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ORIGINAL PAPER

Technological and sensory pork quality in relation to muscle and drip loss protein profiles

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Abstract Fifteen meat samples collected from pigs (Neckar hybrid line) were selected from 75 animals on the basis of their technological quality traits, and the samples were classified as normal, PSE, and acid meat. Sensory analysis was performed on the three meat categories. Total meat protein and drip loss protein were analyzed by electrophoresis (SDS-PAGE), mass spectrometry, and image analysis. From a sensory point of view, PSE meat was characterized by lower color intensity, and acid meat was characterized by the lowest score of juiciness. Certain soluble proteins derived from the drip loss were associated with meat quality, especially phosphoglucomutase and the B chain of hemoglobin in the case of PSE and acid meat. Low quantities of myofibrillar proteins (myosin LC1, troponin T (TnT) and troponin C (TnC)) in meat with high glycogen levels and low pH levels resulted in a higher rate of proteolysis of myofibrillar proteins due to higher enzymatic proteolysis activity in the meat. The results of this study showed that the TnC/TnI ratio may be a pertinent marker of *postmortem* muscle metabolism and that this ratio is related to textural properties.

Keywords Pork · Electrophoresis · PSE meat · Acid meat · Drip loss

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Introduction

Pork represents 60 % of the total amount of meat (70 kg/capita/year) consumed in Poland and half of this meat is sold as culinary fresh meat. Therefore, it is important to satisfy the consumer's expectations of high quality products. The properties of meat are strictly dependent on the range and intensity of glycolytic and proteolytic changes, which are affected by the enzymes present in the sarcoplasmic fraction of meat proteins. The amount and activity of enzymes in the glycolysis pathway, and level of muscle glycogen at slaughter are conditioned by the range of glycolytic changes, which, in turn, influence the decrease in meat pH levels and activation of proteolytic enzymes. As a result of the enzyme activity in meat, new components with molecular weights of 110, 95, and 55 kDa as well as molecular weights less than 30 kDa appear in meat and they are indicators of the degree of proteolysis of myofibrillar proteins [1–4]. Generation of a protein/polypeptide profile determines many traits that influence the technological (water capacity, color intensity, color homogeneity, stability, losses in cooking, and losses in processes) and sensory qualities (appearance, tenderness, juiciness, flavor, and odor) of pork meat.

Several studies have referred to meat quality. In these studies, researchers tried to find a critical factor influencing drip loss [5–11] and tenderness [6, 11, 12] or to identify biochemical mechanisms responsible for meat color variability [11, 13, 14].

Drip loss can be predicted on the basis of pH and temperature measurements within the first 2 h *postmortem* [5].

The rate of *postmortem* pH decline influences the rate of activation/autolysis of μ-calpain, which may have a pivotal role in the degradation of proteins that tie the myofibril to the sarcolemma (such as desmin and talin) [10], which ultimately affect drip loss and the rate of *postmortem* tenderization [6–8]. Desmin is likely to be a protein marker for drip loss because its levels are higher in muscle with low drip loss [9]. Another potential marker is creatine phospho kinase (CPK). A high level of CPK has been observed in muscle with high drip loss [9]. Differences in water-holding capacity may also be related to differences in myosin HC isoforms [6, 11, 15, 16]. Muscles with the lowest percentage of myosin HC IIb and greatest percentage of myosin HC I have less drip loss [6, 17] and better tenderness, and these myosin isoform percentages in muscles are positively related to color characteristics [11]. Lactate dehydrogenase (LDH) also affects the color stability of muscles. LDH has a specific role in metmyoglobin reduction by regenerating NADH, which affects color stability [13, 14].

Moreover, the RN^- gene affects meat quality [18–20]. *Postmortem* meat is a carrier of the RN^- gene and this meat is called acid meat because it has a lower ultimate pH. Although these genes have been eliminated from the selection lines in several countries, PSE (pale, soft, exudative) and acid meat is still found (Niemyjski, personal communication).

There is currently no data on the characteristic of the protein/polypeptide profile in PSE, acid, and normal meat. Joo et al. [21] analyzed the protein compositions of PSE, RSE (reddish-pink, soft, exudative), RFN (reddish-pink, firm, non-exudative), and DFD (dark, firm, dry) meat and they reported that the degree of protein denaturation affects the drip loss and meat color. The aim of the present study was to find differences in the protein/polypeptide profile among PSE, acid, and normal meat and to analyze their relations with technological and sensory qualities. This pilot study will help to better understand the influence of range and intensity of glycolytic and proteolytic changes in meat on its culinary and technological suitability.

