled bulls (DM).

In the DM bulls the number of type II fibres shifted from IIA to IIB. They have a significantly lower percentage type IIA fibres (22 %) and a significantly higher percentage type IIB fibres (49 %). This same tendency is found for the area percentage. In general the myofibres of the DM animals have a smaller cross-sectional area in comparison to the N bulls. The obvious consequence of this characteristic myofibre composition is that the DM bulls show a very significantly lower aerobic factor and a very significantly higher anaerobic factor. These characteristics of metabolic capacity are also present in the oxidative metabolic capacity figures. The N BWB bulls have a significantly lower oxidative metabolic capacity ($Q = LDH/MDH$) compared with the DM BWB bulls. This higher anaerobic capacity enables the DM bulls to obtain the post mortem glycolysis at a higher rate for a longer time. This explains the lower pH₁ of this group. As far as the meat colour is concerned, it can be concluded that the more anaerobic myofibre composition results in significantly paler meat with a significantly higher brightness and paler hue. The outnumbering presence of anaerobic myofibres in the DM BWB bulls results in a significantly lower pigment content and this is in some extent responsible for the paler meat colour. On the other hand, it is possible that because of an accelerated post mortem glycolysis within these DM animals the sarcoplasmatic proteins deteriorate and so cause a more compact structure of the meat, which reflects more the incident rays.

Further study is required to confirm this possibility of reaction, known from PSE porc meat.

DEGRADATION OF TRIPOLYPHOSPHATE IN THE MEAT MATRIX

E. BIANCHI, A. CANTONI, C. CONSIGLIERI, L. IPPOLITI

Chair of Food-Chemistry and Technology, University of Parma

Research has been conducted simulating conditions present in the meat transformation industry to affirm the degradation of tripolyphosphate in the meat matrix. An investigation was also carried out under conditions similar to those found in the industry using a mixture of mortadella and wurstel as a substratum.

For the analysis it was adopted the ion exchange chromatography and the results demonstrated the following:

- 1 - The cause of the degradation is clearly enzymatic.
- 2 - All of the tripolyphosphate is degraded to orthophosphate within a shorter period of time than that which is required for the product to reach the temperature that inactivates the tripolyphosphatase.
- 3 - Pyrophosphate is the intermediate product of degradation.
- 4 - Any nutritional doubts about the presence of tripolyphosphates or pyrophosphates in cooked meat products are unfounded since only orthophosphates can be found.

Degradation of several high molecular weight muscle proteins by calpains I and II mimics changes occurring throughout lamb meat aging.

P. CEÑA, J. A. BELTRAN, I. JAIME and P. RONCALES.

Dep. Producción Animal y Ciencia de los Alimentos. Facultad de Veterinaria. Universidad de Zaragoza. 50013 Zaragoza. Spain.

SDS - PAGE analysis using 6.5% acrylamide was used to reveal if both calpains I and II were able to degrade high molecular weight muscle proteins, when myofibrils were incubated with the enzymes at optimum pH and Ca^{2+} concentration. Protein degradation throughout meat aging was assessed using the same technique.

It was found that at least five different proteins showing a molecular size larger than that of myosin heavy chain were degraded to varying extent by effect of incubation with either calpains I or II. Two of them are likely to be titin and nebulin while the remaining three other protein bands are as yet unidentified. An incubation time of five hours was enough to cause a degradation of 20-40% of initial densitometric peak areas.

The same proteins were found to be hydrolysed throughout meat aging, although their rate of degradation following conventional storage conditions were much lower. Seven days of aging were necessary to result in a degradation of about 20%.

Several bands of lower molecular weight appeared evident in the same electrophoretic region as a result of proteolytic activity brought about by either calpain I, calpain II or meat aging.

It is concluded that both calpains I and II mimic degradative changes of large size proteins occurring throughout lamb meat aging.

Post-mortem shortening of lamb Longissimus dorsi oxidative and glycolytic fibres as affected by temperature, muscle region and skeletal restraint; its relation to meat tenderness.

P. CEÑA, I. JAIME, J.A. BELTRAN and P. RONCALES

Dep. Producción Animal y Ciencia de los Alimentos. Facultad de Veterinaria. Universidad de Zaragoza. 50013 Zaragoza. Spain.

The proportion of oxidative and glycolytic fibres as well as sarcomere shortening were measured by microscopic observation in six regions of lamb Longissimus dorsi (L. d.), either excised from the carcass or not, held post-mortem at various temperature conditions.

Percentages of fibre types were about 33 glycolytic and 67 oxidative. No significant differences were found among muscle regions. In isolated muscles sarcomere shortening of oxidative fibres was in all cases significantly higher. Temperature had a significant effect on fibre shortening, which differed depending on their metabolic type. Whilst shortening of glycolytic fibres was gradually more intense as treatment temperature decreased from 20 to 0°C, in oxidative fibres a maximum contraction of around 40% was reached at 10°C and lower temperatures did not cause any further shortening.

Even though skeletal restraint did not fully prevent the development of cold shortening, it was in fact significantly lower than in excised muscles. Besides this, by conventional suspension of carcasses the L. d. was not subject to the same tension along its whole length. This was evidenced by the fact that following usual refrigeration conditions fibres of all muscle regions were contracted to some extent, with the exception of those having a caudal-ventral location, which were stretched. Differences were highly significant. However, carcass chilling led to a slight decrease of the average sarcomere length, not affected by muscle region.

Sensory meat tenderness was also evaluated and related to the corresponding sarcomere lengths. According to the negative correlation coefficient found ($r = -0.60$) a higher fibre shortening resulted in lower tenderness of meat.

Protein Extraction of Pig Muscle in Concentrated Salt Solutions

E. DILBER-VAN GRIETHUYSEN* and P.J. KNIGHT†

* Nestlé Research Centre, Vers-chez-les Blanc, 1026 Lausanne, Switzerland

† Dept. of Veterinary Medicine, University of Bristol, Langford, Bristol BS18 7DY, United Kingdom

During the curing of meat the distribution of salt influences protein extraction and therefore the functional properties of the final product. Following brine immersion or injection, some parts of the meat experience a high salt concentration which then declines by diffusion into the rest of the tissue. In order to study the influence of changes of salt concentration on protein extraction, experiments were designed to follow the protein extraction of small (~8 g) blocks of pig semitendinosus. Each block was soaked in one of a series of salt concentrations (0.1, 0.5, 1, 2, 3, 4 and 5 M NaCl) with and without pyrophosphate (PPi 10 mM) pH 6.0 and 1 °C. In a second experiment, the meat blocks were soaked for 24 h either in 0.1 or 5.0 M NaCl solution and were transferred each following day into the next solution of the series to obtain monotonic increase or decrease of salt. After determination of the amount of protein in the brine, SDS-PAGE was performed to identify the proteins. The greatest extraction of myofibrillar proteins occurred at 1 M NaCl. Myosin was not extracted at high salt concentrations (3, 4 and 5 M). Addition of pyrophosphate increased the amount of myosin extracted and strong bands were present in the gels for brine samples of 0.5, 3 and 4 M. Monotonic increase of the salt concentration allowed myosin extraction at 3 M NaCl. Monotonic decrease of salt showed poor myosin extraction even at 1 and 2 M NaCl. The experiments showed that myosin extraction occurs mainly at 1 and 2 M NaCl. High salt inhibits extraction and subsequent lowering of the concentration only slightly improves the extraction. The addition of pyrophosphate enhances myosin extraction and enlarges the range of NaCl concentrations where myosin is extracted. Myosin extraction never occurs at 0.1 and 5 M NaCl.

K.H. EGGEN and W.E. BUER

MATFORSK, Norwegian Food Research Institute, Osloveien 1, N-1430 Ås, Norway

Several studies have been performed on *post mortem* degradation of meat proteins using gel electrophoresis. It has been suggested that *post mortem* ageing of meat may involve changes in the glycoconjugates of the ground substance. The aim of the present study was to develop methods to study *post mortem* degradation of glycoconjugates in meat. *M.semimembranosus* from young bulls was minced, vacuum packed and stored for 0, 7, 14 and 21 days respectively at 15 °C. The samples were homogenized in liquid nitrogen, extracted in a guanidine-HCL solution and applied to ultracentrifugation in a CsCl gradient for enrichment of the glycoconjugates. The density of the harvested fractions were measured, as well as the content of uronic acid and protein. In addition SDS polyacrylamide gel electrophoresis was carried out on the extracts before ultracentrifugation and on the harvested fractions by use of a gradient gel ranging from 3 to 12 %. The results showed that ultracentrifugation in a gradient of CsCl was a convenient method of obtaining glycoconjugates. Two types of glycoconjugates were detected; one of large molecular size which appeared in the fractions of the highest density and another one which appeared in the fractions of lower density. The latter had a molecular size consistent with small proteoglycans. Furthermore, the results indicated a reduction in the size of the high density glycoconjugate after 14 days of ageing.

Adenine nucleotide breakdown products in muscle at slaughter in pigs with different halothane genotypes and relation to meat quality.

B. ESSEN-GUSTAVSSON¹, K. KARLSTRÖM¹, K. LUNDSTRÖM² AND R. PÖSÖ¹

¹Dept. of Medicine and Surgery, ²Dept. of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, S-750 07 UPPSALA, Sweden.

