DIVERSITY OF MEAT QUALITY OF HEAVY PIGS WITH RESPECT TO THE RATE OF POST-MORTEM GLYCOLYTIC AND PROTEIN CHANGES

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The aim of this study was to evaluate meat quality of first-farrowing sows differing in regard to the rate of *post-mortem* glycolytic and protein changes. Normal quality meat (RFN) (n=48) was compared with meat characterised by slower glycolytic changes (RFN-s) (n=9). The evaluation of meat quality comprised measurements of the pH value, IMP/ATP ratio, electrical conductivity, proximate composition of meat, its selected physico-chemical properties and changes of proteins in centrifugal drip (2-DE and in association with Western-blotting and MALDI-TOF MS). The results showed that different acidification rate of meat modified its physico-chemical properties. The tenderness of RFN-s meat was lower than the RFN one. Mass spectrometry measurements indicate a possible association of creatine kinase with slower pH decline. Lower acidification led to a smaller thermal drip but deteriorated tenderness of meat from heavy pigs.

INTRODUCTION

Buying meat, consumers are interested, primarily, in its sensory value. At the moment of purchase, meat quality assessment is confined to its external appearance and, as a rule, is focused on the colour, shape and meat to fat ratio of a given piece of meat. Purchase decisions are also influenced by information concerning meat nutritive and caloric value as well as health safety. With regard to fresh meat numerous authors [Gil *et al.,* 2008; Barbut *et al.,* 2008; Kristensen & Purslow, 2001] claim that the most important quality parameters for the consumer comprise: appropriate colour, tenderness, juiciness and palatability.

The key role in the process of meat tenderisation is played by protein degradation taking place after slaughter. In this respect, special role is attributed to myofibrillar protein proteolysis (actin, myosin heavy and light chains, troponin T, tropomyosin α1 and α4), including cytoskeletal proteins such as titin, nebulin, desmin, vinculin [Lametsch *et al.*, 2002, 2004]. Their degradation reduces the loss of capability to hold water in the tissue [Huff-Lonergan & Lonergan, 2005; Bee *et al.*, 2007; van de Wiel & Zhang, 2007] and, consequently, additionally influences other meat properties.

Investigations carried out in recent years have revealed that many metabolic proteins undergo changes in the course of meat storage after slaughter [Lametsch *et al.*, 2003; Jia *et al.*, 2006]. Proteases play an important role in these transformations, more precisely calpains are suspected to initiate cell structure destabilisation, while others – mainly lysosomal enzymes – are responsible for further degradation [Koohmaraie & Geesink, 2006].

Various initiatives are undertaken in order to intensify activities aiming at quality improvement of meat and to increase its quantities on the market. They are connected with securing specific genetic material as well as with ensuring appropriate conditions for animal breeding and rearing [Grześ *et al.*, 2005; Sosnicki *et al.*, 2003; Kristensen & Purslow, 2001]. Other factors such as: transport conditions, handling of animals before slaughter and stunning procedure as well as the choice of the best way – from the technological point of view – of carcasses chilling and raw material processing are also important [Łyczyński *et al.*, 2006]. Furthermore, attention is also drawn to possibilities of rearing heavier animals supplying large quantities of meat of acceptable and sometimes of special quality [Berry *et al.*, 1970; Candek-Potokar *et al.*, 1998; Kuhn *et al.*, 1997; Strzelecki *et al.*, 1999; Fischer *et al.*, 2005].

The aim of the present investigation was to evaluate meat quality of heavy pigs, more precisely first-farrowing sows differing with respect to the rate of *post-mortem* glycolytic changes, and to try and find causes of this phenomenon on the basis of protein analysis. When found, they could be used to develop quality markers which could be employed to select meat raw material in the same way as it was done in other experiments [Carbonaro, 2004; Morzel *et al.*, 2004; Mullen *et al.*, 2006]. The described investigations employed the technique of two-dimensional electrophoresis allowing observa-

tions of proteins with high molecular weight complemented by mass spectrometry to analyse selected proteins.