Materials and methods

Raw material

This study was performed on 15 pigs previously selected from 75 animals (pure Neckar hybrid line). The Neckar line was produced by the PenArLan French Company to be crossbreed with the Pietrain breed. The Neckar line is characterized by high daily gain and good carcass conformation (especially a large area of the loin "eye"), which enables the production of heavy pigs with high meatiness. Pigs originating from the herds were included in the program of elimination of disadvantageous genes' influence on meat quality ($RYR1^T$ and RN^-). Among these 75 pigs, 5 produced PSE meat and 5 produced meat with a low ultimate pH. Out of the remaining 65 pigs were selected 5 animals similar to the average of the group (typical for normal meat). All animals came from the same farm and were kept under identical environmental conditions.

All animals were transported to the meat plants with the same transport conditions. The fatteners were slaughtered at a meat plant in Stanisławowo (Poland) in accordance with legally binding procedures, including automatic electric stunning and bleeding in a horizontal position. After slaughter, the backfat thickness and *Longissimus* muscle thickness (at the height of the last rib) were measured using a CGM apparatus (Sydel, France), and the percentage of lean meat content was estimated according to Borzuta [22].

Meat quality traits

The meat quality parameters were evaluated in the samples taken from the *Longissimus* muscle. The samples were taken at the height of the last rib after the carcasses were cooled for 24 h after the slaughter. At the meat processing plants, pH measurements were taken at 45 min and 24 h *postmortem*. The samples were transported in ice-chilled polystyrene refrigerators at 4 °C. All remaining analyses were made at the laboratory. The pH value was measured at 1 (pH_1), 24 (pH_{24}), and 48 (pH_{48}) h after slaughter with a WTW 330i pH meter (Germany). Meat color was measured according to the CIE L*a*b* system using a CR310 Minolta Chroma Meter with a D_{65} light source (Osaka, Japan) at 48 h *postmortem*. The loin chops (length of 2 cm) were cut and bloomed for 1 h at 4 °C with no surface covering prior to color measurements (in triplicate). The drip loss percentage was determined 48 h after slaughter according to Prange et al. [23]. Muscle glycogen, glucose, and glucose-6-phosphate were determined according to Dalrymple and Hamm [24], and lactate was determined according to Bergmeyer [25]. The glycolytic potential (GP) was calculated according to Monin and Sellier [26]. Drip and meat samples were frozen at −80 °C until subsequent analysis.

Meat selection

The acid, PSE, and normal meat samples were grouped according to pH_1 and pH_{24} as follows: PSE meat had pH_1 values less than 6.0; acid meat had pH_1 values greater than or equal to 6.0 and pH_{24} values less than 5.5; and normal meat had pH_1 values greater than 6.0 and pH_{24} values greater than 5.5. A total of five samples per type of meat (acid, PSE, and normal) were selected according to the

method presented by Koćwiń-Podsiadła et al. [27]. In each group, there were three gilts and two hogs (castrated males).

Sensory analysis

The eating qualities were evaluated in meat aged 96 h. A meat sample (approximately 600 g) was heated in a salt solution (0.8 % NaCl) to reach a core temperature of 72 °C according to Baryłko-Pikielna et al. [28]. After cooking, the meat was cooled down at room temperature (24 °C) and prepared for sensory assessment.

The meat samples were cut into portions (cubes) of approximately equal size and weight (ca. 25 g), and the samples were then placed in plastic, odorless, and disposable boxes covered with lids. The flavor, color intensity, color homogeneity, fat perception, texture, and juiciness were evaluated according to the sensory QDA method [29] with an unstructured, linear graphical scale of 100 mm, which was later converted to numerical values (0–10 conventional units c.u.). The assessment was performed by a formally trained panel of 10 people (3–8 years of sensory evaluation practices). All samples were separately coded for the assessment using three digit codes and were passed in random order to avoid the carryover effect. The condition and assessment mode were determined in accordance with Meilgaard et al. [30].