It has recently been shown by us that stress-susceptible pigs with genotypes Hal^a Hal^a (nn-pigs) have a lower initial muscle pH and lower glycogen, ATP and CP concentrations at slaughter compared with non stress-susceptible pigs with genotypes Halⁿ Halⁿ (NN-pigs), raised and handled under similar conditions. The aim of this study was to further investigate muscle metabolic response in these pigs at slaughter and especially adenine nucleotide breakdown products and their relation to initial muscle pH and meat quality. Analyses of ATP, ADP, AMP, IMP, hypoxanthine, xanthine and uric acid were performed with HPLC-technique on muscle samples obtained from *M. longissimus dorsi* immediately at exsanguination from 9 nn-pigs and 10 NN-pigs. Reflectance (EEL) and drip loss measurements were made the day after slaughter.

The total adenine nucleotide pool was 27% lower in nn-pigs than NN-pigs and an inverse relationship was seen between adenine nucleotide content and IMP levels. High IMP and ammonium levels were observed in those nn-pigs where initial muscle pH was 6.2-6.4. Values for EEL and drip loss showed a close relationship with adenine nucleotide and IMP concentrations. IMP levels were inversely related to capillarisation (cap/mm²), while a positive correlation was found to the percentage of glycogen depleted fibres (previously measured). Muscle fibre properties thus seem to play an important role to avoid a decrease in adenine nucleotide concentrations in stressful situations. These results further underscore that a sufficient capacity of the muscle to regenerate ATP in connection with stress-situations preslaughter is of importance for obtaining meat of good quality.

Ultrastructural *post mortem* changes in myofibrillar structure of normal and halothane sensitive pigs

M. ESTRADE, E. ROCK and X. VIGNON

Institut National de la Recherche Agronomique, S.R.V., Theix, 63122 Ceyrat, France

To determine the myofibrillar lysis that occurs in ageing meat from normal and halothane sensitive pigs, ultrastructural modifications were studied in relation with the analysis of calcium localization at different *post mortem* time. *Longissimus dorsi* muscle from pigs assessed for their halothane susceptibility was used. Samples were taken periodically for pH measurements, conventional electron microscopic examination and intracellular calcium precipitation. As expected, the rate of *post mortem* pH fall was higher in muscles from halothane sensitive pigs. The striation pattern of normal muscles exhibited numerous transversal fragmentations of myofibrils as soon as 24 h *post mortem*. This phenomenon is much more discrete in muscles from halothane sensitive pigs even after 8 days *post mortem*. This difference can be related to the lack of tenderization during the maturation processes and to the low organoleptic properties described by others authors as characteristic of PSE meat.

Enhancement of myoglobin autoxidation induced by phospholipids extracted from beef muscles of various metabolic types.

C. GENOT¹, M.N. BORREL¹, B. METRO¹, G. GANDEMER¹ and M. RENERRE²

1- LEIMA, INRA, BP527, F-44072 NANTES Cédex 03, FRANCE

2- SRV, INRA, THEIX, F-63122 CEYRAT, FRANCE

The aim of this work was to investigate the influence of muscle phospholipids (PL) on the oxidation of oxy-myoglobin (MbO₂).

Beef MbO₂ was extracted from *Longissimus dorsi* muscle. Phospholipid extracts from beef *Longissimus dorsi* (glycolytic muscle), *Psoas major* (intermediate) and *Diaphragma* (oxydative) muscles were added as liposomes to MbO₂ solution (Phosphate buffer, pH=5.8 ; 0.05 M ; 0.05 M NaCl ; 0.1 to 2 mg PL/mg MbO₂). Phospholipid classes and fatty acid composition of PL were determined. Oxidation of MbO₂ was followed at 25°C by measurement of absorbance at 580 nm during 3 hours.

Upon liposomes addition, an increase of MbO₂ oxidation rate proportionnal to PL/Mb ratio is observed. For identical PL/Mb ratios, the extent of the increase of the oxidation rate induced by phospholipids depends on the muscle origin. The oxidation rate in presence of PL extracted from *Longissimus dorsi* is the smaller, this observed with *Diaphragma* PL is the greater and *Psoas major* PL led to intermediate oxidation rate. These results can be related to the phospholipid characteristics of muscles of different metabolic types : *Longissimus dorsi* contains less phospholipid having a smaller content in phosphatidyl-ethanolamine and cardiolipids and polyunsaturated fatty acid than *Diaphragma*, and *Psoas major* has a roughly intermediate composition.

Both lipid oxidation and PL-Mb interactions (as demonstrated by increases of turbidity of Mb+PL mixtures) have to be involved to explain the observed enhancement of MbO₂ oxidation induced by muscle phospholipids.

CATHEPSIN D ACTIVITY AS AN INDEX OF CURING TIME FOR SPANISH DRY-CURED HAM

GIL, M., GISPERT, M., SARRAGA, C.

Institut de Recerca i Tecnologia Agroalimentaries (IRTA). Centre de Tecnologia de la Carn
Monells. Girona. Spain.

It is generally accepted that the African Swine Feber virus (ASFV) loses its viability at 150
180 days of curing. Nevertheless, no clear correlation can be established between curing time
and the physico-chemical parameters usually analyzed. Previous studies suggested that cathepsin
D activity could be a suitable parameter to guarantee a minimum curing time of six months.
In the present study we have quantified the evolution of cathepsin D activity monthly,
series of hams manufactured according to three different technologies: Series A, 4 months
curing process; Series B, 8 months curing process and Series C, 12 months curing process.
Series A was analyzed to evaluate if activity changes were influenced by the curing "per se"
Data on Cathepsin D activity determined-using denatured hemoglobin as substrate - at 3,
5, 6 and 7 months after the beginning of curing in Semimembranosus and Biceps femoris muscle
from series B and C were analyzed by the Scheffe's Test (SAS, 1985) (18 hams per month).
Results suggest that cathepsin D activity is not influenced neither by the curing "per se"
no by the curing time. In both processes - B and C - cathepsin D activity shows significant
differences between the six months (180 days) and the three months (90 days) and four months
(120 days) from the beginning of the curing process.

Meat Tenderness of Different Muscles Cooked to Different Temperatures and Assessed by Different Methods

A.E. GRAAFHUIS, K.O.HONIKEL*, C.E.DEVINE and B.B.CHRYSTALL

Meat Industry Research Institute of New Zealand, Box 617, Hamilton, New Zealand,

* Federal Centre for Meat Research, D-8650 Kulmbach, Germany.

The assessment of tenderness is of increasing importance to guarantee product consistency in the market-place. Although a trained
taste panel can assess the toughness of cooked meat, mechanical shear force devices (tenderometers) give more objective measurements.
Mechanical devices commonly determine shear force, either by compression with an increasing force (MIRINZ) or by using shear plates
with force applied at a constant velocity (Warner-Bratzler).

In this paper we report on the influence of cooking temperature endpoint, the differences between the muscles studied, and the
differences between tenderometers (MIRINZ, Warner-Bratzler and the Instron Materials Testing Machine using the MIRINZ and
Warner-Bratzler shear heads). The muscles used were *longissimus*, *biceps femoris*, *psoas*, *supraspinatus* and *sternomandibularis*.
Unaged and aged muscles were cooked to endpoint temperatures, ranging from 55-75°C.

The relationships between the results obtained by the different test methods, within the temperature range employed and for the
different muscles will be discussed.

Relationships between pH and extracellular space in veal muscle

F. GUIGNOT, X. VIGNON, and G. MONIN

Station de Recherches sur la Viande, INRA, Theix, 63122 Ceyrat, France

The aim of this study was to evaluate the relationship between the rate of pH fall and the changes in the extracellular space, and the relationship between the ultimate pH and these changes in the extracellular space in veal.

Two experiments were designed. In experiment 1, large variations in the rate of pH fall were observed in the *Psoas major* muscle. At 4 hours *post mortem*, pH varied between 5,60 and 6,40 ; these values corresponded to values of extracellular space (measured at 29 hours *post mortem*) between 15% and 6%. PH fall and extracellular space were negatively correlated ($p < 0,01$). In experiment 2, variations in ultimate pH were induced by adrenaline administration. Ultimate pH values varied between 5,45 and 6,35 in the *Psoas major* muscle, and between 5,50 and 6,80 in both *Longissimus dorsi* and *Trapezius* muscles. Extracellular space, measured at 29 hours *post mortem*, varied between 6,7 and 13% in the *Psoas major* muscle, between 4,5 and 10% in the *Longissimus dorsi* muscle and between 5 and 11% in the *Trapezius* muscle. Extracellular space was not correlated with ultimate pH, whereas it was with the rate of pH fall ($p < 0,01$).

It can be concluded that the extracellular space is influenced by the rate of pH fall, but not by the ultimate pH in veal muscle.

Stress testing effects on blood lipid concentrations and organ lipid composition of different genotypes of swine.

S. HARTMANN, W. OTTEN and H.M. EICHINGER

Versuchsstation Thalhausen, Techn. Univ. München, W-8051 Kranzberg, Germany

The evaluation of stress susceptibility in pigs can be greatly improved by direct measurements of physiological reactions. It was the aim of this study to investigate the effects of stress testing in pigs on blood lipid concentrations and their relations to organ lipid composition.

12 German Landrace pigs were tested to be homozygous Halothan-negative, heterozygous Halothan-negative and homozygous Halothan-positive. After reaching an average body mass (BM) of 116 kg, blood samples were taken after a treadmill run (20 min.) and after a glucose tolerance test (oral 2g glucose/kg BM). The blood sampling for controls was performed twice after 12 h fasting. Total plasma cholesterol and free fatty acid (FFA) levels were measured enzymatically. The animals were slaughtered at an average body mass of 127 kg and samples of liver, m. long. dorsi (MLD) and m. supraspinatus (MSP) were removed. Lipids were extracted and dried and total lipid contents were determined. Samples were further saponificated and total cholesterol was measured colormetrically as its ironoxyde-complex.