MATERIALS AND METHODS

Animals and preparation of muscle samples

The experimental material comprised the *Longissimus dorsi* muscle (LD) which was obtained from pigs referred to as "first-farrowing sows", *i.e.* heavy pigs which were used only once for reproduction purposes and next culled and slaughtered. Experimental pigs derived from crossings of the Large White with Polish Landrace. Both feeding and rearing conditions of the animals were controlled. Experiments were conducted on meat differing with respect to the rate of *post--mortem* glycolytic changes. The first group comprised raw material which was characterised by red, firm and non-exudative normal meat quality (RFN) (n=48). This meat was characterised by pH_1 value (45 min *post mortem – p.m.*) above 5.8 and pH_2 value (24 h *p.m.*) below 6.0. It was compared with meat characterised by slower *post-mortem* changes and which was described as RFN-s (n=9). The cut off value for pH_1 value was similar to RFN meat, but pH_2 value was above 6.0. Investigations started directly after slaughter by analysing the rate of glycolysis and collecting samples for protein studies which were continued later, on chilled raw material. The above-mentioned muscle was cut out from carcasses between the 5th thoracic and the last lumbar vertebrae on the day after slaughter, after chilling, vacuum-packed into polyamide-polyethylene bags and stored for 2 and 5 days at the temperature of 2°C.

Determination of the chemical composition of meat and its quality

Muscle tissue chemical composition (contents of water, fat, dry matter and protein) was determined after 48 and 144 h of storage. Measurements of the pH value of the muscle were taken 45 min as well as 3, 24, 48 and 144 h *post-mortem* with a portable pH-meter type Handylab 2. Electrical conductivity (EC) was estimated 90 min, 3 and 24 h *post-mortem* using an LF STAR device [Strzelecki *et al.*, 1995]. In addition the IMP/ATP ratio, characterising the rate of nucleotide changes associated with the process of glycolysis in the tissue, was measured immediately after the slaughter [Honikel & Fischer, 1977]. Additionally, objective measures of pork colour (L*, a*, b*) were performed on the LD muscle using a Minolta CR200b spectrometer (Minolta Camera, Osaka, Japan) 24 hours *p.m.* after carcass chilling. An average of two random readings on the LD were used to measure lightness (L*), redness (a*) and yellowness (b*).

Drip loss was determined as the difference in sample weight before and after storage at 48 and 144 h *p.m.* In order to determine thermal drip, 25 mm thick slices weighing around 120 g were prepared (connective tissue removed) and placed in a convection furnace of Rational Combi type. Heating was carried out in hot air at the temperature of 160°C for about 15 min, *i.e.* until the core temperature reached 72°C [Grześ *et al.*, 2005]. Tenderness was estimated on meat slices as shear force using an Instron 1140 apparatus equipped with a Warner-Bratzler attachment.

2-DE and Western blotting

The analysis was carried out on centrifugal drip proteins obtained by centrifugation (20 min, 25,000×*g*, 2°C) [Honikel, 1987] of 6 g of ground, slightly thawed meat taken from samples collected 45 min, 3, 48 and 144 h after slaughter (n=3) and immediately frozen in a liquid nitrogen. Prior to the two-dimensional separation (IEF and SDS-PAGE), the drips were desalted on mini-columns filled with the Sephadex G-10 gel, because salt deteriorates proteins separation. Before the separation, Protease Inhibitor Mix (GE Healthcare) and DTT were added to the drip. Aliquots of 4 μL of the sample were loaded to the gel and protein concentration in it was 10-15 μg/mL. The samples for investigation were selected randomly within selected groups and each sample was evaluated twice.

2-DE is a technique allowing the separation of hundreds different proteins in one gel. The proteins were separated in two dimensions: in the first dimension they were separated by a charge up in a pH 3-10 gradient according to their isoelectric point (*pI*), in the second dimension the proteins were separated according to their molecular weight (MW) by SDS-PAGE. A combination of these two separation methods results in the two-dimensional map on which proteins are visible as spots determined by their pI and MW.

The IEF separation (first dimension) was conducted on 1% (w/v) agarose gels with the assistance of FBE-3000 apparatus (Pharmacia). Electrophoretic conditions were as follows: 1200 V, 15 mA and 2 W. Protein bands separated on the pI base were further equilibrated in the pH 8.6 buffer (25 mol/L Tris, 1.92 mol/L glicyne, 3% (w/v) SDS, 0.1% (w/v) bromophenol blue sodium salt). SDS-PAGE electrophoresis was conducted in 15% (w/v) polyacrylamide gel (14.925% acrylamide, 0.075% N,N'-methylenebisacrylamide) with the addition of 8 mol/L of urea (8 mol/L urea, 2 mol/L thiourea, 0.05 mmol/L Tris, 75 mmol/L DTT, 3% (w/v) SDS, 0.05% (w/v) bromophenol blue, pH 6.8) [Pospiech *et al.*, 2000]. Ready-to-use commercial gels are 12.5% (12.125% acrylamide, 3.375% N,N'-methylenebisacrylamide). The use of agarose and further of PAGE gels with a changed ratio of T:C, enabled the observation of high molecular weight proteins because of agarose large pores and the formation of large pores in the acrylamide/methylenebisacrylamide matrix.