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

SDS-PAGE of drip loss and muscular tissue was performed according to the method of Bollag and Edelstein [31] using the STANDARD system (Kucharczyk TE, Poland). Proteins were resolved on a 12 % separation gel and 5 % stacking gel. Myofibrillar proteins were extracted from 20 mg of muscle, homogenized with 800 µL of a Tris–HCl buffer (pH 6.8) containing 0.375 M 2-mercaptoethanol, 3 % SDS, 8 M urea, and 2 M thiourea. Muscle protein concentration was determined as total nitrogen using the AOAC method [32]. The concentration of soluble protein from the drip loss was determined using the Biuret procedure. Myofibrillar and soluble proteins from the drip loss were dissolved 1/1 (v/v) in Tris–HCl sample buffer (pH 6.8) containing 0.375 M 2-mercaptoethanol, 3 % SDS, 8 M urea, 2 M thiourea, and 0.05 % bromophenol blue. The mixture was then heated for 3 min at 95 °C, and 25 µL of the mixture was then placed in each well. Gels were first run for approximately 1 h at 75 V followed by 5 h at 150 V. Gels were stained with Coomassie Brilliant Blue R250. Image analysis and quantification were performed using GelScan v. 1.45 software (Kucharczyk TE, Poland).

Protein identification by mass spectrometry

Coomassie-stained spots of interest (W12) were manually removed using pipette tips. The gel spots were then destained with 100 µL of 25 mM NH_4HCO_3 with acetonitrile 95/5 (v/v) for 30 min followed by two washes in 100 µL of 25 mM NH_4HCO_3 with acetonitrile 50/50 (v/v). The gel spots were then dehydrated in 100 % acetonitrile. Gel spots were completely dried using a Speed Vac before trypsin digestion at 37 °C for 5 h with 15 µL of trypsin (10 ng/µL; V5111, Promega) in 25 mM NH_4HCO_3. Peptide extraction was optimized by adding 8 µL of acetonitrile, followed by 10 min of sonication. Peptide mass identification of trypsin-digested spots was attempted using nano LC–ion trap MS/MS analysis. HPLC was performed with an ULTIMATE LC SYSTEM combined with a Famos autosampler and Switchos II microcolumn switching for preconcentration (LC Packings, Amsterdam, The Netherlands). The supernatant-containing peptides (6 µL) was loaded on the PEPMAP C18 column (5/75 µm ID/15 cm column; Dionex, Labège, France) using a preconcentration step in a microprecolumn cartridge (300 µm ID/1 mm) at 30 µL/min. After 3 min, the precolumn was connected to the separating column and the gradient was commenced at 200 nL/min. The following buffers were used: (A) 5 % ACN and 0.5 % HCOOH in water; and (B) 5 % H_2O and 0.5 % HCOOH in ACN. A linear gradient from 10 to 90 % B for 45 min was applied. For ion trap MS, an LCQ DECA with a nanoelectrospray interface (Thermo Fisher Scientific, Les Ulis, France) was used. Ionization (2.2 kV ionization potential) was performed with a liquid junction and a non-coated capillary probe (New Objective, Cambridge, USA). Peptide ions were analyzed by the data-dependent "triple play" method as follows: (1) full MS scan (m/z 400–1,400), (2) zoomscan (scan of the major ion with larger resolution), and (3) MS/MS of this ion. Identification of peptides was performed with Mascot 2.2 restricting the taxonomy to *mammalia* (20080417 and 1177111 sequences) in the National Center for Biotechnology Information (NCBI) nr protein database. Mass deviation tolerance was set at 1.5 Da for parent ions and 0.8 for fragment ions. Protein identification was validated when at least two peptides originating from one protein showed significant identification scores.

Data analysis

All values were reported as the means ± standard deviation (SD). A one-way analysis of variance of the type of meat as a fixed effect was performed. Pearson correlation coefficients between meat quality parameters and protein quantification obtained by electrophoresis were measured. Statistical analysis was conducted with Statistica 9.0 software (Stat Soft, Inc. version 9.0).

Results and discussion

Meat quality

All the pigs showed a high level of meatiness (58.5 %) with a medium hot carcass weight of 90 kg. This carcass characteristic is close to the one of fatteners obtained from crossing Polish Landrace and Polish Large White sows with P76-PenArLan hybrid boars and which were used to obtain the Neckar line [33].

