SESSION 1. MUSCLE BIOLOGY AND BIOCHEMISTRY

1:1

INTERRELATIONSHIPS BETWEEN VARIOUS COMPOSITIONAL AND QUALITY PARAMETERS OF PORK FROM PURE AND CROSS-BREEDS

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Meat Quality has become a widely felt problem in Italy during the last few years in spite of the fact that pigs are slaughtered at an higher weight, which means also an older age, than elsewhere. The introduction of new breeds and breeding systems has been followed by an increase in processing difficulties of traditional pork products. An investigation has therefore been set up to study various compositional and quality parameters commonly employed for the evaluation of pork. 54 pigs of pure breed,evenly distributed between Cinta Senese, Belgian Landrace, Italian Landrace, Large White, Duroc and Pietrain, and 45 animals from crossbreeds, randomly distributed between various crosses of Italian Landrace, Large White and Duroc, were employed in the research. Type of feeding, animal age at slaughter and warm carcass weight were recorded.

In the case of pure breeds the following studies were carried out: colour assessment (Hunter L,a,b) at 90 minutes post mortem in semimembranosus muscle and at 48 hours p.m. in l. dorsi, semitendinosus, semimembranosus ans biceps femoris; pH determination at 45' p.m. in l. dorsi and semimembranosus, at 48h p.m. in l.dorsi, semitendinosus, semimembranosus and biceps femoris; proximate composition, soluble proteins and non protein nitrogen in semitendinosus; glycogen in l. dorsi and semimembranosus.
In the case of crossbreeds: colour and pH at 48 h p.m. in semitendinosus, semimembranosus and biceps femoris; proximate composition, soluble proteins and non protein nitrogen in semitendinosus.
The results suggest that final pH has a marked influence on protein solubility and non protein nitrogen. The former decreases while the latter increases with lower final pHs. Correlation coefficients and significance vary between pure and cross-breeds but are high in both taken as groups. Breed is responsible for variations of 45' and final pH, glycogen and intramuscular fat content. Among colour parameters the "L" value seems to be the most consistenly related with final pH and between thigh muscles.

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AGE-RELATED CHANGES IN THE COLLAGEN OF BOVINE CORIUM

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OBJECTIVE

Collagen constitutes the major protein component of skin, bone, tendon and other forms of connective tissue. The objective of this study was to obtain better understanding of the properties of native bovine corium collagen as related to biological aging of the tissue.

EXPERIMENTAL METHODS

Bovine skin from freshly slaughtered Holstein cattle was used. Four age groups were included: (1) fetal, (2) 3 to 6 weeks, (3) 18 months, and (4) 40 months. The effect of acid, neutral salt, and enzyme on the extractability, solubility, and molecular size distribution was investigated. Relationship to histological and ultrastructure changes was observed using transmission electron microscopy (magnification of 10,000X to 50,000X) and scanning electron microscopy (magnification of 3,000X to 25,000X).

RESULTS

Citrate-soluble collagen content of corium from fetal skin was 30.9%, while that of 3- to 6-week old calf skin was 6.4% and of 18-month old steer skin was 3.2%. Similar trends were also noted in sodium chloride--soluble and pepsin-treated, acid-soluble fractions. The greater resistance to degradation in biologically older bovine collagen is thought to be directly related to increased cross-link formation. Gel permeation chromatography, using a newly developed u-Bondagel column, was found to provide a rapid means for separation and determination of molecular size distribution.

The micrographs of fetal corium and calf corium reveal a rather loose arrangement of fibers and fiber bundles with considerable matrix spacing. This is in contrast with that of 18-month and 40-month samples which show massive, tightly-packed fiber bundles and increasing diameter of the fibrils. These results are reviewed in light of existing knowledge which has established that, with advancing age, collagen becomes increasingly more resistant to both mechanical and chemical degradation.