The separation was carried out using an SE 250 type apparatus (Hoefer Scientific Instruments). The current intensity which was applied during electrophoresis was 20 mA/gel. Proteins separated with the assistance of the above-mentioned techniques were assessed employing western blotting (semi-dry transfer) and immunostaing using titin (anti -titin clone 9D10), myosin (anti-myosin clone MF-20) and troponin-T (anti-troponin T clone 9D) monoclonal antibodies [Fritz & Greaser, 1991]. The gels stained with Coomassie blue R-250 were scanned and analysed using Image Master® 2D software. The means of two readings of the size of the spot expressed as its percentage share in relation to all proteins in the sample were used for the statistical evaluation.

MALDI-TOF MS

Selected protein spots were subjected to analysis using a MALDI-TOF mass spectrometer. To this end, stained bands were cut out from the gel, destained, exposed to reduc-

tion and alkylation reactions (DTT and iodoacetamide) and dried in a vacuum centrifuge for 20 min at the temperature of 45°C. Then, 10 μL of trypsin solution at the concentration of 10 ng/μL in 25 mmol/L NH$_4$HCO$_3$ were added to each sample, which were then incubated overnight at the temperature of 37°C. After that time, 1 μL of 100% acetonitrile was added to samples and the samples were incubated for 5 min in an ultrasound bath.

The analysis was carried out using the method of peptide mapping in a MALDI-TOF type spectrometer (Autoflex, Bruker Daltonics Company). A sample was placed on a MALDI plate and mixed with the saturated solution of the matrix (α-cyanic-4-hydroxycinnamic acid) at 1:1 ratio. Ionisation occurred following sample excitation with laser rays of 337 nm wave length. Analyses were conducted in positive ion mode employing a reflector extending the ion track. For each sample, mass spectra were registered obtained as a result of summing up at least 50 laser impulses.

Data obtained following analyses in the mass spectrometer were utilised to search available protein and genomic databases with the assistance of a MASCOT program (www.matrixscience.com). The following parameters were used for this purpose: tolerance of mass measurement error ±0.2 Da, allowable 1 place in the peptide which did not undergo enzymatic cutting, carbamide methylation of cysteine residues, possible methionine oxidation.

Statistical analysis

Data from all comparisons were evaluated using Statistica Program v. 7.1. It was used to calculate the means, standard deviations and to detect differences between groups of means values. Significant differences between them (p≤0.05) were determined with the assistance of Fischer test.

RESULTS AND DISCUSSION

Assessment of meat quality, rate of post slaughter changes and its culinary value

The meat of heavy pigs was characterised by slow after slaughter changes. That is why PSE as well as DFD meat defects were not observed. On the other hand, basically two groups of muscles differing in the rate of pH drop were observed: muscles characterised by normal (RFN) and slow acidification process. The latter muscles were designated as RFN-s, *i.e.* those in which the pH value below 6.0 appeared

slowly, only at 48 h (Table 1). The fastest rate of pH value changes was observed up to the 48 h after slaughter and these changes during the remaining 4 days of meat cold storage were relatively small. In the case of the RFN-s meat, the average pH value 45' and 24 h after slaughter was 6.71 and 6.13, respectively. These values differed significantly (p<0.05) in comparison with the average pH value 45' and 24 h after slaughter found in the RFN meat (Table 1). At consecutive terms of analysis, the acidification in the RFN-s meat continued and the final pH values obtained were typical for normal meat. Despite differences in the rate of the pH drop, the value of the IMP/ATP ratio determined 45 min after slaughter failed to differ significantly between muscles of the two compared groups. It should be mentioned here that the variation in the meat acidification rate was not caused by differences in the pre-slaughter weight of pigs as it was similar in both groups and, on average, amounted to 143-144 kg.

Values of electrical conductivity (Table 1) of the RFN meat were usually higher than of the RFN-s meat and, after 24 h, the determined differences were statistically significant. In the case of the RFN meat, electrical conductivity increased to the value of 2.86 after 24 h in relation to values registered after 3 h, whereas in the RFN-s meat – the EC dropped to the value of 1.66 reaching values similar to cattle meat or in DFD pigs [Strzelecki *et al.*, 1995]. Both values were lower in comparison with those reported by Fischer *et al.* [2005], even though the measurements concerned pigs of similar pre-slaughter weight.