Meat quality defects are strongly linked to major genes in pigs and environmental conditions. Despite that the pigs originated from herds belonging to the program of elimination of disadvantageous genes' influence on meat quality ($RYR1^T$ and RN^-), approximately 10 % of the 75 meat samples were classified as PSE and a similar percentage was found for acid meat. However, PSE meat and meat with low pH levels have been observed in races lacking these two genes [17, 19, 34, 35], which may have been due to stress conditions of preslaughter trade, slaughter, and feeding [19, 36]. The electrical low voltage stunning may contribute to the occurrence of PSE meat [37, 38]. The meat quality parameters for acid, PSE, and normal meat are shown in Table 1. The rate of pH (pH_1) differed according to meat category. As expected, PSE had the highest rate of pH decline. The ultimate pH was the lowest for acid meat with PSE being in the middle between acid and normal meat. These results were in accordance with the higher GP measured in acid meat and confirmed the negative relationship between glycogen levels and ultimate pH. Compared to the other meat types, more lactate was produced during *postmortem* glycolysis and fourfold more residual glycogen was present in *postmortem* acid meat (Table 1). Similar results were reported by Immonen [39] and Przybylski et al. [40] who reported that high glycogen content just after slaughter leads to a higher degree of acidification. At the same time, however, a considerable amount of residual glycogen was observed. Despite the absence of the RN^- gene, there was relatively high GP in the studied groups. Resenvold et al. [36] reported that the level of glycogen at slaughter is largely dependent on animal nutrition. In addition, there have been recent reports about the impact of other genes on the level of glycogen and GP. Sieczkowska et al. [35] showed a significant effect of PKM2 gene (pyruvate kinase muscle gene in the glycolytic pathway that catalyzes the conversion of phosphoenolpyruvate into pyruvate, which is later reduced to lactate in anaerobic conditions) on GP, pH, and drip loss. The average GP values reported by these authors for each of the three possible genotypes were similar to those obtained in this study. Moreover, Kamiński et al. [34] reported the effect of the DECR1 on GP, glycogen metabolism, and meat quality and the reported values of these parameters were similar to those obtained in the present study. DECR1 is a nuclear gene encoded by a mitochondrial enzyme that participates in the fatty acid beta-oxidation pathway. According to Stefanon et al. [41], DECR1 is involved in the control of fat and protein deposition. In Landrace pigs, Amills et al. [42] showed the effect of DECR1 on *Longissimus thoracis* pH, lightness, and redness of meat. Kamiński et al. [43] also showed the effects of DECR1 on the growth rate of Landrace boars.

With regard to the color parameters (L*, a*, and b*), the luminosity of normal meat differed from the luminosity of the acid and PSE meat. Moreover, a lighter intensity of color was observed for the PSE and acid meat compared to the normal meat. Lightness of meat color is characteristic of PSE and acid meat [21] and is partly explained by the degree of pH decline [19, 20, 26, 27].

The drip loss varied according to *postmortem* metabolism as a result of ATP degradation and the rate of acidification, and the drip loss also varied with the chilling conditions. Compared to other studies in pigs [15, 21, 44], the measured drip loss remained low. The drip loss was lower than 3 % in normal meat, and the increase was by about 1.08 and 0.71 % for PSE and acid meat, respectively (Table 1). Among the quality classes, however, there was no significant difference in the size of drip loss. Similar ultimate pH, glycogen level, and drip loss results were obtained by Josell et al. [19] in pigs carrying the RN^- gene and pigs lacking the RN^- gene. Similarly, Choe et al. [17] reported that the drip loss results in pigs at high and low GP with low lactic acid and varying ultimate pH levels are not significantly different. However, Van Oeckel et al. [44] reported a significant difference (approximately 20 %) in drip loss between normal and PSE meat.

Sensory attributes

The results of the sensory analysis are shown in Table 2. The three types of meat did not differ in terms of odor or color homogeneity. The PSE meat had lower color intensity, compared to the acid and normal meat. The juiciness was lower in the acid meat compared to the other meat types, and the tenderness did not differ between the studied materials (Table 2). The literature concerning the relationship between ultimate pH and tenderness and the relationship between ultimate pH and juiciness is controversial. Josell et al. [18] and Miller et al. [45] showed that meat with low pH is characterized by higher tenderness and juiciness, but Le Roy et al. [46] reported a negative effect of low ultimate pH on the tenderness of meat. Moreover, Toldrá and Flores [47] did not find any effect of ultimate pH on the tenderness or juiciness of meat. Josell et al. [18] reported that the higher tenderness and juiciness values of meat from pigs with higher GP (RN^- carriers) are

Table 1 Meat quality traits from normal, PSE, and acid meat (Mean value ± SD of five separate samples)