CONCLUSIONS

The implications of this work to those involved in the processing of bovine corium for use in preparing regenerated collagen structures are quite profound. Since the physico-chemical properties of bovine collagen are directly related to age-induced cross-links, one should be able to predict the suitability of a collagen material for use in the regenerating process. Hide corium from a very young animal would be more easily solubilized, but would lack sufficient stable, intermolecular cross-links necessary to produce a structure of relatively high tensile strength. The gel permeation chromatography method described in this study provides a rapid means for analysis of collagen solubles in native tissue. The procedure may also be useful for following the collagen solubles which develop in these tissues as a result of the processing treatments which they are subjected to.

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BIOCHEMICAL DISTINCTION OF *DIAPHRAGMA PARS LUMBALIS* FROM OTHER BEEF MUSCLES.

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The biochemical composition of the muscle *diaphragma pars lumbalis* (D)has been studied comparatively to that of other muscles of the beef carcass in order to propose a simple and accurate method for distinguishing this type of meat from other cuts of beef.

The following characteristics were considered on samples of completely trimmed muscles : amount of haem iron (Fe, in µg/g of fresh meat), frequence of each of the five isoenzymes of the LDH (ISO1, ISO2, ISO3, ISO4, ISO5), ratio of Heart to Muscle form of the LDH (H-M), amount of total nitrogen(NT), amount of soluble nitrogen (NS), amount of sarcoplasmic protein nitrogen (Nps), amount of non-proteic nitrogen (Npn), all in g of nitrogen per 100 g of fresh meat.

Analyses were made on meat taken at 3-4 days *post-mortem* from ten beef carcasses chosen to represent a commercial sample of the type of carcasses existing in the French market (carcass weight = 316.2+66.9 Kg, age = 51.2+23.7 months, conformation score (EAAP method) = 9.1+3.3).

In each carcass 26 muscle locations were analyzed.The average of the whole results of the 260 determinations for each trait was considered as representative of the "beef" composition (B).

The comparative composition of B and D was respectively for :

	Fe	NT	NS	Npn	Nps	ISO1	ISO5	H/M
for B(n=260) :	18.25,	3.428,	0.824,	0.387,	0.437,	8.42,	49.28,	0.447
for D(n=10) :	31.85,	3.012,	0.634,	0.304,	0.329,	29.93,	13.97,	1.572

The comparison between D and each of the 26 muscles showed that for the ratios Fe/NS, Fe/ISO5, ISO5/ISO1, Fe/Npn, Fe/Nps, H/M, differences were all highly significant ($P < 0.01$ to 0.001).

To distinguish D from other types of beef muscles it is suggested to use the value of the ratio :

$$\frac{ISO5 \times NS}{Fe}$$

whose value was found to be 0.278 ± 0.098 for D compared to 2.463 ± 1.134 for B.

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LACTATE DEHYDROGENASE ACTIVITY IN PORCINE MUSCLE AS INFLUENCED BY FREEZING, THAWING, AGING, CURING AND HEATING

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OBJECTIVES

Protective measures by the USDA to ensure the importation of disease-free pork products have included the acid phosphatase test to verify an internal temperature of 69°C. Adequacy of heating could be better substantiated if another test were available. The objectives of this study were to: identify and quantify porcine muscle enzymes which maintain activity up to 69°C under various processing conditions; and develop an enzyme assay to accurately determine the heating endpoint achieved.

EXPERIMENTAL METHODS

Lactate dehydrogenase (LDH) was selected from previous studies for development as a potential biological indicator to identify heating endpoints. In the first experiment, semimembranosus (SM) semitendinosus (ST), biceps femoris (BF) and rectus femoris (RF) muscles were dissected from hams (6.4 to 7.7 kg) three days post-slaughter (PS). LDH activity was monitored for the following muscle treatments: fresh at 4°C for 5 days PS; frozen at -10°C for 8 days; frozen at -10°C for 8 days, then thawed 24 hrs at 4°C; and aged at 4°C for 10 days PS. In a second experiment, four porcine muscles from three fresh hams were heated to 65°, 69° or 71°C and held for 8, 23 or 38 min. LDH activity was determined for each muscle at each temperature/time combination. For the third experiment, the SM-adductor (AD) and BF-ST muscle groups were each bisected and one half brine-cured. Both cured and uncured samples were heated to 65° or 69°C for 8 or 23 min and the LDH activity determined.