Both meats were characterised by almost identical lightness, while the RFN-s meat was characterised by a smaller value of redness and yellowness (Table 1). In the case of the red colour, differences were statistically significant. In both groups, meat colour was appropriate, pink-red and without distinct discolourations. It is evident from studies conducted by Joo *et al.* [1999] that pork meat colour can be associated with denaturation of soluble proteins of sarcoplasm, primarily – phosphorylase, creatine kinase, triosephosphate isomerase and myokinase. However, the above differences are observed mainly in the case when meat defects associated with rapid acidification occur leading to the development of watery meat defect (PSE), which was not observed in this experiment.

Very small variations were also noted when analysing meat proximate composition (Table 2). It was found to contain a relatively high content of protein and small quantities of fat. The concentration of the latter meat constituent fluctuated from 1.74% to 1.97% and did not differ from values

TABLE 1. Selected meat quality traits of heavy pigs differing in the rate of *post-mortem* changes.

Meat quality	pH value of meat					IMP/ATP
	45'	3 h	24 h	48 h	144 h	
RFN	6.50^{a*}±0.22**	6.12^a±0.43	5.62^a±0.16	5.49^a±0.06	5.55^a±0.06	0.99^a±0.06
RFN-s	6.71^b±0.20	6.54^b±0.39	6.13^b±0.08	5.52^a±0.09	5.56^a±0.08	0.99^a±0.03

Meat quality	EC of meat (mS/cm)			Meat colour		
	90'	3 h	24 h	Lightness (L*)	Redness (a*)	Yellowness (b*)
RFN	3.10^a±0.94	2.56^a±1.05	2.86^b±1.26	54.65^a±3.88	3.83^b±1.67	9.49^a±1.55
RFN-s	3.00^a±1.17	2.26^a±0.83	1.66^a±0.54	54.12^a±4.79	2.37^a±1.42	8.55^a±1.00

*^{a,b} – means with different letters in the same column are significantly different (p<0.05), ** values after "±" mark represent standard deviation.

TABLE 2. Proximate composition of meat of the examined pigs at different time *post-mortem* (%).

Meat quality	48 h			
	Water	Fat	Dry matter	Protein
RFN	74.66[a]±1.05	1.81[a]±0.79	23.85[a]±1.10	22.77[b]±0.76
RFN-s	74.73[a]±0.81	1.97[a]±1.00	23.30[a]±0.44	22.09[a]±0.51

Meat quality	144 h			
	Water	Fat	Dry matter	Protein
RFN	74.33[a]±1.51	1.92[a]±1.29	24.28[a]±1.91	22.82[a]±0.66
RFN-s	74.51[a]±0.92	1.74[a]±0.83	23.75[a]±0.72	22.39[a]±0.38

*[a,b] – explanations see Table 1.

determined in light pigs slaughtered at the weight of about 100 kg [Wood *et al*., 1986, 1995].

Drip loss was slightly lower in the case of RFN-s compared to RFN meat at 48 h and 144 h *p.m.* (0.33 and 0.13% point, respectively), however these differences were not significant (Table 3). The observed lack of variability was probably connected with relatively small differences in the final acidification of both groups (in this case 48 h after slaughter) which, according to some researchers [Honikel, 1987; Joo *et al*., 1999], exert a strong impact on this meat trait.

The comparison of the size of the tissue thermal drip in the experimental groups revealed that, in the case of the RFN-s meat, it was significantly lower (Table 3) and differences between them were statistically significant and their average values, in the case of measurements taken 48 and 144 h after slaughter, reached 4.78 and 5.87%, respectively. The recorded drip levels were smaller towards the end of the aging process but the differences with respect to the second day were statistically non-significant. Smaller drips, favourable from the point of view of meat quality [Pospiech & Ender, 2006; Warner *et al*.,1997], were registered in the case of the RFN-s meat. A reverse situation was observed when meat tenderness was measured which showed that RFN-s meat was characterised by lower tenderness when compared with the RFN meat. On the first term of analysis, the mean shear value amounted to 83.24 N/cm² in the case of the RFN-s meat and was by 16.01 N/cm² higher than that determined in the RFN meat (Table 3). Following 6 days of storage, values of the shear force decreased but – in the case of the RFN-s meat – the mean value amounted to 61.85 N/cm² which shows that, on that term, this meat was still tough, even though the drop was greater than in the RFN meat (21.39 N/cm² against 15.18 N/cm²). Calculating the magnitude of the meat shear force drop during the aging period on the basis of the measurements taken at the two above-mentioned terms, it was found that it would probably be tender only after about 10 days.

A similar rate of tenderisation is usually observed in the case of cattle meat.

