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ANALYSIS OF CHANGES OF THE PROTEIN ISOELECTRIC POINT OF PORK AS A FACTOR AFFECTING ITS TENDERNESS AND WATER HOLDING CAPACITY

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Introduction

One of the phenomena typical of after slaughter changes is the advancing acidification of tissue directly after slaughter followed by its alkalization. It is believed that the observed drop in the pH value is the result of meat anaerobic glycolysis, whereas the alkalization is the consequence of protein decomposition. Modern meat packaging and storage methods slow down alkalization processes but protein degradation, including, in particular, cytoskeleton proteins, continues to take place and contributes to the improvement of meat tenderness. Information characterizing changes in the protein isoelectric point is scarce, especially with regard to the products of protein degradation.

Objectives

The aim of the performed investigations was to analyze protein changes in meat stored in a cold room with regard to the protein isoelectric point (IP) and to ascertain whether the above changes are associated with changes of selected meat properties, especially tenderness and water holding capacity.

Methodology

The experimental material comprised musculus longissimus dorsi (LD), its lumbar and thoracic parts, from 34 pigs. Their origin, nutrition as well as rearing conditions were controlled and their pre-slaughter weight was about 105 kg.

The studies included both normal quality muscles as well as muscles with watery (PSE) and acid (ASE) properties. The quality classification was based on measurements of the pH value taken 45 minutes (pH_1) and 24 hours (pH_2) after slaughter and of electrical conductivity measured 90 minutes (EC_1) and 24 hours (EC_2) after slaughter. Normal quality meat (RFN) required the following characteristics: pH_1 above 5.8, whereas EC_1 and EC_2 - below 8 mS/cm. In the case of acid meat, the EC_1 limiting value was below 8 mS/cm, while meat with PSE defects was characterized by the pH_1 value below 5.8. After 24 hours, in the case of both defects, the EC_2 was above 8. The entire

muscle was divided 24 hr post mortem into portions to be analyzed 48, 120, 168 and 336hr after slaughter which were vacuum-packaged in polyamide-polyethylene bags and stored in refrigerated conditions. At each of the above-mentioned four terms, the pH value was determined and the isoelectrofocusing analysis (IEF) was performed on the proteins from centrifugal drip obtained as the result of tissue centrifugation (25 000 g; 20 min., 2^{o}C). Instrumental measurements of meat tenderness and water holding capacity as well as sensory assessment were performed after 48 and 168 hours of storage. IEF of the proteins of centrifugal drip was carried out using agarose gels (1.2%) at the pH 3-10 gradient (Pospiech et al., 2001). Before separation, samples were desalted using Sephadex G-10. The identification of titin, myosin and troponin T (Tn-T) in the centrifugal drip was confirmed using Western blotting analysis according to the method of Fritz and Greaser (1991) with one modification referring to the type of the used membrane. Samples subjected to the IEF were blotted onto nitrocellulose membrane instead of Immobilon (Fritz and Greaser 1991). Anti-myosin monoclonal (A4335 from Sigma), anti-titin (9D10) and anti-Tn-T (9D) were used as the primary antibodies. The secondary antibody was goat anti-mouse IgG (H&L) conjugated to the alkaline phosphatase from Organon Teknika. Titin 9D10 and troponin T –D10 antibodies were obtained from the Muscle Biology Laboratory University of Wisconsin.

The water binding capacity was determined by measuring the amount of weight losses of the meat samples (around 500g) stored for 48 or 168 hr at 2^{o}C. The sensory assessment was performed according to the method of linear scaling and comprised the evaluation of taste, flavor, juiciness and tenderness. It was carried out on meat heated to the temperature of 70^{o}C. Cooked samples measuring 10 x 10 mm were also used to assess tenderness with the assistance of the Instron type 1140 apparatus.