RESULTS

LDH activity rates of SM and RF muscles were 742.3 and 753.4 mol/min x g, respectively, while ST and BF muscles were slightly lower at 652.8 and 460.7 µmol/min x g. Aging the porcine muscles 10 days PS increased BF and RF activities by 61.2 and 28.6%, but had little effect on SM and ST. Freezing caused decreases in LDH activity of 32.4, 61.6, 29.1 and 53.5% for the SM, ST, BF and RF muscles. Freezing and thawing resulted in further loss of LDH activity by 76.2, 91.7, 80.2 and 64.4% for the same muscles. Heating fresh tissues for 8, 23 or 38 min at 65°C did not have an appreciable effect on LDH activity. ST, BF and RF muscle activity declined to marginal or non-detectable levels at 68°C. Curing alone had little or no effect on LDH activity in SM-AD and BF-ST muscles while the combined effect of heating (8 or 23 min) at 62°-64°C and curing lowered activities by 85.4 and 97.1%, respectively.

CONCLUSIONS

When compared to fresh tissue, aging of porcine muscles increases LDH activity rates while freezing and thawing depress activity. Heating fresh muscles (SM, ST, BF, RF) to 68°C regardless of heating duration results in virtually no LDH activity. Curing alone minimally affects LDH activity, but curing and heating cause loss of activity at 62°-64°C. Because the loss of LDH activity in cured, heated porcine tissues occurs well below the minimum endpoint of 69°C, LDH would not likely serve as a biological marker for testing imported, cooked pork.

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INVESTIGATIONS ON THE NUCLEOTIDE BREAK-DOWN AND SENSORIC AND TECHNOLOGICAL EVALUATION OF NORMAL AND PSE PIG MEAT DURING STORAGE

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Storage of meat is accompanied by a temperature dependent decrease of adenosin-nucleotides and a subsequent augmentation of inosin and hypoxanthin. Storage is also associated with variations in technological and sensoric criteria. As the original quality determines the shelflife of stored meat it was the objective of this investigation to compare the effects of different storage conditions on the nucleotide break down and further technological and sensoric parameters of normal and PSE pig meat.

Methods

From routine slaughterhouse measurements of pH and conductivity 49 pig carcasses were selected and accordingly grouped in three classes: "PSE", "intermediate" and "normal" meat quality, with pH_1-values below 5.6, from 6.0 to 5.6, and above 6.0 respectively. After cooling, 2 cm thick slices of longissimus dorsi were then distributed on 3 storage conditions: 1 day at +4°C, 5 days at +4°C and 28 days at -18°C. Subsequently from all samples the nucleotides were extracted, separated and analysed by HPLC with UV detection. Further measurements included water binding capacity (GRAU HAMM), shear values (WARNER BRATZLER), reflexionvalue (GÖFO) and weight losses during storage and heating.

A 12 persons test panel scored tenderness, juiciness, taste and overall acceptability after heating the samples to an internal temperature of 74°C.

Results and conclusions

Already 24 h p.m. AMP was below 10 ppm. IMP (3492 ppm) decreased, Inosin (404 ppm) increased during 5 days storage at +4°C, whereas freezer storage had no significant effect. Hypoxanthin level was lowest after freezer storage (49 ppm). Analysis of variance showed significant effects for storage, but not for pH class.
Technological criteria were significantly affected by both, pH-class and storage. All sensoric evaluations showed significant lower values for PSE meat. Storage at -18°C had positive effects on tenderness and overall acceptance. Shear values and scores for tenderness were closely related, but no correlations were found between the nucleotid contents and sensoric evaluations.
So after an initial postmortem phase the nucleotide breakdown is by far influenced by storage, even highly significant differences in initial pH-values do not create further differences.
Normal freezer conditions reduce nucleotide breakdown efficiently. A pronounced amelioration of sensoric evaluation especially of PSE pig meat occurs along with the freezing storage, which is independent from nucleotide pattern.