The obtained results indicating slower tenderisation process in the meat of the examined pigs are corroborated by the literature [Kauffman *et al*., 1964; Berry *et al*., 1970; Candek-Potokar *et al*., 1998; Kuhn *et al*., 1997; Strzelecki *et al*, 1999; Fischer *et al*., 2005]. Usually meat derived from heavier and older animals requires longer time to reach its full maturity level, especially with regard to tenderness. One of the probable causes of lower meat tenderness in heavy pigs may be greater cross-linking of the connective tissue. This phenomenon intensifies with age of animals and is observed in all species. Another cause may be associated with smaller activity of tissue enzymes. It is suggested that their activity may undergo changes depending on the intensity of processes associated with protein accumulation in the animal body [Goll, 1991; Sárraga *et al*., 1993].

Greater toughness of the raw material obtained from the heavy pigs in comparison with that obtained from standard fatteners indicates that, when it is intended to be used for culinary purposes, it should be allowed a longer period of aging. This meat could probably provide excellent raw material for the production of dry-cured meat products submitted to long ripening periods. However, the above suggestion requires more precise investigations.

Evaluation of protein separation of meat centrifugal drip on the basis of 2-DE analysis and mass spectrometry

Over 50 protein spots were identified on two-dimensional electrophoretic separations of which 15 were selected for detailed analysis (Figure 1). It should be mentioned here that
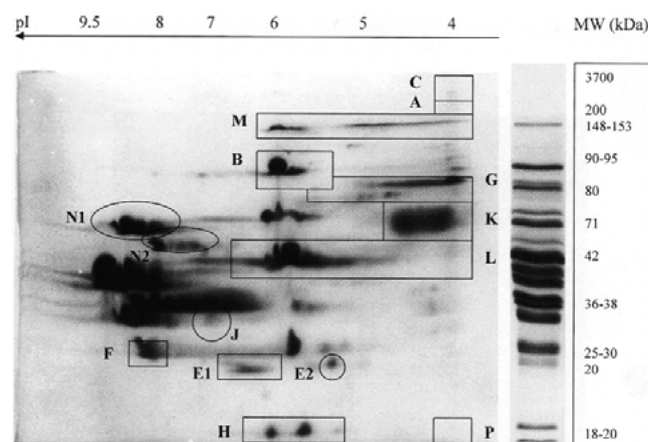

FIGURE 1. 2-DE of centrifugal drip proteins from the *Longissimus dorsi* muscle of heavy pigs (all selected proteins are marked in the text with additional subscript 2D, *e.g.* C_{2D}).

TABLE 3. Selected properties of meat from heavy pigs differing in quality.

Meat quality	Drip loss (%)		Thermal drip (%)		Shear force (N/cm²)	
	48 h	144 h	48 h	144 h	48 h	144 h
RFN	1.40[a]±1.09	2.29[a]±0.57	30.88[b]±4.00	29.24[b]±3.33	67.23[a]±17.67	52.05[a]±15.09
RFN-s	1.07[a]±0.57	2.16[a]±1.01	26.10[a]±6.98	23.37[a]±6.04	83.24[b]±32.59	61.85[a]±21.36

*[a,b] – explanations see Table 1.

the protein isolated on gel could be an aggregate containing, apart from a specific protein, also other proteins. Some blots reacted not only with one but sometimes with two or even three antibodies. This could have been caused by identical migration pathways of different proteins and/or their gradual degradation.

From among a number of proteins of the centrifugal drip which were isolated during separation with the assistance of the 2-DE, 10 protein spots were subjected to analysis with a mass spectrometer. The results obtained are shown in Table 4.

Proteins of centrifugal drip comprised mainly those which take part in cell metabolic processes such as glycolysis or fatty acid biosynthesis, because centrifugal drip contains, primarily, sarcoplasmic proteins. According to literature data, this group of proteins constitutes 25.5% – according to Bouley *et al.* [2004] of even 40% – according to Chaze *et al.* [2006] of all determined proteins and is classified most frequently with the method of mass spectrometry. However, there are also other proteins observed in the drip such as structural proteins (titin, vinculin, alpha-actinin, desmin, actin) or proteins of the contractile apparatus (myosin light chains, tropomyosin, troponin) which can appear following *post-mortem* changes [Pospiech *et al.*, 2000; Morzel *et al.*, 2004].

The largest protein whose molecular weight corresponded to 2400-3700 kDa interval and its *pI* reached 4 was the protein described as C_{2D}. Its highest proportion – 1.1% – was found in the RFN-s meat 144 h after slaughter. It was recognised as titin degradation product with the aid of the 9D10 antibody.