Oral glucose application enhanced significantly the levels of total plasma cholesterol ($\bar{x} = 96.1$ mg/100ml) compared to the controls ($\bar{x} = 84.3$ mg/100ml). Treadmill running showed no influence on total plasma cholesterol levels. FFA in plasma were elevated by running on the treadmill (0.59 mmol/l) and decreased by glucose application (0.37 mmol/l) compared to the controls (0.50 mmol/l). Analyses of variance also revealed significant influences of the different genotypes. Blood cholesterol levels were negatively correlated with muscle total cholesterol (max. $r = -0.61$) and positively correlated with liver total cholesterol (max. $r = +0.68^*$). Correlations between intramuscular total lipid concentration and intramuscular total cholesterol content were not significantly negativ.

Our results proof the very significant general influence of physical activity and nutritional factors on blood lipid concentrations in pigs, which is further modified by genetical disposition.

Effect of Beta-agonists on Composition of Pork Longissimus Muscle

H.B. HEDRICK¹, K.J. FENNEWALD¹, M.E. BAILEY¹, H. HEYMANN¹, T.P. MAWHINNEY¹, C.H. CHANG² and D.H. WALLACE²

¹University of Missouri, Columbia, Missouri. ²Merck, Sharp and Dohme Research Laboratories, Rahway, New Jersey.

The objective of this study was to determine the effects of beta-agonist compounds in the diet of pigs on qualitative and quantitative characteristics of pork muscle. Seventy pigs were randomly assigned to five treatments by litter and sex (male castrate and female). All treatment groups were fed a corn-soybean meal diet. Four groups received beta-agonist compounds in their diet. Pigs were slaughtered at approximately 100 kilograms live weight. Subcutaneous fat depth and longissimus muscle area were measured at the 10th rib. Chemical analyses were performed on the longissimus muscle for moisture, ether extractable constituents, protein, cholesterol and hydroxyproline. Beta-agonist treated pigs had larger longissimus muscle areas and less subcutaneous fat than control pigs. Longissimus muscle from beta-agonist treated pigs contained less ether extractable constituents, cholesterol, insoluble hydroxyproline and total hydroxyproline and more moisture than muscle from control pigs. Female pigs had less subcutaneous fat, larger areas of longissimus muscle than male castrates. Muscle from females contained more moisture, less ether extractable constituents and less cholesterol than muscle from male castrates. Results from this study showed beneficial improvements in increased muscle, less subcutaneous and intramuscular fat and lower cholesterol levels of beta-agonist treated pigs compared to litter mate non-treated pigs.

Influence of Age, Sex, and Feeding Regimes of Cattle on Biochemical Changes post mortem, Sarcomere Length and Water-Holding Capacity of Various Muscles

K.O. HONIKEL and K. POTTHAST

Federal Centre for Meat Research, D-8650 Kulmbach, Germany

The velocity and the extent of the post mortem breakdown of energyrich compounds in muscles of a carcass, like glycogen and ATP with its metabolites, are of paramount importance for quality characteristics of meat. Shelflife, tenderness, colour, flavour and water-holding capacity are affected. In this paper the effect of age (150 to 700 days old), sex (bulls, steers, heifers), feeding (ad libitum and restricted) on post mortem changes in about 140 animals of the breed "Deutsches Fleckvieh" and in 4 muscles (longissimus dorsi, psoas major, semitendinosus, supraspinam) are reported.

Age does not influence the velocity nor the extent of post mortem biochemical changes; drip losses in steer muscles, however, increase quite often with age. Sex of the animals has no further influence on post mortem changes and the quality characteristics measured. There exists also no influence on the characteristics by the feeding regimes used. The main differences exist between muscles. M. psoas major has the fastest post mortem changes in all age groups and sexes, M. longissimus dorsi the slowest changes. The glycogen concentration at 1 hour post mortem of M. supraspinam is with about 4 - 8 mg glycogen/g muscle only half the concentration of that of M. longissimus dorsi with 8 - 11 mg glycogen/g muscle. In both muscles, however, the pH values at 1 hour and 48 hours post mortem are similar.

The drip losses of M. supraspinam are in general lower than those of the other 3 muscles. Psoas major muscles with pH values of 5.8 and lower at 1 hour post mortem exhibit no sign of PSE characteristics.

Various methods for the determination of water-holding capacity have been employed. Drip losses at 1, 3, 6, 8 and 14 days correlate rather close with each other ($r > 0.6$), with centrifugation loss and capillary volumeter measurements, however, the relationships are rather loose ($r < 0.5$). Sarcomere length shortening has a minor influence on centrifugation loss and capillary volumeter readings.

From the results it can be concluded that the post mortem changes of cattle of various sexes and ages are similar. In comparison muscle type, chilling conditions and other exogeneous factors, applied during the period of conditioning, are likely to be more important for the quality of beef.

Meat colour in loin and ham muscles of normal meat quality from Hampshire, Swedish Landrace and Swedish Yorkshire pigs.

G. JOHANSSON, E. TORNBERG and K. LUNDSTRÖM¹

Swedish Meat Research Institute, POB 504, S-244 24 Kävlinge, Sweden

¹Department of Animal Breeding and Genetics, Swedish University of Agricultural Sciences, S-750 07 Uppsala, Sweden

The colour of pork is an important quality property, that may be influenced by the level and physical status of the meat pigment, as well as the fat content and the structure of the muscle. The aim of this study was to evaluate the colour of longissimus dorsi (LD) and biceps femoris (BF) muscles of normal meat quality from pure bred Hampshire, Swedish Landrace and Swedish Yorkshire in relation to meat pigment, fat content, ultimate pH and meat structure (FOP-values). The colour was measured as surface reflectance by a Hunterlab Color Quest Instrument using the CIELAB colour scale as well as the reflectance at different wavelengths. The relative amount of oxymyoglobin (MbO) was calculated from the reflectance values.

The highest content of pigment and fat, as well as the highest FOP value, was found in pork from the Hampshire breed. Pork from Swedish Yorkshire had a lower and pork from Swedish Landrace the lowest FOP value, pigment and fat content. The pH was lower in pork from Hampshire compared to Swedish Yorkshire and Swedish Landrace. There were colour differences between the breeds and the muscles but not between the sexes. The colour was redder and yellower in LD as well as in BF from Hampshire compared to the other two breeds, due to more MbO at the surface. The lower pH in pork from Hampshire can explain the differences in MbO. On the other hand, there were no differences between the breeds in lightness of LD and only small differences in BF. A higher pigment content was not correlated to a darker colour because the higher FOP values counteracted the higher pigment content. The colour of BF was darker and redder than the colour of LD, mainly due to a higher pigment content.

The effect of sample/water -ratio and added salt on the buffering capacity of meat

RIITTA KIVIKARI

Department of Meat Technology, University of Helsinki, SF-00710 Helsinki, Finland

The effect of sample/water -ratio used when titrating and the effect of added salt on the buffering capacity of meat was studied.

Samples of beef trimmed free of visible fat and connective tissue were used. Samples were homogenized and two 10 g aliquots were weighted out and separately homogenized with distilled water. Sample/water -ratios used were 1:10, 1:5 and 1:1. The homogenates were titrated using 0.1 N HCl and 0.1 N NaOH. The titration curve for pH range 4-9 was obtained by combining data from these two titrations.

When studying the effect of NaCl the samples were first homogenized with salt, left to equilibrate for 30 minutes and then mixed with salt solution. The concentrations of NaCl used were 1%, 2%, 3%, 4% and 5%. Sample/salt solution -ratios used were 1:10 and 1:1.

Curves were fitted to datapoints using the spline smoothing procedure (SAS/GRAPH).

With a sample/water -ratio 1:10 a buffering capacity maximum occurred around pH 6.8. When the amount of water used for diluting the sample was decreased this maximum moved to lower pH values. With a sample/water -ratio 1:1 it occurred around pH 6.4. Increasing of the NaCl concentration also caused the maximum to move to lower pH values (2% NaCl, maximum at pH 6.6, 4% NaCl, maximum at pH 6.4) and the minimum at pH 5.5 to disappear.

R.E. KLONT

Research Institute for Animal Production "Schoonoord", Dribergseweg 10 D, 3700 AM Zeist, The Netherlands

Preslaughter handling causes stress which, together with genetic components, will influence meat quality. Important meat quality parameters which are related to stress and exhaustion before slaughter are pH, rigor mortis, temperature, color and water binding capacity of the muscles. The effects of minimal stress before slaughter on these muscle quality characteristics were conducted by using a combination of azaperone and metomidate anaesthesia.

Three lines of Belgian Landrace pigs differing in their susceptibility to stress (nn, Nn and NN) were anaesthetized and kept in a "steady state" during 45 minutes. Blood-gas analysis was performed at different times. After this period the animals were bled and slaughtered. No differences could be found in the pH and the temperature at 45 minutes and 24 hours after slaughter in both the m. semimembranosus and the m. longissimus dorsi between the three genotypes. Heterozygous animals had intermediate values. Hunter L* values and drip loss were also a little bit higher for the nn-genotypes compared with the NN-pigs. However none of the examined animals showed PSE conditions.

It may be suggested that due to the low preslaughter stress the pH at 45 minutes after slaughter is high and no differences in pH can be seen between pigs differing in their susceptibility towards stress. Color and water binding capacity still seem to be related to the genetic background. More research should be performed to determine the effect of preslaughter handling and genotype on metabolism and meat quality of the different muscles.