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VARYING TECHNOLOGICAL BEEF MUSCLE PROPERTIES AND THE INTRAMUSCULAR FATTY ACID PATTERN

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Fat content influences almost all technological and sensoric properties of meat. These variations occure between different muscles and can be largely modified by feeding and time of slaughter, there are also variations in the fatty acid pattern.
It was the aim of this study to investigate the effects and dependencies of the fat content and fatty acid pattern on the technological properties of different muscles of differently reared cattle.

Methods

From six early weaned and five milk replacer fed, four months old male Simmental cattle carcasses, the longissimus dorsi and the m. supraspinatus were removed and 24 h p.m. the pH, waterbinding capacity, shear value and reflexion value measured. After methanol/chloroform extraction the intramuscular fat content, and after further transesterification and gaschromatography, the fatty acid pattern was analysed.

Results and conclusions

Mean and standard deviation for intramuscular pH was 5,63 ± 0,21, for GÖFO-reflexion value 70,5 ± 16,4, for water binding (GRAU-HAMM, rel. m:f) 36 ± 5,3, and for shear value 8,6 ± 3,8. Total intramuscular fat content was 1,29 % ± 0,22. Most prominent fatty acids were C_{16} (22,1%), C_{18} (14,0%), $C_{18:1}$ (23,5%), $C_{18:2}$ (26,0%) and $C_{20:4}$ (6,9%) which accounted for 92,5 % of the whole pattern. Total fat content was different between the two muscles (1,35 vs 1,08%), and there were significant differences in their fatty acid patterns (C_{16}, C_{18}, $C_{18:2}$ and $C_{18:3}$). Also reflexion value and shear value differed between muscles. Analysis of variance confirmed a general influence of feeding on technological criteria and data of lipid analysis. Correlations were found between the reflexion values and pH (.77), as well as total fat content (-.64). Reflexion values were further correlated with the relative amounts of the C_{12} and C_{14} fatty acids. Water binding was associated with C_{12}, C_{14}, $C_{18:1}$, $C_{18:2}$ and $C_{20:4}$ and shear values showed significant correlations with C_{16}, C_{18} and $C_{18:3}$ fatty acids.

It is concluded, that the differences in technological properties and fatty acid pattern found in this material are not independent and can be largely influenced by feeding.

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Fiber Number and Type Composition in Muscle of Aging Rats

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Fisher 344 rats have a uniform genetic background, are reared under rigidly controlled conditions and are used specifically for aging studies. Histochemical (M-ATPase) fiber typing was done on extensor digitorium longus (EDL), soleus (SOL) and diaphragm (DIA) muscles at ages 3, 9, 28 and 30 months. In the EDL there was no difference in the percent of type I fibers among the four age groups, and there was no apparent trend for an increase or decrease in the percent of type IIa or IIb fibers between the four age groups. The percent of type I fibers was greater in the aged than in the younger groups in both SOL and DIA muscles. Total fiber number per cross section of muscle did not change in the EDL over this age range or in the SOL after 9 months of age. Published results generally indicate that decreasing fiber number and preferential loss of type II fibers are aging phenomena, but our results do not substantiate these previous findings.

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MODES OF ACTION OF REPARTITIONING AGENTS IN SHEEP

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There is increasing interest in the efficient production of lean meat from domestic livestock. The long term approach to this goal has been the development of genetic selection and crossbreeding programmes. The advent of transgenic animals could increase the rate of progress of such genetic programmes. However, the development of pharmaceutical compounds, known as repartitioning agents, presents a possible short term solution to the production of lean meat from existing domestic livestock.