A similar situation was observed in the case of protein described as A_{2D} (200 kDa and *pI* – 4) (Figure 1). It was identified only during the first 3 h after slaughter and its mean share was never higher than 0.47%. This spot also reacted with the titin 9D10 antibody. Numerous researchers [Taylor *et al.*, 1995; Pospiech *et al.*, 2000] reported that titin degradation products also showed molecular weight of about 200 kDa. Studies using a mass spectrometer indicated several possible configuration corresponding to this spot, namely, a mixture of DEPGG3, AAA91804, G3P_SHEEP, Q9TUY2_PIG peptides corresponding, among others, to glyceraldehyde-3-phosphate dehydrogenase (EC 1.2.1.12) whose molecular weight is 35914, its different forms and, sometimes, from another species than swine (G3P_SHEEP). Okumura *et al.* [2003] indicate that glyceraldehyde-3-phosphate dehydroge-

nase shows an amino acid sequence that is similar to the P2 peptide which is probably a product of its hydrolysis. The P1 peptide sequence, which is a product of Tn-T degradation, is similar to P2. The sequence of this protein probably belongs to very frequently repeated sequences.

When analysing meat centrifugal drip using SDS-PAGE, the changes in the protein with mass of about 38 kDa are observed. In spectral analysis these proteins are referred to the above-mentioned dehydrogenase [Mullen *et al.*, 2000; Okumura *et al.*, 2003]. Therefore, the disappearance of proteins of about 38 kDa is associated with their degradation, which corresponds very well with the research results obtained so far indicating Tn-T as the protein undergoing degradation during storage and contributing to meat tenderisation [Okumura *et al.*, 2003].

Protein described as G_{2D} (80 kDa, *pI* 4 – 5.5) whose structure corresponds to the sequence of 6-phosphofructokinase of muscle type is probably a protein from the metabolic group. This enzyme exhibits allosteric properties, is activated by ADP, AMP or fructosebiphosphate and participates in glycolysis and phosphorylation.

Depending on meat quality, changes in the proportion of protein described as L_{2D} (42 kDa, *pI* 4÷7) were observed. In the case of the RFN meat, its share changed from 12.43 to 15.02%, respectively 45 min and 144 h after slaughter. In the case of the RFN meat, a slight change in the content of this protein was observed towards the end of the storage period from 10.75% to 10.21% on the last term of analyses. On the two last terms of analyses, differences between the two quality groups were statistically significant. The sequence of this spot revealed the highest matching to creatine kinase type M, with the matching coefficient of 166.

In the case of the spot described as M_{2D} (148-153 kDa, *pI* 4-6), generally speaking, a continuous increase of its share was observed. This protein was recognised as a peptide derived from amylo-α-1,6-glucosidase enzyme. However, this spot can also be associated with the C and M protein of myofibrils [Mikołajczak *et al.*, 2005] which control the integrity of the thick filament, although it can also be a product of its degradation [Sawdy *et al.*, 2004]. The observed growing share of this spot in the drip appears to indicate that the advancing meat tenderisation process is also probably connected with the structure loosening of this muscle filament.

TABLE 4. Main characteristics of proteins identified using mass spectrometry.

Identified spots	Protein identity	Mr[1]	pI[2]	MASCOT results[3]	Accession no.	Calculated MW[4] (kDa)
M_{2D}	Amylo-α-1,6-glucosidase	179 823	4-6	94	S38758	148-153
L_{2D}	Creatine kinase M-type	43 260	4-7	166	Q45EW9_PIG CKM	42
F_{2D}	Adenylate kinase	21 739	8	82	KIPGA_PIG	25-30
K_{2D}	Serum albumin precursor	71 362	6	315	ABPGS	75
G_{2D}	6-phosphofructokinase	85 939	4-6	104	K6PF_MOUSE	80
$E1_{2D}$	Adenylate kinase	21 739	6.5	105	KIPGA_PIG	25-30
$E2_{2D}$	Adenylate kinase	21 739	5.5	84	KIPGA_PIG	20
J_{2D}	Carbonic anhydrase 3	29 574	7	118	CAH3_PIG	35
H_{2D}	Myoglobin A	16 902	6-7	145	1MYHA	18

[1] Molecular weight of spot. [2] Calculated isoelectric point of spot. [3] MASCOT identification scores. [4] Calculated molecular weight of full-length protein.

It is evident from experiments conducted by Hwang *et al*. [2005] that the acid part of the gel is dominated by contractile proteins of the actomyosin complex, *i.e.* heavy myosin chains, light myosin chains 1 and 2, tropomyosin and actin, whereas the alkaline part – by metabolic enzymes among which we can distinguish: enolase, creatine kinase, fructose-biphosphate aldolase, glyceraldehyde-3-phosphate dehydrogenase. Among alkaline proteins, we were able to identify 6-phosphofructokinase, creatine kinase as well as carbonic anhydrase. These results are in keeping with investigations carried out by Chaze *et al*. [2006] in cattle skeletal muscles. Similarly, albumin precursor of swine serum, identified in our studies, was also found in cattle [Bouley *et al*., 2004].