Ultrastructural Changes in Normal and DFD Beef Muscle Tissue during Electro-Mechanical Treatment

L.S.KUDRYASHOV, L.V. GORSHKOVA

Kemerovsky Institute of Food Industry, Kemerovo, USSR

T.P. BUSLAEVA, A.S. BOLSHAKOV, V.G. BORESKOV

Institute of Applied Biotechnology, Moscow, USSR.

Results of ultrastructural research of meat, characterized by normal course of autolysis (NOR), electrostimulated and cured under conditions of electro-mechanic influence, and also of DFD beef muscle tissue, treated by sodium chloride under conditions of mechanical massaging, showed significant changes in myofibrillar structure caused by above-mentioned treatment and by proteolytic enzymes of tissue. Differences in structural changes of protein macromolecules of NOR and DFD meat in process of curing under conditions of electromassaging and further mechanical treatment evidence about destruction of myofibrillar structure about disruption of myofibrils in Z-line zone, and about damage of sarcolemma integrity. Destructive changes of DFD muscle tissue during curing with the use of mechanical massaging are less profound, this being confirmed by lesser degree of destruction of myofibrillar Z-lines.

Crosslink Type and Tensile Strength of Collagen

L.B. KURTH and D.J. HORGAN

CSIRO Division of Food Processing, Meat Research Laboratory, Cannon Hill, 4170, Australia.

In order to determine the relationship between the types of crosslinks stabilising collagen chains and the tensile strength of the collagenous tissue, bovine tendons with different collagen crosslink pathways were utilised to provide a variety of crosslink profiles and thermal stabilities. Tendons of the Psoas major muscles were divided in half, and the posterior half again bisected to yield a segment PM A (posterior portion) and a segment PM B. Longissimus dorsi (LD) tendons were also used. Tendon strips were heated in buffer for 1 h at temperatures in the range 20 to 80°C, and their tensile strengths measured after cooling to room temperature. Both PM B and LD tendon strips had significantly more tensile strength than PM A at 20 and 50°C. After heating at 60 or 80°C there were no significant differences in tensile strengths between the PM A and PM B segments. LD tendon had a lower tensile strength than PM A and PM B segments after heating at 60 and 80°C.

The concentrations of the multivalent crosslinks pyridinoline (PYR), Ehrlich chromogen (EC), and histidinohydroxymerodesmosine (HHMD) were measured in the tendons. PM B had more strength than PM A and LD tendon at 20 and 50°C because of the combined effect of the high HHMD and EC concentration. After heating at 60 and 80°C, HHMD was destroyed whereas the EC and PYR remained as heat stable crosslinks in the denatured network. LD tendon had the lowest tensile strength. PM A, with higher levels of PYR and EC had a greater mean tensile strength than PM B.

Estimation of the hydrophobicity variation of meat proteins upon thermal treatment

M.L. MARIN, C. CASAS and I. CAMBERO

Departamento de Nutrición y Bromatología III, Facultad de Veterinaria, Universidad Complutense, 28040 Madrid, Spain

Heat treatment is a very used and, sometimes, essential process to produce many meat products. However, this treatment induces protein denaturation and modifications of their functional properties. The quantitation of protein hydrophobicity and its variations by heat treatment can be an essential step for accurate prediction of meat proteins functionality.

A fluorescence probe method using 8-anilino-1-naphthalenesulphonique acid (ANS) and retinol (RET) has been used to study the effects of heat treatments on the hydrophobicity of meat proteins. The number of ANS binding sites per unit protein increased from 0.75 for unheated samples to 2.12 for meat proteins heated at 100°C for 30 min. The number of RET binding sites increased from 0.13 to 0.46, with the same heat treatment. This is discussed in terms of % increase in aromatic and aliphatic hydrophobicity of meat proteins. Linear regression analysis showed that the heat treatment was significantly correlated with the % increase of aromatic ($r=0.970$, $n=7$, $P<0.005$) and aliphatic ($r=0.985$, $n=7$, $P<0.005$) hydrophobicity of meat proteins.

The following regression equations were obtained:

$$\% \text{ increased of aromatic hydrophobicity} = -119.442 + 2.789 \text{ Heat treatment.}$$

$$\% \text{ increased of aliphatic hydrophobicity} = -183.247 + 4.313 \text{ Heat treatment.}$$

J. MIELNIK, E. POSPIECH* and K.I. HILDRUM

MATFORSK - Norwegian Food Research Institute, 1430 Ås, Norway

*Institute of Food Technology of Animal Origin, Agricultural University, Poznan, Poland

The investigation was carried out on the bovine Semitendinosus muscle, which was excised directly after slaughter from carcasses subjected to low voltage electrical stimulation (60 V, 2 min). The conditioning of the meat was performed in water of different temperatures. Four conditioning variants were applied: 30 °C, 4 hours (II), 40 °C, 4 hours (III), 50 °C, 2 hours (IV) and 60 °C, 2 hours (V). Chilled muscles were used as control samples (I).

The muscles were assessed after 2 and 7 days of storage at +4 °C. Tenderness was analysed by sensory and instrumental methods. Denaturation of proteins, both sarcoplasmic proteins and washed myofibrils, was studied by differential scanning calorimetry (DSC) and Page-SDS-electrophoresis.

Distinct improvements in meat tenderness were observed in the samples heated to 60 °C for 2 hours (V). Significant denaturation effects were noticed, particularly in sample V. Muscle weight losses were moderate. The incorporation of high temperature conditioning in hot processing of meat will be discussed.

Effects of Electrical Stimulation on Peptides, Amino Acids and ATP Related Compound of Beef Muscle

M. MIKAMI, M. SEKIKAWA and H. MIURA

Laboratory of Meat Preservation, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080, Hokkaido, Japan

During conditioning of meats, peptides and amino acids increase by the action of proteases. On the other hand ATP decrease rapidly after slaughter and IMP (inosine monophosphate) is produced. The purpose of this work is to investigate the amount of peptides, amino acids and ATP related compounds in the meats treated with electrical stimulation (ES). Holstein cows were slaughtered and ES (40 V, 13.8 Hz, 60 sec) was carried out within 5 min after slaughter. The muscle of Biceps femoris were obtained at 48 hr after slaughter. The sample for peptides and amino acids was homogenized and the sample for ATP related compounds was cut into small pieces and vacuum-packed. These samples were stored at 1°C for 7, 14 and 21 days after slaughter. Peptides were determined with the size exclusive column on HPLC and biuret method. Amino acids were determined by ninhydrin method. ATP related compounds were by reversed-phase type column on HPLC. In the supernatant of homogenate, high and low molecular peptides in ES treated muscle were larger than in control, and middle size of peptides were larger in control after 7 days. The contents of IMP at 2 days were the highest on both treatments and they decreased gradually until 21 days. However, the contents of IMP in ES muscle were higher than that of control after 10 to 21 days and the difference was about 0.6-1.0 $\mu\text{mols/g}$ between ES and control muscle. These facts mean that ES contributes to conditioning of beef.

Histological characteristics of different muscles of pigs influenced by dietary levels of lysine.

F. NICASTRO^a and G. MAIORANO^b.

^aDipartimento di Produzione Animale, 70126 Bari;

^bDipartimento di Scienze e Tecnologie Agro-Alimentari e Microbiologiche, 86100 Campobasso, Italy.

The dietary amino acid requirements for pigs are commonly expressed as a percentage of the diet. On this basis, differences in the needs of the animal for maintenance and growth are frequently ignored.

The purpose of this research was to study the influence of two levels of dietary lysine on histological characteristics of two different muscles in pigs.

Forty pigs YorkshirexHampshire (Barrows and sows) were randomly allotted to two dietary lysine levels [.65 (A) and .95 (B)]. Pigs were slaughtered at approximately 95 Kg.

Samples for histological evaluation were obtained 24h postmortem from the 10th rib longissimus thoracis (LT) and the trapezius (pars thoracica). Duplicate muscle samples were immersed in liquid nitrogen and evaluated for fiber type using enzyme substrates (DPNH-Tr and ATPase) to demonstrate presence of aerobic (β R) and anaerobic (α W) fibers. Oil-red-O was used to detect the presence of intramuscular fat cells. Photomicrographs of the muscle fiber were taken. Measurements from the photomicrographs were made and area for each cell was obtained. The sows in group A had a significantly larger ($P \leq .05$) α R fibers and conversely smaller intramuscular fat cells in LT muscle. The trapezius muscle showed in the barrows smaller β R and α W fibers ($P \leq .05$). Statistical significance for percentage of α W and α R fiber type in LT ($P \leq .05$) between the sex.

Different influences of the dietary lysine levels in the muscles and between the aerobic and anaerobic fibers.

Oxidative changes in vacuum packed cured and salted meat products

H.-J.S. NIELSEN and M.K.B. KEMNER

Biotechnical Section, The Engineering Academy of Denmark, DK-2800 Lyngby, Denmark

Objective The purpose of this study was to investigate oxidative changes developing in vacuum packed meat products, one with added nitrite, the other without nitrite addition. Further to examine any relationship between number of microorganisms and oxidative changes.

Experimental methods Two commercially produced meat products were used, a cured porc loin sliced and packaged at the manufacturer and a Bologna-type sausage made without nitrite addition. The latter was sliced in the laboratory using aseptic procedures and inoculated with a suspension comprising a "normal" bacteriological flora.

Packages of cured porc loin were stored at 5 and 10°C, and Bologna sausage at 5, 10 and 20°C.

Bacteriological examinations were done on duplicate samples homogenized in peptone-salt water using a Stomacher. Total counts and numbers of lactics were measured. Additionally, numbers of *Brochothrix thermosphacta* and Gram negative bacteria were followed on pork loins.