In two previous studies, one on pen-fed weaners and another on suckling/grazing lambs, small quantities (50-100μg/day) of the β_2-adrenergic agonist, clenbuterol, were injected subcutaneously. Control animals received an equal volume of physiological saline. Clenbuterol treatment had no significant effects on growth rate and carcass weight, but the amount of fat was decreased and the amount of lean increased, both by as much as 30%, in the carcass meat of treated sheep, relative to controls (see Thornton et al. 1985).

To further study the regulatory mechanisms of clenbuterol on ovine lipid metabolism, in vivo and in vitro (isolated adipocytes) experiments were conducted. From these experiments it is evident that clenbuterol could influence carcass meat composition directly through effects on the metabolism of adipocytes, such as (1) reduced acetate incorporation into lipid (lipogenesis), (2) reduced long chain fatty acid incorporation into lipid, and (3) increased glycerol release (lipolysis), and/or indirectly by altering the serum levels of regulatory hormones, eg. increased growth hormone (5.1 vs 4.0 ng/ml) and decreased insulin levels (1.4 vs 2.0 ng/ml), in clenbuterol treated sheep.

Other workers have shown that clenbuterol has little effect on whole-body protein synthesis but reduces muscle protein catabolism and amino acid oxidation in sheep (Macrae et al. 1986). Whether or not reduced muscle protein catabolism is a direct effect of clenbuterol, or is induced by some associated hormonal response, eg. the 25% increase in growth hormone, and/or the 30% decrease in insulin level, reported here, is yet to be clarified.

It is evident that β_2-adrenergic agonists have considerable potential for the production of leaner meat from sheep. Clenbuterol treatment is an effective model for the study of metabolic interactions and repartitioning of substrates between muscle protein and adipose tissue lipid.

Macrae, J.C., Lobley, G.E., Skeme, P.A. and James, S. (1986). J. Anim. Sci. Supp. 1:453

Thornton, R.F., Tume, R.K., Payne, G., Larsen, T.W., Johnson, G.W. and Hoehenhaus, M.A. (1985). Proc. NZ Soc. Anim. Prodn. 45:97-101.

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CHANGES IN PERIRENAL FAT FROM PIGS WITH PSE AND DFD MUSCLES

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Some processes of accelerated glycolysis develop in the PSE and DFD muscles just before or during slaughter, and the quality of the muscle tissue changes chemically and physically.

The aim of this paper is to find out if the fat from pigs with normal process of glycolysis and that from pigs with PSE and DFD muscles demonstrates the same course of hydrolytic and oxidation changes during 30 days' storage.

The perirenal fat from 27 pigs of pre-slaughter weight ± 120 kg was studied. This number included 12 pigs with normal muscles, 8 pigs with PSE muscles, and 7 pigs with DFD muscles. The fat was stored for 30 days at 20°C. The oxidation and hydrolytic changes were measured 1, 10, 15, 20 and 30 days after slaughter. The basic chemical composition of the fat was determined as well as the acid number, peroxide number, TBA number higher fatty acid and higher free fatty acid content.

The water content in fat from pigs with normal course of post-slaughter glycolysis was 3,09 %, in that from PSE pigs 4,42 %, and in that from DFD pigs 6,75 %. During storage the water content decreased by about 1 % in all fat samples studied, the greatest was in fat from PSE pigs.

The determination of fat stability cleary revealed the changes due to the activity of lipases and lipooxidases. After 30 days of storage the acid number of fat from PSE pigs was higher by about 8.0 and from DFD pigs amouted to 37,7 as compared with the normal pigs. The fastest hydrolysis was obserwed in fat from pigs showing DFD meat. The oxidation processes were also faster in fat from pigs with PSE and DFD meat than in that from pigs with normal glycolysis. Untill 15 days of storage the peroxide number in fat from the PSE and DFD pigs increased by 4,4 and 6,6 and an increase of malon-aldehyde content as compared to the perirenal fat from the normal pigs was noted. After 15 days of storage the peroxide number as well as the TBA number distinctly lowered in all fat samples studied, probably due to a shift in the dynamic balance of the reaction towards decomposition and formation of secondary products resulting from further fat oxidation.