2-DE separations also revealed the occurrence of proteins of similar parameters but whose shares, both during storage and depending on meat quality, varied fairly strongly although these differences were not statistically significant. These included $E1_{2D}$ ($25 \div 30$ kDa, *pI* 6.5) and $E2_{2D}$ (20 kDa and *pI* 5.5) proteins. The proportion of the first of them exhibited a declining tendency, while in the second one – a reverse dependence was noted. The first protein showed a weak reaction with myosin (light myosin chains) and titin, while the second one – rather with troponin T and titin. The growth in the proportion of Tn-T degradation products during meat storage is a typical phenomenon [Penny & Dransfield, 1979; Ho *et al*., 1994]. The amount of the so-called P1 peptide, which turned out to be troponin T degradation product, increased in the course of storage of vacuum-packed meat [Okumura *et al*., 2003]. A reaction with myosin antibody can indicate weakening in the myosin filament itself or loosening of the bond between actin and myosin which – if it did occur – would indicate an additional cause of the increased meat tenderness and water holding capacity. Both of the above proteins were recognised as peptides corresponding to adenylate kinase from pigs. Since the same matching corresponded to the F_{2D} protein, it can be presumed that the separation of these proteins can be the result of their somewhat different isoelectric point and is the consequence of after slaughter changes in meat which may affect protein changes, including their *pI*.

The amount of structural proteins titin and troponin T degradation products increased with the passage of time. From among 103 protein spots analysed by Lametsch *et al*. [2003] after 72 h of pork meat storage, 26 exhibited links with meat tenderisation. They included, among others, heavy myosin chains, titin, light myosin chain 1 and 2, CapZ protein,

cofilin 2 as well as triosephosphate isomerase I. Also actin degradation occurring during the process of meat aging can influence tenderisation [Lametsch *et al*., 2003; Hwang *et al*., 2005]. Analysing cattle meat 14 days *post mortem* Muroya *et al*. [2007] came to the conclusion that 9 fTnT (fast type) isoforms and 2 sTnT (slow-type) isoforms underwent degradation and molecule breakdown took place, primarily, in the glutamic acid-rich amino-terminal region.

Data presented in Table 5 show that percentage proportions of the L_{2D} protein (48 and 144 h after slaughter) correlated significantly with indicators of the glycolysis rate, *i.e.* changes of pH and EC values. On the other hand, significant correlations between pH determined 45 min and 24 h after slaughter and the proportion of A_{2D} protein were found 45 min and 3 h after slaughter. A very high canonical correlation coefficient for this protein was observed also in the case of thermal drips. Despite high values of canonical correlation coefficients, the relationships between percentage proportions of the separated proteins and the value of the shear force and drip losses were statistically non-significant.

The comparison of the two meat groups, varying with regard to the rate of acidification, revealed a lower thermal drip from the RFN-s meat and higher tenderness of the RFN meat, although in the case of the latter feature, the difference decreased with the duration of the meat storage process. The performed canonical analysis showed that the highest correlations were obtained for the relations between the A_{D2} protein and pH_1 and pH_2 values, although in the case of the L_{D2} protein, its proportion correlated significantly with the acidification level and EC determined 24 h after slaughter. Moreover, in the case of the A_{D2} protein, a significant correlation was also found with the size of the thermal drip on both terms of analyses. Commonly, lower meat quality is associated with accelerated or flat after slaughter changes, primarily, glycolysis. However, the course of pH changes in the swine meat with flat acidification is rather a rare phenomenon (DFD meat). Its exceptionality could consist, among others, in the fact that after the period of slowing down of the changes during the first 24 h, pH values taken at later terms are similar to those found in the RFN meat. Despite similar pH values 48 and 144 h after slaughter, water binding capacity and tenderness were different in two groups of meat from this study (Tables 1 and 3).

Referring these observations to shares of proteins determined with the assistance of the 2-DE, two proteins described as N_{2D} and L_{2D} draw our attention as their quantities varied

TABLE 5. Values of correlation (C_R) coefficients for selected proteins of the centrifugal drip analysed with the assistance of 2DE (independent variables) as well as explaining sets of variables – values of pH, EC and size of thermal drip.