Oxidative changes were followed using material from the same packages as used for microbiological examinations.

Fluorescent compounds were extracted using chloroform-methanol, thiobarbituric (TBA) reactive compounds were examined using distillation methodology and total carbonyl compounds were measured as 2,4-dinitrophenylhydrazones. Further the concentration of free fatty acids were examined.

Results On porc loin, total counts, numbers of lactics and *B.thermosphacta* reached 10^5 /g within 6-7 weeks at 5°C and within 3-4 weeks at 10°C. Total counts and lactics on Bologna sausage exceeded 10^8 /g after 12 days at 5°C and within 5 days at 10 and 20°C.

TBA reactive compounds whether measured in water solution or acetic acid solution were increasing (app. 5 times) in Bologna sausage during storage. However, in porc loin, higher levels could only be measured in acid solution after 6 weeks. Levels of fluorescent compounds in the two products varied during storage.

The concentrations of total carbonyl compounds showed only minor changes during storage.

Levels of free fatty acids on Bologna sausage were app. 3-4 μ mol/g and 10 times less on porc loin. However the levels were relatively constant during storage.

Conclusion The microbial flora developed more or less as expected for these kinds of products. Oxidative changes, however, were scarce. Neither levels of free fatty acids, total carbonyl compounds or fluorescent compounds changed significantly during storage, nitrite present or absent, it may be concluded that oxidative changes are very small as only an increase, and yet at very low concentrations, in TBA reactive substances could be observed, when measured fluorometrically indicating that these kinds of products are very stable towards oxidative changes.

TAKAHIDE OKAYAMA, MINORU YAMANOUE and ISAO NISHIKAWA

Faculty of Agriculture, Kobe University, Nada-ku, Kobe 657, Japan

Two experiments were conducted to clarify the content and behavior of proteins and peptides in sarcoplasm during conditioning of beef (*M. semimembranosus*) from Japanese Black Cattle. In Experiment 1, the influence of conditioning (7, 14, 21, and 28 days at 0°C) on the content and behavior of proteins in different subcellular sarcoplasmic fractions of beef was investigated. The content of mitochondrial and lysosomal proteins decreased during conditioning. The SDS-PAGE patterns of the conditioned beef samples revealed the components of the cytoplasmic fraction which had changed. In Experiment 2, the influence of conditioning (0, 2, 4, 6, 8, 10, 14, 17, and 21 days at 4°C) on the content and behavior of peptides (phenol reagent positive materials) in the low-molecular weight sarcoplasm fraction (the dialyzate of sarcoplasm) and heated soup (supernatant solution obtained by boiling meat homogenate for 20 min) of beef was investigated. The amount of peptides in the low-molecular weight sarcoplasm fraction and soup increased during conditioning, particularly in the soup. The SDS-PAGE patterns for low-molecular weight components of the sarcoplasm and soup of the conditioned beef samples exhibited the appearance of components below 10,000 daltons. Size exclusion (SEC) high performance liquid chromatography (HPLC) profiles showed some condition sensitive fractions in the low-molecular weight sarcoplasm fraction and soup during conditioning. SEC/HPLC of the low-molecular weight sarcoplasm fraction showed that the first three elution peaks in the stage from 0 to 6 days of conditioning practically disappeared when conditioned for 8 days.

The influence of low temperature, type of muscle and electrical stimulation on the course of rigor and tenderness of two beef muscles

U. OLSSON, C. HERTZMAN and E. TORNBERG

Swedish Meat Research Institute, POB 504, S-244 24 Kävlinge, Sweden

The course of rigor, ageing and tenderness have been evaluated for two beef muscles, *M. semimembranosus* (SM) and *M. longissimus dorsi* (LD), when entering rigor at constant temperatures of 1, 4, 7 and 10°C, respectively. Additionally, the influence of low-voltage electrical stimulation was compared with non-stimulated muscle. The course of post mortem changes was registered by isometric tension shortening of unrestrained muscle strip and by following the pH-decline and the changes in metabolites such as ATP and CP. After fully developed rigor, the events during ageing at +4°C were recorded by measuring the Warner-Bratzler shear-force values 2, 8 and 15 days post mortem. The sensory properties of the cooked meat (74°C, 1 h) were assessed 15 days post mortem.

At 1 and 4°C the so called cold-shortening started immediately, in contrast to the less contracted samples at 7 and 10°C, which had a delay period of up to 10 hours. The results suggest that the shorter the time to rigor onset, the higher the ATP-level, the more shortening will occur. Tenderness decreased with increasing degree of shortening. The only effect of electrical stimulation on tenderness was obtained at 1 and 4°C for LD. The tenderness of LD was acceptable for the stimulated 4°C-samples and for all 7- and 10°C-samples. In the case of *M. semimembranosus* only the 10°C samples were tender. The results from the shear-force measurements during ageing showed that the 1°C-samples could not be tenderized when stored up to 15 days, whereas this was the case for those muscles entering rigor at +4°C.

These results suggest that for both the muscles investigated and at temperatures in the cold-shortening region the course of rigor is more important for the ultimate tenderness, than the course of ageing. However, having the same degree of shortening, LD was more tender than SM.

Effects of n-3 fatty acid supplementation on lipid composition in muscle, subcutaneous fat, liver and kidney of swine

W. OTTEN, C. WIRTH and H. M. EICHINGER

Versuchsstation Thalhausen, Techn. Univ. München, W-8051 Kranzberg, Germany

Reportedly n-3 fatty acids are discussed to prevent thrombosis and atherosclerosis in humans. They serve, as well as n-6 fatty acids, as precursors for prostanoid and leukotriene synthesis, and the extent of incorporation in membrane lipids may affect membrane fluidity and function of membrane proteins. It was the aim of this study to investigate the influence of dietary n-3 fatty acid supplementation on the fatty acid pattern in muscle, subcutaneous fat, liver and kidney of swine.

6 male German Landrace pigs received a diet supplemented with 5% fish oil rich in n-3 fatty acids (eicosapentaenoic acid, EPA, C 20:5 n-3; docosahexaenoic acid, C 22:6 n-3). Another 6 male German Landrace pigs were fed a diet counterbalanced with 5% coconut fat. The supplementations were given over a period of 13 weeks starting at an average body mass of 28.7kg. The animals were slaughtered at 100kg body mass, tissues removed and lipids extracted. Fatty acids were transesterificated to fatty acid methyl esters and analysed by Gas Chromatography.

The total lipid content of tissues did not differ between the two feeding groups. N-3 fatty acid supplementation enhanced significantly the relative amounts of EPA and DHA in all examined tissues and reduced the amounts of arachidonic acid (C 20:4) in heart muscle, liver and kidney. The relative amounts of lauric acid (C 12:0) and myristic acid (C 14:0) were also reduced in most tissues. Highest amounts of EPA and DHA were found in heart muscle, liver and kidney, where EPA ranged in the fish oil group from 12.9% to 16.1%, DHA ranged from 4.5% to 13.0%. The lowest relative amounts of n-3 fatty acids were found in the subcutaneous fat.

Our results indicate that n-3 fatty acid supplementation alters fatty acid pattern of muscles, subcutaneous fat, liver and kidney in different extents.

Phospholipid- and fatty acid pattern influenced by genetic and nutritional factors in pig heart muscle

W. OTTEN and H. M. EICHINGER

Versuchsstation Thalhausen, Techn. Univ. München, W-8051 Kranzberg, Germany

Phospholipids as the most important structural components of cell membranes are also involved in transmembranal cell signaling. It was the aim of this study to investigate the influence of n-3 fatty acid supplementation on the phospholipid composition and fatty acid pattern of pig heart muscle from different genotypes.

39 German Landrace pigs were tested to be homozygous Halothane-positive, heterozygous Halothane-negative and homozygous Halothane-negative. 18 animals received a diet supplemented with 5% fish oil rich in n-3 fatty acids (eicosapentaenoic acid, EPA, C 20:5 n-3; docosahexaenoic acid, DHA, C 22:6 n-3); the diet of the control animals was counterbalanced with 5% coconut fat. The animals were slaughtered at 100kg body mass and the left ventricle was removed. Lipids were extracted for further analysis by High Performance Liquid Chromatography and Gas Chromatography.

Halothane-positive animals showed significantly higher amounts of lyso-phosphatidylethanolamine (LPE) compared to the homozygous Halothane-negative animals. Phosphatidylethanolamine (PE) showed the reverse results for the same genotypes. The fatty acid pattern of the main phospholipids phosphatidylcholine (PC), PE and LPE was significantly different in the three genotypes. Heterozygous Halothane-negative and Halothane-positive animals showed significantly higher relative amounts of linoleic acid and lower relative amounts of arachidonic acid in these phospholipids compared with the homozygous Halothane-negative animals. N-3 fatty acid supplementation enhanced the amounts of EPA and DHA significantly whereas the amounts of arachidonic acid were significantly reduced. Halothane-positive animals showed no difference between feeding groups in their relative amount of n-3 fatty acids in PC, whereas heterozygous and homozygous Halothane-negative animals strongly depend on supplementation.

Our results indicate the influence of n-3 fatty acid supplementation on the phospholipid- and fatty acid pattern of pig heart muscle, which can be modified by the genotype.

A. OUALI, X. VIGNON and M. BONNET

Meat Research Station, INRA de Theix, 63 122 CEYRAT, France.