The percentage content of higher fatty acids also demonstrated some changes during storage. In the perirenal fat from normal pigs a distinct decrease in the palmitic acid content was observed while in DFD pigs, among other things, an increase of higher saturated fatty acid content accompanied by a drop in that of the unsaturated ones was found.

When compared with the perirenal fat in the normal pigs, the fat in the DFD pigs after 10 days ofstorage and the fat in the PSE pigs after 15 days of storage both demonstrated pronounced detrimental changes.

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IMMUNIZATION AGAINST 5α-ANDROSTENONE IN BOARS USING ANDROSTENONE-KLH AND GnRH-KLH AS IMMUNOGENS

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INTRODUCTION. The steroid 5α-androst-16-en-3-one (androstenone, And) is a causative factor of boar taint or sex odour in uncastrated male pigs. It accumulates in the adipose tissue during growth and may cause an offensive aroma in the meat during cooking and eating. Recent experiments aimed at reducing the accumulation of androstenone in the fatty tissue have involved immunization procedures; bovine serum albumin (BSA) was used as carrier protein, and results from that study showed a statistically significant ($P<0.05$) reduction in the mean level of androstenone in the backfat of treated boars compared with controls. In the study now reported, keyhole limpet haemocyanin (KLH) was used as carrier protein; being of crustacean origin and with molecular weight of ca 4 million it should theoretically be more immunogenic than the bovine protein (M_r ca 70,000) and hence produce a better antibody response in the boar.

In addition to immunization against androstenone, some boars were also immunized against gonadotrophin releasing hormone (GnRH). Neutralisation of GnRH causes a reduction in testosterone secretion, and like testosterone, androstenone is secreted in response to gonadotrophic stimulation, so suppression of the GnRH stimulus should reduce androstenone production.

The purpose of the study therefore was to compare the effectiveness of two immunogens, And-KLH and GnRH-KLH, singly and in combination, in the control of boar taint.

EXPERIMENTAL. Sixty Large White x Landrace boars and 20 gilts were penned in 20 littermate groups of 3 boars and one gilt. Ten boars were allocated to each of four treatments, the remaining boars and gilts being controls. The gilts were included as stimulants for the boars. The following immunization treatments were applied at 40 ± 5 days of age, followed by 'booster' at 103 ± 5 days: a) 1.0 mg And-KLH, b) 1.0 mg And-KLH + 0.5 mg GnRH-KLH, c) 0.5 mg GnRH-KLH and d) 1.0 mg GnRH-KLH. Performance during growth was recorded and, after slaughter at 95 kg weight, carcass parameters were measured.

Samples of blood at slaughter and subcutaneous fat were taken for analysis; androstenone present in the subcutaneous fat and serum were measured by an enzyme immunoassay, and the levels of testosterone, luteinising hormone and free androstenone in serum measured by radioimmunoassay.

RESULTS. The GnRH-KLH immunization treatment reduced significantly ($P<0.05$) the accumulation of androstenone in the back fat; mean values for the 0.5 mg and 1.0 mg treatment levels were 0.69 µg/g (n=10; range: ND-3.0) and 0.80 µg/g (n=10; range: ND-5.0) respectively, compared with a mean of 2.87 µg/g (n=37; range: ND-15.0) for the controls. The combined And-KLH/GnRH-KLH treatment also gave a significantly lower mean of 2.08 µg/g (n=10; range: ND-5.0), but the And-KLH treatment alone was not effective (mean 2.93µg/g; n=10; range: ND -8.5). Boars immunized with GnRH had a significantly higher feed:gain ratio than the controls and greater fat thickness at the shoulder and P2 positions.