Results & Discussion

The statistical analysis of the pH values measured 45 minutes after slaughter revealed significant variations in the obtained results, depending on meat quality. The obtained mean pH_1 value at the level of 5.37 for PSE muscles was considerably lower than values observed in the case of the RFN (6.5) and ASE (6.25) muscles. On the other hand, statistically non-significant pH value relationships were found between the muscles of different quality at later terms, i.e. after 24, 48, 120, 168 and 336 hours of storage. Mean pH values ranged from 5.32 to 5.44, irrespective of the meat quality. The ASE muscles showed a tendency towards the lowest pH value, especially after 24 h storage. The results recorded at the first two terms of measurements correspond with literature data which characterize properties of the LD muscle of varying quality (Feldhusen et al. 1987, Borzuta and Pospiech 1999, Lee et al. 2000,).

It was observed that the value of the electrical conductivity, when measurements were taken 90 minutes after slaughter, was significantly diverse between the PSE muscles (14.06) and the ASE (3.75) and RFN (3.58) muscles. After 24 h of storage, differences between all quality groups were statistically significant and those values amounted to: 11.43 for the PSE meat, 9.31 – for the ASE meat and 4.57 – for the RFN meat.

Weight losses during storage were affected significantly both by the quality and time factors. In the case of the watery meat, the size of the drip, both at the first (48hr) (3.46%) as well as the second (168hr) (6.79%) term of measurement was significantly higher in

comparison with the ASE and RFN samples (respectively: 1.97% and 4.66% for the ASE and 1.34% and 3.54% - for the RFN). After 168 hr of storage, a significant increase of the juice loss from the meat tissue was observed in comparison with the first date of measurements, irrespective of the quality of the raw material.

In comparison with the normal and watery meat, acid meat, after 48hr of storage, was characterised by a slightly better tenderness (41.58N/cm^2) and its tenderness was found improved only slightly (39.51N/cm^2) after 168hr of storage. A significantly greater shear force was found for normal muscles (44.09N/cm^2) and PSE muscles (45.86N/cm^2) at the first date of measurements in comparison with the results obtained after 168hr of storage (36.32N/cm^2 and 36.04N/cm^2, respectively).

The separated protein samples were analyzed within the following 5 IP ranges, which values were adopted arbitrarily: 1 - IP<4.69; 2 - IP 4.7÷5.59; 3 - IP 5.6÷7.69; 4 - IP 7.7÷8.79; 5 - IP>8.8. Within the implemented IP ranges, either the greatest concentrations of protein bands and/or significant changes in their quantities found in the course of meat storage were observed.

It was then found that the amount of proteins from the first range increased gradually during the storage period from the value of 13.16% 48 hr after slaughter to 14.82% - 336 hours after slaughter. The statistical evaluation of the percentage share of proteins from the second IP range (4.7÷5.59) revealed significant differences between the storage times of 48 and 336 hr. In the case of the 48 hr, the mean value of the share was 24.34% and increased to the value of 27.91% at the last date of analysis. After 120 and 168 hr of storage, the mean values amounted to 24.95% and 25.62%, respectively. As regards the third range (IP 5.6÷7.69), a significant decrease in the proportion of the separated bands after 336hr storage were recorded, from 17.44% to 12.94%. In the case of two intermediate terms of analyses, identical proportions (15.94%) of these proteins were found. Proteins of the fourth IP range were characterized by the highest proportion, on average 37.6%, of that after 48hr, and it increased by about 1.8% at the last term of analyses. Bands of the fifth range with IP>8.8 constituted the lowest proportion, on average from 7.14 to 7.65% at the analyzed terms. The above remarks indicate that in the course of meat storage the greatest changes took place in the case of proteins found in the second and third range, i.e. those which were characterized by the isoelectric point at the 4.7÷7.69 interval.