In beef important osmotic pressure changes occurred in postmortem muscle especially during rigor onset. The present work attempted to describe the time course change in muscle osmotic pressure in relation with pH, chilling conditions and muscle type. We also tried to identify the nature of the ions contributing mostly to these changes. Osmotic pressure was measured on intact frozen muscle samples and the value derived from the melting point of indigenous water determined by Differential Scanning Calorimetry (DSC). Muscles used were beef *Longissimus* (L), *Tensofascia latae* (TFL), *Rectus abdominis* (RA) and *Masseter* (M).

As rigor proceeds, osmotic pressure increased from the physiological value which is close to 300 milliosmoles to a final value ranging from 480 to 560-600 milliosmoles depending mainly on chilling conditions and muscle type, this maximum value being attained at the completion of the rigor process. Muscle osmolality was highly related to the rate and the extent of pH fall ($r > 0.90$); hence, pH was assumed to play a major role in the observed changes in osmotic pressure.

The rate of osmotic pressure changes decreased as temperature of rigor onset raised from 10 to 30°C. Conversely, an increase in the maximum value achieved with the temperature of rigor onset was noted for all muscles investigated especially between 15 and 30°C whereas a wholly comparable value was obtained at 10 and 20°C. This important temperature effect was strengthened by the high enthalpy of activation found between 15 and 30°C, an enthalpy estimated to about 300 Kcal/mole.

In rigor and post-rigor muscles, the maximum osmotic pressure attained greatly varied between muscles according to their metabolic and contractile types. Thus, a close relationship was observed between osmotic pressure and muscle contraction speed assessed through measurement of the Mg-Ca activated myofibrillar ATPase activity, highest values being observed in fast twitch glycolytic muscles (LD>TFL>RA>M). Concentration of various monovalent and divalent cations (Na^+ , K^+ , Mg^{++} and Ca^{++}) was measured in extractable muscle juice obtained from the different muscles by centrifugation which exhibited similar osmolality than muscle itself. Principal component and Discriminant factor analysis of the whole set of data showed that the most active ions were Na^+ , K^+ and probably Ca^{++} .

Protein Changes on Heating and Their Influence on Water Binding Capacity of Meat

E. POSPIECH* and K.O. HONIKEL

Federal Centre for Meat Research, D-8650 Kulmbach, Germany

and*) Agricultural University, PL-60-621 Poznan, Polen

Besides cellular membrane breakdown, protein changes with regard to their change in three-dimensional structures are responsible for the heating (cooking) loss of meat. Various heating regimes result in different cooking losses. The faster the heating, the lower is the cookout. The cause for this behaviour was the aim of the study.

The experiments were carried out on porcine and bovine *M. longissimus dorsi*. Pig muscles were divided into normal quality ($\text{pH}_1 > 6.0$ and $\text{pH}_{24} < 5.8$) and watery ($\text{pH}_1 < 6.0$ and $\text{pH}_{24} < 5.8$). In the case of bovine muscles normal ($\text{pH}_{24} < 5.8$) muscles were compared with DFD muscles ($\text{pH}_{24} > 6.4$). Lean meat samples 2-3 days post mortem were heated at various rates to the final temperatures ranging from 55°C to 95°C. Denaturation changes in muscle proteins with solubility changes, calorimetric studies and PAGE (molecular weight differences) were measured.

In the temperature range to 65°C on slow heating and up to 75°C on rapid heating the weight losses in the muscle were dependent mainly on the denaturation of sarcoplasmic and myofibrillar proteins. This heating range was clearly separated on protein changes from phenomena connected with tissue shortening at higher temperatures. Release of soluble proteins during the initial stage of the thermal processing had a significant effect on the water binding capacity of the muscle tissue. Myosin/actomyosin proteins released from the muscle which was rapidly heated to about 60°C, could retain their "native" features. Lower weight losses found in the rapidly heated muscles might be explained with the different denaturation patterns of proteins. The experiments indicate that the course of the thermal processes in the muscle proteins is highly diversified and dependent upon many factors associated with the physico-chemical and structural changes of the muscle as well as upon the method of the heating.

Muscle Structure, Protein Metabolism, and Nucleic Acid Content in Response to pST in Pigs

CH. REHFELDT, R. WEIKARD and K. ENDER

Research Centre of Animal Production, 0-2551 Dummerstorf, Germany

The effects of a longterm application of porcine somatotropin (pST) to Landrace pigs on the development of muscle structure characteristics, protein metabolism and nucleic acid concentrations were examined in finishing pigs from about 120 to 200 days of age. Biopsy samples from the longissimus muscle of each 60 barrows, gilts and boars were taken at the initiation of treatment and after 5 and 10 weeks. Transverse sections were reacted for DPNH-tetrazolium reductase and acid-preincubated ATPase. Furthermore samples were analyzed for cell-free translation, Ca-dependent proteases, DNA and RNA. The injection of 2 mg and 4 mg pST/d caused a stimulated hypertrophy of muscle fibres resulting in 6% to 11% thicker fibres at the end of treatment. The muscle fibre type frequencies were not affected by pST. The cell-free translation was stimulated by 10% to 30%. The proteolysis studied in barrows was reduced by 7% to 12%. In gilts higher RNA concentrations (by 16% and 26%) and RNA/DNA ratios were found after 10 week treatment. The DNA concentration, DNA/protein ratio and the muscle fibres' nucleus-plasma-ratio declined by age and remained unchanged by pST with a partly decreasing tendency.

The results suggest, that the pST induced muscle fibre hypertrophy is caused by an increase of protein synthesis, both on the level of translation and transcription. In parts proteolysis in muscle is inhibited by pST.

Post Mortem Evolution in the Pectoralis Superficialis Muscle from Two Turkey Breeds : A Relationship between PH and Colour

Véronique SANTE, G. BIELICKI, M. RENERRE and A. LACOURT

Institut National de la Recherche Agronomique, S.R.V., 63122 Ceyrat, France

An experiment was designed to evaluate the rate and the extent of *post mortem* pH fall in the *Pectoralis superficialis* muscle of turkeys from two breeds (high and low performance production) in relation to meat color.

Changes in A.T.P. and P.C. levels were followed using ³¹P N.M.R.. Colour coordinates were determined using spectrophotometry in the CIELAB system.

In the first assay, as soon as possible after slaughter, *Pectoralis superficialis* muscle of 12 week old turkeys were placed in decreasing temperature simulating slaughterhouse conditions. The initial rate of pH fall was 2,2 pHunit/hour; ultimate pH was reached 35min *post mortem*.

In the second assay, *Pectoralis superficialis* muscle of 32 week old turkeys were placed at a constant temperature of 25°C. In the high performance production breed, the rate of pH fall was 1,4 fold faster and the color less stable than in the low performance production breed. The ultimate pH was inversely correlated to redness a* (R = 0,80).

These experiments show that the onset of *rigor mortis* in the *Pectoralis superficialis* turkey muscle was extremely rapid and is similar to the onset of *rigor mortis* observed in the PSE pig muscle but with an higher ultimate pH.

F. SCHWÄGELE

Federal Centre for Meat Research, D-8650 Kulmbach, Germany

Since many years meat scientist are looking for fast and reliable methods for the detection of meat quality at various times after slaughter. Most of the methods known are either limited in their detection period to very narrow times post mortem or allow only limited conclusions on some meat quality aspects. The more reliable methods in this context are e.g. the measurement of pH, colour and electric conductivity (EC). The latter can be detected over a period of time with satisfactory results. Thus during the last few years the determination of EC became an important tool in this respect with the increase in percentage of "quality-pork" programmes offered by different cooperatives on the German meat market.

The aim of the performed studies was to investigate the influence of post mortem changes, transport and cutting on the EC measurements in pork.

In "normal" glycolizing pig carcasses the chilling rate is the most decisive factor for the resulting pork quality, as the biochemical processes in the muscles slow down with decreasing temperature. This fact is reflected in the development of the final EC readings in the primal cuts. The EC₂₄-values in loin and top round were lower (≤ 7 mS/cm) after a sufficient chilling process including a blast freezer period of 60 min than after silent and slow chilling procedures. Furthermore before transport, carcasses should be cooled down to the legally required core temperatures of 7°C.

Chilled transport at low temperatures had no influence on the resulting EC. The final EC-values in top round and loin of pig were also independent from the time of cutting (hot or cold boning) after slaughter.

Halothane and putative second messenger agents enhance the release of intracellular Ca²⁺ in hepatocytes prepared from swine susceptible to malignant hyperthermia

M.J. SEEWALD (1,*), H.M. EICHINGER (2), G. POWIS (1) and P.A. IAIZZO (3)

(1) Dep. of Pharmacology, Mayo Clinic & Foundation, Rochester MN 55905, USA; * Present address: Abt. Allgemeine Physiologie, Universitaet Ulm, 7900 Ulm, Germany; (2) Versuchsstation Thalhausen, TU-Muenchen, 8051 Kranzberg, Germany; (3) Dep. of Anesthesiology, University of Minnesota, Minneapolis, MN 55455, USA

The effects of halothane and several agents involved in intracellular signal transducing pathways on the release of Ca²⁺ were monitored in primary cultures of hepatocytes from normal swine and those susceptible to malignant hyperthermia (MH). Two different methods were used to ascertain the Ca²⁺ mobilization: 1) by measuring the release of ⁴⁵Ca²⁺ from non-mitochondrial stores of saponin-permeabilized cells; and 2) by recording changes in luminescence from intact hepatocytes loaded with the Ca²⁺-sensitive photoprotein aequorin. In general, halothane in a dose-dependent manner, induced the release of intracellular Ca²⁺. This release was increased in hepatocytes prepared from swine susceptible to MH compared to those from the control group. It was also observed that 1,4,5-inositol trisphosphate (IP₃), guanosine-5-trisphosphate and arachidonic acid all induced a significant release of ⁴⁵Ca²⁺ from permeabilized swine hepatocytes, only the quantities of ⁴⁵Ca²⁺ released by IP₃ were significant higher for the hepatocytes prepared from the susceptible animals. These data indicate an abnormal Ca²⁺ homeostasis in hepatocytes isolated from swine susceptible to MH which supports the hypothesis that membrane systems from multiple organs may be affected in MH.

