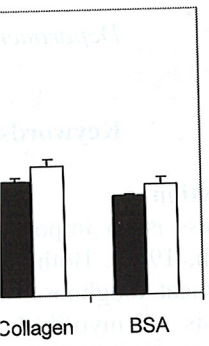

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DIFFERENTIATION OF ACID MEAT FROM OTHER QUALITY GROUPS (RFN AND PSE) THROUGH THE ANALYSIS OF PROTEINS FROM ITS DRIP

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Introduction

Processes taking place in the muscle tissue directly after slaughter cause protein changes resulting in specific sensory and functional properties of the muscle tissue. Changes in proteins in the case of muscle defects are of particular interest. The observed reduced water holding capacity in the case of PSE meat is associated primarily with protein denaturation, especially myosin (Stabursvik *et al.*, 1984) as well as with a slower degradation of cytoskeletal proteins, including, in particular, titin (Pospiech *et al.*, 2004). In the case of acid meat, which is also characterised by low water holding capacity, the degradation of titin is faster than in PSE meat (Pospiech *et al.*, 2004), but is distinguished by usually greater acidification. There is no information whether this process is associated with other proteins or if they can differentiate muscle in regard to their quality. In order to find the answer to this question, a comprehensive analysis of proteins of meat drip was performed using 2-dimensional electrophoresis (2DE) and mass spectrometry (MS).

Materials and Methods

The experimental material comprised *longissimus thoracis* and *lumborum* muscles derived from 12 pigs of known origin and pre-slaughter weight. Experiments were carried out on muscles of varying quality, which was assessed on the basis of the following measurements: pH value 45 m and 24 h after slaughter, determination of the R value which corresponds to the ratio of IMP to ATP nucleotides (Honikel and Fischer 1977) as well as the electrical conductivity (EC) measured 90 minutes and 24 h after slaughter. Employing criteria recommended by Borzuta and Pospiech (1999), the following three quality muscle groups were distinguished: RFN, PSE and ASE. Samples from the carcasses of the slaughtered pigs were collected four times: 45 m, 3, 48 and 144 h after slaughter. The samples were then frozen to allow subsequent assessment of protein changes in the drip from meat which was obtained by centrifugation of slightly defrosted meat samples, employing a method typically used to determine water holding capacity (Honikel 1998). Simultaneously, 24 hours after slaughter, muscles were cut from carcasses and divided into portions to estimate water holding capacity and meat tenderness 48 and 144 h after slaughter. Samples were vacuum packed and stored in refrigerated conditions at 2-4°C. Tenderness and water holding capacity were assessed instrumentally employing methods suggested by Honikel (1999) and Grześ *et al.* (2005) respectively. Electrophoretic analysis of muscle proteins was performed using the method of 2DE (Mikołajczak *et al.*, 2005) and the separated proteins were evaluated with the assistance of a scanning densitometer of the Image Master® VDS type. Some proteins whose presence varied in relation to the muscle quality were subjected to further analysis with the aid of a mass spectrophotometer using the MALDI-TOF method (Lametsch *et al.*, 2001) and the Bruker Autoflex mass spectrometer. Selected spots were cut from the polyacrylamide gel, digested using trypsin and their MS MALDI TOF spectra were registered. Proteins were identified on the basis of comparison of the obtained peptide maps with the maps developed for protein registered in databases. The final analysis took into consideration only those proteins for which the protein score was greater than 76 and was significant at $p<0.05$. All the results obtained were subjected to statistical single and two-factorial analysis of variance using the STATISTICA program.