In order to achieve a more comprehensive identification of the selected proteins (titin, myosin and Tn-T), western blotting was performed. It was found that these proteins were characterized by the IP of a wide range of pH values. In the case of titin, it was observed that, with the passage of meat storage time, both the number and intensity of bands with higher IP values increased. The proportion of titin bands of IP<4.69 decreased from 8.55% after 48hr storage to 1.17% at the last date of analyses. The greatest, almost double, increase in the proportion of this protein, together with the advancing process of proteolysis, was observed in the fourth IP range (7.70÷8.79). The mean share of this band amounted to 18.84% after 48hr and reached the value of 37.35% after 336hr. Western blotting with the antibody against myosin was characterized by the smallest changes in the proportions of the separated bands. The amount of proteins from the first IP range (<4.69) increased from 8.26% to 15.14% after 336hr. An increasing trend was also recorded in the second IP range (4.7÷5.59). In the case of the IP>7.7, the proportion of proteins derived from myosin was the least diversified and, on average, amounted to

41.47%. In the case of the Tn-T antibody, its bands were found a little shifted towards higher IP values during storage but this shift was somewhat different from that observed in the case of the titin. The proportion of bands with IP<4.69 decreased from 12.11% after 48hr to 2.73% after 336hr of storage. The proportion of proteins from the last range (IP>8.80) increased, in relation to their amount at the first date of analyses, by 9.5% after 120hr and by 7.22% and 7.27% at the consecutive storage periods.

The variations concerning the meat of different quality for the three proteins were relatively small. However, the above observations indicate that in the case of each of the analyzed proteins, changes in their proportions were observed within specific ranges and they were probably the result of the on-going proteolysis. It is worth noting that, in the case of titin and Tn-T, major proteins associated with the meat tenderization process, the storage process led to the decrease in the proportion of proteins with low IP and increase of those with high IP. In the case of myosin, the observed changes were, generally speaking, reverse but changes in its proportions were more restricted. The meat pH value is a resultant of actions of many factors (Pösö & Puolanne 2004). After slaughter special role may be probably played by proteins of the drip. Since the tissue pH value underwent relatively small changes in the course of the 2-week long cold storage, it can be assumed that changes associated with the protein degradation resulting in the disappearance of some and the development of others – as products of these changes – counterbalanced changes in the pH value of the tissue and, consequently, contributed to the described phenomenon. However, it did not mean that some meat properties could not undergo changes. The increased amount of the drip obtained from it could have resulted from the decreased share of myosin, or its structural sub-units, characterized by a higher IP since water holding capacity is strongly related with IP and increases as the pH value moves away from the IP of the given protein. The observed increased proportions of titin and Tn-T bands of higher IP may, in turn, indicate that tenderization leads to the liberation from the muscle structure of these proteins characterized by high IP. Perhaps these changes are associated with the changes of the PEVK region of titin, the protein responsible for actin-myosin interactions and meat tenderization (Niederlander et al. 2004, Boyer-Beri & Greaser 1998, Greaser et al., 2002). It can be presumed that their more comprehensive characterisation, both as regards the moment of their appearance as well as the determination of relationships between the occurrence of given proteins and meat properties, especially tenderness could lead to a better understanding of mechanisms affecting them.

Conclusions

IEF separations of the proteins from centrifugal drip of the pig LD muscle indicated that they were dominated by proteins with the IP ranging from 7.7÷8.79 (about 37.6% on average) and the range 4.7÷5.59 (on average about 25.7%). The smallest proportion of proteins derived from IP over 8.8 (7.4%).

In the course of the two-week long storage of meat, the greatest changes in the amount of proteins bands were observed in the range 4.7÷5.59 (increase) and 5.6÷7.69 (decrease).

Myosin immunoblotting indicated the smallest changes from among the three analyzed with regard to IP. The proportion of this protein increased for the range of IP less than 5.59 which could have been associated with the increasing drip from the tissue.

In the case of titin and troponin T, the proportion of bands with higher IP (>7.7) increased. Since meat aging is associated with fractures in the structure of these proteins, it can be presumed that a more comprehensive characterization of relationships between their appearance and meat tenderization and water holding capacity could lead to the development of markers of these processes.

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