Selected proteins	Sets of explaining variables				
	pH_1 (45 min), pH_2 (24 h)	pH_2, PE_{24}	Thermal drip 48 & 144 h	Shear force 144 h, thermal drip144 h	Shear force 48 & 144 h
A_{2D} protein 200 kDa, 3 h	C_R=0.9832**	C_R=0.6653	C_R=0.7091	C_R=0.6939	C_R=0.3284
A_{2D} protein 200 kDa, 45 min & 3 h	C_R=0.9844*	C_R=0.7958	C_R=0.9984**	C_R=0.9722	C_R=0.6869
L_{2D} protein 42 kDa, 48 h	C_R=0.9461*	C_R=0.9784**	C_R=0.8066	C_R=0.7906	C_R=0.3981
L_{2D} protein 42 kDa, 144 h	C_R=0.9600*	C_R=0.9662*	C_R=0.8507	C_R=0.8740	C_R=0.3710
L_{2D} protein 42 kDa, 48 & 144 h	C_R=0.9602	C_R=0.9792*	C_R=0.8874	C_R=0.9787	C_R=0.8158

*- values significantly different (P ≤ 0.05), ** – values significantly different (p≤0.01).

significantly between the meats of the two groups. Regarding the latter of the above-mentioned proteins, its quantities were smaller in the RFN meat beginning with the first hours after slaughter. The situation was slightly different in the case of the first of these proteins. Initially, its level was lower in the RFN meat but on the last term of analysis its quantities in this meat exceeded those in the RFN-s meat.

Apart from these significant differences in the share of proteins of the compared muscle groups, fairly large, albeit statistically non-significant, differences were determined in the case of M_{2D}, F_{2D} and H_{2D} proteins.

However, frequently meat quality does not depend only on changes observed at later stages of analyses but rather on these occurring shortly *p.m.* It is likely that just in the case of the RFN-s meat, the continually higher share of the L_{2D} protein found in it and determined using the mass spectrometer as creatine kinase – an enzyme whose activity is strongly associated with the rate of glycolysis [Ryu *et al.,* 2005]. Higher activity of this enzyme and lower concentration in drip loss is typical for PSE meat [Fischer & Hamm 1980]. It is possible that its higher share in RFN-s muscles was related with its lower activity leading consequently to the deceleration of glycolysis (slower pH decline). It is possible that lower acidification for a longer period of time directly after slaughter, by favouring greater calpain activity, led to more extended degradation changes in myofibrillar proteins and they resulted in a smaller thermal drip.

This hypothesis appears to be confirmed by observations made by Bee *et al.* [2007], who associated lower drip losses with higher pH values shortly *p.m.* as well as with greater desmin and talin degradation. However, from among all the above-mentioned proteins usually their higher shares (with one distinct exception of L_{2D} protein) were observed, especially during the first 48 h of storage in the case of the RFN meat whose tenderness was better and thermal drips higher in comparison with the RFN-s meat. The question is if it is possible to explain these two contradictory phenomena, *i.e.* higher protein degradation leading to meat tenderisation and at the same time leading to increase in the thermal drip from it. The explanation can be simple and relates to the level of meat acidification. Lower acidification (higher pH value) usually results in lower drip loss [Bee *et al*., 2007] and lower thermal drip. Such an effect was partly observed especially during the first 48 h also in this study. However, it is also known that lower acidification of muscles can slow down changes in connective tissue, which in turn may lower meat tenderness. This situation corresponds to the case of meat from heavy pigs, whose collagen can be more cross-linked and probably is responsible for lower tenderness of RFN-s meat 48 h after slaughter.

CONCLUSIONS

Meat chemical composition of heavy pigs is similar to that of typical fatteners and is characterised by low fat and relatively high protein contents. The results showed that this meat exhibited a different acidification rate which modified its physico-chemical properties including, in particular, tenderness and the level of thermal drip. The tenderness of meat

with slower acidification process (RFN-s) was lower than that of normal meat (RFN).

Changes in the pH as well as electrical conductivity values were correlated significantly with changes in the shares of centrifugal drip proteins A_{2D} and L_{2D} separated with the assistance of 2DE. Shares of A_{2D}, L_{2D} and N_{2D} proteins differed significantly between the meats of both quality groups; the quantity of the first of these proteins was greater in the RFN meat, of the second – smaller and of the last one – initially smaller reaching higher levels on the last term of analyses. It is likely that the greater share of the L_{2D} protein in the RFN-s meat, determined using mass spectrometry as creatine kinase, was associated with a lower pH decline.

The performed investigations indicate that lower acidification for a longer period of time directly after slaughter, perhaps by favouring higher calpain activity, led to such changes in proteins that they resulted in a smaller thermal drip of RFN-s meat. At the same time, lower acidification of muscles from heavy pigs rich in more cross-linked collagen could slow down changes in the connective tissue, which in turn might deteriorate tenderness of this type of meat.

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