CONCLUSION. Active immunization using KLH as carrier protein was successful when coupled with GnRH but not with androstenone; the five-fold difference in the size of the two molecules may have been partly responsible. Immunization against GnRH significantly reduced androstenone accumulation in the subcutaneous fat, but also increased fatness. Immunization with KLH-androstenone conjugate alone was not effective.

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THE SUBSTANTIATION OF THE USE OF RAW MEAT AS RELATED TO THE EXTENT AND NATURE OF AUTOLYSIS

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The purpose of the work was to study the activity of muscle proteases (as exemplified with cathepsin D) in relation to the nature and extent of meat autolysis, and to substantiate meat ageing time on the basis of the results obtained. For the experiments the l.dorsi m. from beef sides within 1 hour post slaughter was taken. In the process of meat autolysis pH values, WHC and cathepsin D activity were recorded. Histochemical detection of the acid phosphatase, the basic enzymic marker of lysosomes, was performed by the Gomori method. Previous results indicated that, for DFD- and PSE-meat, a higher proteolytic activity at the initial stage of autolysis is typocal, as compared to Normal meat, characterized with a traditional course of post-slaughter changes. The above results evidence changes in the functional activity of lysosomal enzymes, which is connected with the state of lysosomal membranes. Comparinf histochemical results on the activity of acid phosphatase and biochemical data on the activity of cathepsin D in the muscle tissue, it can be assumed that a high tissue protease activity in DFD-meat is a consequence of the metabolic stress in the live animals, which destabilizes considerably the lysosomal membranes and helps releasing higher amounts of enzymes from lysosomes. In DFD-meat a high level of muscle protein hydration is maintained throughout the test period of autolysis.

Hence, a relatively high proteolytic activity of cathepsins at the initial stage of autolysis in DFD-meat, a fast rate of attaining the maximum enzymic activity and stable processing characteristics (pH and WHC), as compared to Normal meat, allow to process DFD-meat at the early stage of autolysis (1-24 hr). Judging by cathepsin D activity, pH and WHC, it is rational to process Normal meat at the third day of autolysis.

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THE ACTIVITY OF PORK MUSCLE PROTEASES AS EFFECTED WITH SODIUM CHLORIDE

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The purpose of this work was to study the effect of sodium chloride on the free activity of porcine muscle proteolytic enzymes. Muscle cathepsin activity was determined by incubating muscle homogenates at 37°C for 1 hour; homogenates were prepared from the l. dorsi m. with or without NaCl according to Anson. Cathepsin activity was found with a standard straight line after measuring the optical density of the reaction mixture at 280 nm in a spectrophotometer. NaCl was established to inhibit cathepsin D, the greatest activity reduction being observed at the NaCl concentration of 5%. The enzyme activity is reduced by 50% at this concentration. Increasing NaCl concentration from 5 up to 15% slows down the inactivation rate, and the activity of cathepsin D is decreased by 8-15%. The analysis of the data obtained indicates the non-competing type of proteolysis inhibition in the presence of NaCl. The non-competing inhibition of pork muscle cathepsin D with NaCl shows that NaCl is linked both with the free enzyme and with the enzyme-substrate complex. Due to the fact that in this case the inhibitor differs from the substrate in its structure, it may be suggested that NaCl interacts with the centre which is different from that of substrate binding. Some authors think that enzymic activity inhibition is due to the distortion of its three-dimensional structure caused by the inhibitor. In addition, bound NaCl as an inhibitor can influence the catalytic process, screening partially the active centre of the enzyme. Non-competing inhibition is characterized with a lower maximal reaction rate as compared to that in the absence of the inhibitor.

Thus, the experimental results allow to assume that NaCl used in meat curing can inhibit the free activity of tissue proteases. NaCl was shown to be a non-competing inhibitor. The inhibition constant K_i was found to equal 1.07 M.