Results and Discussion

Data from the Tables 1 and 2 showed that the measurement results of the pH, PE and R values differentiated the examined muscles with regard to their quality. The slowest *post mortem* changes occurred in RFN meat. The best tenderness characterised the acid meat, but the largest drip loss was observed in case of this and PSE meat (Table 3). Depending on the type of sample, 2D gels of separated proteins were revealed from about 70 to 80 spots. It was found that 14 of them were not found in each sample. However, protein "D" drew special attention (Table 4) as it was characteristic only for acid meat. It was characterised by the molecular weight ranging from 25÷30kDa and pI 9.5. It was found only in those samples which showed acid meat properties and derived from pigs which were crosses of [(Polish Landrace x Polish Large White) x (Duroc x Pietrain)]. It was not found in the meat of the Danish Landrace crossed with the Danish Yorkshire. Investigations carried out with the aid of the mass spectrometer revealed that the domains of this protein corresponded to the basic cytosolic protein (Schoentgen *et al.*, 1992) (protein score of 86). It takes part in processes associated with the transmission of neurohormonal signals, although its precise role has not been elucidated completely. The finding that the basic cytosolic protein belongs to the group of protein kinases is of particular interest. The AMP-stimulated protein kinase responsible for the development of acid meat also belongs to this group. The observed lack of significant differences in the amount of the D protein at different times after slaughter

(Table 4) may point to the fact that its occurrence is associated with the influence of a factor of pre-slaughter nature. Protein D, being characteristic for acid muscles, sets them apart from other quality groups. However, this differentiation is by no means perfect since only 67% of all muscles showing ASE meat properties contained this protein.

Table 1: Changes of pH value of various quality muscles during their storage in chilled room.

Meat quality	No. of samples	Term of measurement 45'	3 h	24 h	48 h	144 h
RFN	3	$6.66^{c}\pm0.20^{*}$	$6.35^{b}\pm0.31$	$5.70^{b}\pm0.21$	5.47 ± 0.01	$5.57^{bc}\pm0.04$
PSE	3	$5.75^{a}\pm0.06$	$5.39^{a}\pm0.10$	$5.44^{a}\pm0.05$	5.45 ± 0.03	$5.49^{ab}\pm0.04$
ASE	6	$6.17^{b}\pm0.22$	$5.76^{a}\pm0.32$	$5.39^{a}\pm0.03$	5.48 ± 0.06	$5.48^{a}\pm0.04$

* standard deviation; ^{a,b,c} - means marked with various letters differ significantly in regard to the muscle quality

Table 2: Electrical conductivity values (mS/cm) and the ratio of IMP/ATP of investigated muscles.

Meat quality	Electrical conductivity 1.5h	24h	IMP/ATP 45'
RFN	$2.72^{a}\pm0.64$	$1.56^{a}\pm0.07$	$0.98^{a}\pm0.01$
PSE	$9.07^{c}\pm3.36$	$10.10^{b}\pm3.13$	$1.11^{b}\pm0.04$
ASE	$5.18^{b}\pm1.51$	$9.47^{b}\pm1.57$	$1.02^{a}\pm0.03$

Table 4: Protein „D" content (pixels) in the centrifugal drip of acid meat.

Term of measurement	Protein D content
45 min.	52.67±60.12
3h	42.17±56.58
48h	57.00±83.91
144h	39.67±54.16

Table 3: Drip loss (%) and tenderness (N/cm^2) of meat of investigated pigs.

Meat quality	Drip loss 48h	144h	Meat tenderness 48h	144h
RFN	$0.86^{a}\pm0.22$	$1.50^{a}\pm0.47$	$62.88^{b}\pm32.87$	$45.77^{a}\pm5.65$
PSE	$3.00^{b}\pm0.51$	$5.09^{b}\pm1.30$	$55.73^{ab}\pm16.61$	$44.73^{a}\pm13.16$
ASE	$3.94^{b}\pm1.46$	$6.77^{b}\pm2.06$	$41.94^{a}\pm9.17$	$32.18^{a}\pm6.61$

Conclusions

The results obtained show that the muscle protein composition variation may be associated with the character of changes that take place after slaughter. The proteins "D" characterised by the molecular weight ranging from 25÷30kDa and pI 9.5 sets acid meat apart from other quality groups. The occurrence of this protein may be associated with pre-slaughter factors. Differentiation of acid meat is not absolute and only 67% of all muscles showing ASE meat properties contained this protein. The results obtained indicate that it is necessary to continue investigations in this area in order to find a more convincing explanation of the role of this protein in the development of acid meat.

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