Porcine skeletal muscle culture: a basic method for physiological and biochemical examinations on the cellular level

M.J. SEEWALD (1), H. BRINKMEIER (1), H.M. EICHINGER (2) and R. RÜDEL (1)

(1) Abt. Allgemeine für Physiologie, Universität Ulm, 7900 Ulm, Germany;
 (2) Versuchsstation Thalhausen, TU München, 8051 Kranzberg, Germany

Muscle cell culture has been a useful tool for the investigation of differentiation and growth of mammalian skeletal muscle. Considering the advantages of tissue culture for basic physiological and biochemical examinations in the field of animal production, we developed a method for establishing porcine skeletal muscle cultures from muscle specimens collected shortly after slaughtering. Approximately 2 g of the *m. supraspinatus* were taken and stored over night in Hanks' salt solution with 2 mM HEPES. The tissue was then mechanically dissected into small pieces and further dissociated by enzymatic treatment with a PBS solution containing 1.25 mg/ml trypsin (Difco) and 1 mg/ml collagenase (Sigma, type I). The suspension was filtered (pore size 20 μ m) for the removal of tissue fragments and subsequently centrifuged for the harvesting of isolated satellite cells. The cells were transferred into growth medium consisting of 15% fetal calf serum (Gibco) and 85% HME medium (1:1, BM 86, Boehringer Mannheim and CMRL 1415, Biochrom). After reaching confluence within 5 - 10 days, the medium was changed to HME containing 5% horse serum. During the next five days the satellite cells fused to multinucleated myotubes. In general, this method is useful to provide tissue cultures from skeletal muscle which is the major interest in meat science. E.g., it offers one chance to examine cellular mechanisms reflecting genetic differences and growth characteristics in pigs. Furthermore, the necessary muscle specimens are easily and economically obtained post mortem and therefore the method is also a very suitable one with respect to animal protection.

The influence of constant rigor temperature and storage time on the internal reflectance (FOP) in pork muscle

G. von SETH, E. TORNBORG and B-M. MÖLLER

Swedish Meat Research Institute, POB 504, S-244 24 Kävlinge, Sweden

One of the most common quality defects in pork is PSE. It occurs in muscles that have undergone excessively fast post-mortem glycolysis either due to stress in the live animal and/or due to too slow cooling of the carcass. There is a need for a fast and reliable method of registering PSE. Instruments for internal light reflectance measurements are often used and the objective of this investigation was to study the influence of rigor temperature and storage time on the internal reflectance, measured with the Fibre Optic Probe (FOP).

The influence of constant rigor temperature was studied for *M. longissimus dorsi* (LD) and *M. biceps femoris* (BF) when $pH_1 \geq 6.1$ and at 2, 12, 20, 25, 30, 35 and 39°C. The FOP-values were registered every half hour during the first 8 h p.m. and at 24 h p.m. At 24 h p.m. the pH, the drip loss and the protein solubility in high and low ionic strength were analysed, too. The influence of storage time was studied for *M. longissimus dorsi* (LD), *M. biceps femoris* (BF) and *M. semimembranosus* (SM) between 24 h p.m. and 6 days p.m.

LD developed PSE at a somewhat lower rigor temperature than BF. At 30°C PSE spots had occurred for LD, but at 39°C both LD and BF had severe PSE. It took between 4 h and 24 h to reach relative constant FOP-values. At temperatures below 25°C it took approximately 8 h and at 39°C it took on average 5 h. The soluble proteins in low ionic strength explained most of the variation in the FOP reflectance value in both BF ($R^2=80.1\%$) and LD ($R^2=65.8\%$). The simple correlations between FOP and drip loss were $r=0.66^{***}$ (LD) and $r=0.86^{***}$ (BF). The internal reflectance increased with storage time up to 6 days p.m. and the increase was greatest in LD (~ 5 units/day) and least in SM and BF (~ 2 units/day). This shows the importance of measuring the internal reflectance at the same time p.m. (>24 h) in order to get comparable values.

A trial for early prediction of PSE and DFD pigmeat by measuring pH and the percentage of PAS-positive fibres

M. SEVERINI, M. TREVISANI and A.R. LOSCHI

Ist. Ispezione degli Alimenti di O.A., Fac. Veterinaria, Università di Perugia, Italia

PSE and DFD are two of the most well known conditions which greatly affect the processing of pigmeat, especially curing and aging. Legs and loins for ham and "lonza" with either these conditions must be discarded or processed separately to avoid a very poor quality, not unacceptable defects, of the final product. Therefore, the early detection of PSE and DFD meat is of utmost importance. The present experiment was set up to evaluate the efficiency of both pH measuring and the PAS-positive fibre count at 2hr after slaughter to predict PSE and DFD conditions in swine Longissimus dorsi muscle.

Samples of L. dorsi muscle were taken from heavy pigs at a commercial slaughterhouse on the basis of the pH detected at 45 min post mortem. The samples were transported to our laboratory where pH and WHC were measured and small pieces were frozen, sectioned and stained according to PAS method to detect glycogen-containing fibres. The pH, WHC and colour were evaluated even 24hr after slaughter.

The results showed that the combined evaluation of pH and percentage of fibres with a strong and weak PAS-positive reaction at 2hr p.m. is a suitable means of predicting whether muscles at 24hr post mortem are PSE and DFD. However, a few samples did not show a very good correlation between WHC and the other parameters measured at each given time. This might cause concern in selecting the proper meat to be processed for aged hams since WHC is an essential factor affecting the curing of ham.

Encapsulation of the Cooked Cured-Meat Pigment and Irradiation of Nitrite-Free Cured Products

F. SHAHIDI¹, R.B. PEGG¹ and K. SHAMSUZZAMAN²

¹Department of Biochemistry, Memorial University of Newfoundland, St. John's, NF, Canada

A1B 3X9

²Radiation Application Research Branch, Atomic Energy of Canada Limited, Pinawa, MA, Canada

ROE 1L0

As part of a program to develop nitrite-free meat curing systems, we have stabilized the prepared cooked cured-meat pigment (CCMP) by its encapsulation in food-grade wall materials. The powdered cooked cured-meat pigment (PCCMP) so produced was stable for ≥ 18 months at refrigerated temperatures. Upon its dissolution in water or in pickle solutions, it acted as a potent colourant in nitrite-free curing systems. Meat emulsion systems which included CCMP were sterilized by a radiation process (0-10 kGy). The products so obtained had colour and flavour stability characteristics equivalent to their nitrite-cured counterparts. Thus, CCMP together with radiation sterilization may serve as a viable alternative to nitrite in the curing of meat products.

Certain influences on the histomorphological properties of muscle fibers in bulls of Simmental breed and crossbreeds with Montbeliarde breed

D.ŠKORJANC¹, A. HRASTE², I. ERŽEN³ and S. ČEPIN¹

¹Zootechnical Department, Biotechnical Fac., Univ. Ljubljana, 61230 Domžale, Yugoslavia

²Department of Anatomy, Histology and Embryology, Veterinary Fac., Univ. of Zagreb, 41000 Zagreb, Yugoslavia

³Institute of Anatomy, Medical Fac., Univ. Ljubljana, 61105 Ljubljana, Yugoslavia

Minimal muscle fiber diameter, area and extent of each muscle fiber were measured with computer - aided method. Samples were taken 24 to 35 hours after death from the middle part of musculus longissimus dorsi after 7th rib and were studied in 31 bulls of Simmental (S) and cross-breed Simmental*Montbeliarde (S*M) bulls. Muscle fibers were determined on the basis of succinat dehydrogenase activity to red - oxidative, and white glycolitic muscle fibers. On the basis of myosin ATPase activity muscle fibers were determined to type I and type II. S bulls had greater minimal diameter, area and extent of type I and red muscle fibers, as well as minimal diameter of white muscle fibers compared to S*M cross-breeds. The studied properties of red, white, type I and II muscle fibers were significantly influenced by the sires of both breeds, weight at slaughter and the interaction of breed * weight influences. The interaction of analysed place on the sample * meat % in the back, and interaction of analysed place on the sample * fat % in the back, fat % in the back and fat % in a carcass half significantly effected type II muscle fiber properties. Meat and fat % in the back significantly effected red and white muscle fiber properties, while meat and fat % in a carcass half had significant effect on white muscle fiber properties. Effects influencing the properties of type I were not the same for red muscle fibers. The same problem was between type II and white muscle fibers.

A Further Look at the Effects of Growth Hormone on Morphological Muscle Characteristics in Pigs

M.B. SOLOMON¹, N.C. STEELE², T.J. CAPERNA² and V.G. PURSEL³

U.S. Dept. of Agr., ARS, Beltsville, MD 20705 U.S.A. ¹Meat Sci. Res. Lab., PQDI, ²Nonruminant Nutr. Lab., LPSI, ³Reproduction Lab., LPSI.

Morphological parameters of the longissimus (IM) muscle from pigs treated with a) exogenous porcine growth hormone (pGH) or b) transgenic pigs expressing a bovine growth hormone gene (T-pigs) were compared. Muscle fiber differentiation was successful for pGH treated pigs, yet unsuccessful for T-pigs when treated with the combination myofibrillar (acid) ATPase and succinate dehydrogenase (SDH) staining procedure described by Solomon and Dunn (1988, J. Anim. Sci. 66:255) for porcine muscle. However, when IM samples from T-pigs were treated with the combination SDH and myofibrillar (acid) ATPase staining procedure described by Solomon and Dunn (1988, J. Anim. Sci. 66:255) for bovine muscle, effective fiber type differentiation was achieved. All three fiber types were present at varying proportions in both the pGH and T-pigs. However, classical porcine fiber arrangement (β R fibers grouped in clumps, surrounded by α R and α W fibers) was less evident for the T-pigs. Hypertrophied (giant) muscle fibers were present in the IM from the pGH treated pigs, yet were not present in T-pigs. Even though T-pigs exhibited signs of stress-sensitivity, there were no indications of pale, soft, exudative (PSE) muscle. There was no indication of stress-sensitivity for pGH treated pigs, however, a few instances of PSE were identified.

S. O.TÖMEK and M. SERDAROĞLU

Ege University, Food Engineering Department, Bornova, İzmir, Turkey

Turkish Sucuk has specific characteristics and a specific production technique. In this study, 3 different levels of carbohydrate and starter culture were practiced in the sucuk combinations consisting of 80% lean meat, 20% cattle fat and of spices like black pepper, red pepper, allspice, cumin and garlic. Starter culture mixtures of *Pediococcus cerevisia* and *Lactobasillus plantarum* were added to each group of sucuk paste in amounts respectively as 0.4 g/kg, 0.5 g/kg and 0.6 g/kg. To the control group, no starter cultures were added.

In order to show the effect of carbohydrates on sucuk fermentation, 2% lactose, 2% saccharose and 2% glucose were added to the sucuk formulation which already contain different amounts of starter cultures. In the control group, no carbohydrates were used. With the usage of different carbohydrates with different amounts of starter culture, 16 different samples were obtained. The samples were awaitened 12 hours at +4°C and then left to fermentation for 42 hours at 35°C and 85% RH. After the fermentation, they were dried for 11 days at 18°C and 60% RH.

The most significant pH decline after the fermentation and the drying was seen in the samples which contain 2% glucose. In these samples, lactic acid amount was found to be higher. It was determined that the starter culture levels and the sugar types have been very effective on the moisture levels of the samples during fermentation and drying. In the sucuk samples, the decreasing pH and the moisture content have caused the aw value to decrease. The starter culture and carbohydrate containin samples had a smaller aw value than the control samples. After the filling, the TBA values of the samples differed in the range 0.26–0.56 mg. malonaldehyde/kg samples. Within all the starter culture samples, the smallest TBA value after drying was found to be in the samples containing glucose. The usage of 2% carbohydrate that can be fermented with starter culture, have shortened the maturing period in Turkish type sucuks. It was determined that 2% glucose addition have caused the pH to decline faster. In Turkish type sucuks, with the usage of starter cultures, a controlled fermentation can be provided.

Muscle biology, post mortem muscle biochemistry and consumer meat acceptance

C. VALIN, G. MONIN, A. OUALI and M. FERRRA

Département de Technologie de la Viande, INRA THEIX, 63122 CEYRAT, France

ABSTRACTS

Regarding meat, a challenge for the ninety's is to know how research can assure survival in a consumer driven industry. It is claimed that there are at the least three factors influencing the perception consumer have of meat out of any ethical considerations. They are **quality**, **safety** and last but not the least **the value for money** which highlights the critical importance in a very concurrent market, of products of constant and certified quality which requires that control of the post mortem changes be achieved, in order to manage the variability observed between animals in their meat quality traits. In this respect, special emphasis has to be put on the post mortem period and on the long term changes affecting meat texture.

In the immediate post mortem, the main biochemical changes are the pH fall and the rigor onset. These changes are affected first by intrinsic muscle characteristics partly dependent on a genetic control and second by the physiological status of the muscle at slaughter which is deeply affected by the animal environment and by the slaughter conditions which through anoxia, nervous stimulation, increased levels of circulating hormones and muscle temperature affect rate and intensity of the pH drop.

Regarding texture, mechanisms of post mortem tenderization is far from being known. Of the different structural changes occurring post mortem only few can be related to meat tenderness. Revisiting sarcomere structure in order to provide structural functions to numerous components is necessary. The enzymology of meat tenderization necessitates in depth investigation of the proteolytic systems involved and of the control of their activities.

More than accumulation of new isolated data the need is for their integration into a coherent structure and their extrapolation towards both basic understanding and practical application.

Fractionation and characterization of proteinase inhibitors from bovine skeletal muscle.

M. ZABARI, M. BERRI, P. ROUCHON and A. OUALI

SRU INRA DE THEIX 63122 CEYRAT, FRANCE

Several proteinase inhibitors were isolated from bovine *Diaphragma* muscle by means of two chromatography steps.

The crude extract was first run on a Sephadex G100 column (100 x 5 cm), which fractionated four papain inhibiting fractions (FI,II,III,IV). Each of them was further loaded on Q Sepharose column (15x 2.4 cm) equilibrated in Tris-HCl buffer of adapted pH and proteins were eluted by a NaCl gradient (0-0.3 M). Throughout this fractionation procedure, all fractions collected were tested for their inhibitory activity against papain, trypsin and chymotrypsin. They were further analysed by SDS PAGE using either Coomassie Brilliant Blue or silver.

F-I: anionic exchange chromatography separated two fractions: (FI a) : eluted with the gradient between 0.2 and 0.25 M NaCl and inhibiting papain and trypsin; (FI b) : eluted with the gradient between 0.25 and 0.3 M NaCl and inhibiting papain, trypsin and chymotrypsin.

F- II: contained only one active fraction eluted at 0.2 M NaCl and inhibiting only papain.

On SDS PAGE, Q Sepharose fractions obtained from FI and FII show bands emerging at position between 40 KDa and 70 KDa. Further studies are now being conducted to purify and characterized these inhibitors.

F-III: was similarly separated into three fractions eluted in the gradient between 0.2 and 0.25 M NaCl and inhibiting only papain. SDS PAGE show only one band of Mr 30 KDa. The fractions were stable over a large pH range (5.0-8.0).

F-IV: anionic exchange chromatography separated three fractions:

(F-IVa): eluted at 0.1M NaCl and inhibiting most effecienly papain and showing no activity against trypsin and chymotrypsin. SDS PAGE show only one band of Mr 14 KDa; (F-IVb): eluted at 0.15 M NaCl and inhibiting highly papain and moderately trypsin. SDS PAGE show only one band of Mr 14KDa; (F-IVc): eluted at 0.2 M NaCl and inhibiting only papain. SDS PAGE show two bands of Mr 14 and 12KD.

The fractions were thermostable over a large temperature range (37-100°C).

Purification and property of glucose-phosphate isomerase from pig skeletal muscle

ZENG SHIH-YUAN

Food Science Department, Hangzhou Institute of Commerce, China

Abstract

The purification procedures and properties of glucose-phosphate isomerase (GPI) were studied. GPI is an important enzyme in carbohydrate metabolism. Based upon our previous studies and literature reports, it was admitted as a promising enzyme in study of meat quality. In order to elucidate its role in this respect, especially in finding out the biochemical mechanisms of enzyme action in meat quality variations, it is necessary to isolate and purify this enzyme in a definite quality and an appropriate quantity. We purified this enzyme from pig skeletal muscles of different anatomical locations (e.g. M.longissimi dorsi, M.psoas major, M.biceps femoris, M.quadriceps femoris, M.supraspinatus etc.) by 4-step purification methods (i.e. extraction by dilute salt solution, precipitation by organic solvents, chromatography using sephadex G-100). The results were that we obtained PAGE-pure GPI with following criteria: purification fold 10.86, specific activity 1.6×10^5 units, PAGE-spectrum showed one sole band, M.W. by SDS-PAGE method was 119,350, isoelectric point by isoelectric focusing method was 6.5-6.6, optimum temperature 35°C., optimum pH 8, Michaelis-Menton constant 6.02mM, activation energy 7735.2 cal. The GPI purified from pig skeletal muscle had antigenic property as identified by immunoelectrophoresis method with rabbit immune serum. The purification procedures are simple, easily manageable, the purity is high and the reproducibility is good. Along with those property criteria, this study makes a good basis for further research in elucidating the relationships between enzymes and meat quality.

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ZHANG HEPING, NA ERSHONG and AN YIN

Department of Food Engineering, Inner Mongolia College of Agriculture and Animal Husbandry

In this study, we determined the contents of total iron, heme iron and nonheme iron in pig longissimus dorsi (LD) and studied the effects of heating and chemical treatment on these contents. The results indicated that contents of total iron, heme iron and nonheme iron in LD were 11.59 $\mu\text{g/g}$, 8.34 $\mu\text{g/g}$ and 3.25 $\mu\text{g/g}$ fresh meat, respectively. After cooking meat at 85°C for 30, 60, and 120 min, the contents of nonheme iron increased by 7.69%, 22.46% and 52.62%, respectively. The increase of nonheme iron induced by heating was possible due to the oxidative cleavage of the porphyrin ring there by allowing release of the iron from the heme complex and this action had linear relationship with the heating time. In addition adding of sodium nitrite appears to protect against the release of iron from heme complex, but the effect was not significant ($p > 0.05$); addition of sodium chloride during the heating of meat had no remarkable effect on the content of nonheme iron ($p > 0.05$). This study can provide some information for estimating the potential bioavailability of meat iron.