	Meat quality classes			
	Normal	PSE	Acid	*P*
pH_1	$6.68^{c} \pm 0.15$	$5.89^{b} \pm 0.07$	$6.35^{a} \pm 0.21$	0.01
pH_{24}	$5.64^{c} \pm 0.04$	$5.46^{b} \pm 0.08$	$5.35^{a} \pm 0.03$	0.01
pH_{48}	$5.61^{c} \pm 0.05$	$5.44^{b} \pm 0.08$	$5.33^{a} \pm 0.03$	0.01
Glycogen (μmol/g)	$3.3^{b} \pm 1.09$	$8.1^{a} \pm 5.54$	$12.9^{a} \pm 2.31$	0.01
Lactate (μmol/g)	$104^{b} \pm 7.80$	$104^{b} \pm 4.10$	$116^{a} \pm 5.40$	0.01
GP (μmol/g)	$111^{b} \pm 8.50$	$120^{b} \pm 14.40$	$142^{a} \pm 6.15$	0.01
Color coordinates				
L*	$51.45^{b} \pm 1.39$	$54.34^{a} \pm 2.84$	$56.20^{a} \pm 0.66$	0.01
a*	15.84 ± 0.97	16.57 ± 0.88	16.37 ± 1.33	NS
b*	4.69 ± 0.22	5.24 ± 1.63	5.92 ± 0.63	NS
Drip loss (%)	2.89 ± 0.86	3.91 ± 1.26	3.60 ± 0.82	NS

[a,b,c] Means with different superscript show significant differences (at $P \leq 0.05$)

Table 2 Means of sensory attributes of cooked normal, PSE, and acid meat (Mean value ± SD of five separate samples)

Sensory attributes (0–10 c.u.)	Meat quality classes			
	Normal	PSE	Acid	*P*
Odor of cooked meat	7.7 ± 0.27	7.8 ± 0.21	8.0 ± 0.22	NS
Acid odor	2.4 ± 0.39	2.4 ± 0.12	2.6 ± 0.24	NS
Fatty odor	2.3 ± 0.65	3.0 ± 1.00	2.1 ± 0.48	NS
Another odor	1.3 ± 0.04	1.4 ± 0.12	1.3 ± 0.08	0.06
Color intensity	$8.4^{a} \pm 0.18$	$8.1^{b} \pm 0.12$	$8.7^{a} \pm 0.29$	0.02
Homogeneity of color	8.1 ± 0.45	7.7 ± 0.42	8.5 ± 0.46	0.09
Tenderness	7.4 ± 1.09	7.6 ± 0.20	7.6 ± 1.01	NS
Juiciness	$6.4^{b} \pm 1.04$	$6.9^{b} \pm 0.25$	$4.9^{a} \pm 0.70$	0.02
Flavor of cooked meat	7.6 ± 0.47	7.6 ± 0.21	7.6 ± 0.36	NS
Acid flavor	2.2 ± 0.43	2.4 ± 0.06	2.5 ± 0.39	NS
Fatty flavor	2.2 ± 0.61	2.7 ± 0.59	1.8 ± 0.10	0.10
Salty flavor	1.8 ± 0.24	1.9 ± 0.35	2.0 ± 0.27	NS
Another flavor	1.2 ± 0.19	1.5 ± 0.06	1.2 ± 0.17	0.08
Overall quality	7.6 ± 0.79	7.3 ± 0.53	6.9 ± 0.65	NS

[a,b] Means with different superscript show significant differences ($P \leq 0.05$)

attributed to the lower isometric tension and higher enzymatic activity, which increase the aging rate and myofibrillar fragmentation. 1 and 4 days *postmortem* meat from RN^- carriers has significantly shorter myofibrils than meat from non-carriers RN^-, which indicates higher proteolytic activity early *postmortem* in the RN^- carriers. The flavor intensity and overall meat quality of the three meat types were similarly estimated.

Protein markers of meat qualities

SDS-PAGE analysis of proteins from muscle tissue

Figure 1 shows the protein profile from muscle tissue in normal, PSE, and acid meat. Table 3 shows the quantification for each protein band. Myosin, α actinin, actin, troponin T (TnT), tropomyosin, myosin LC1, troponin I (TnI), troponin C (TnC), and myosin LC2 represented 15.3, 6.3, 17.1, 7.1, 8.0, 3.0, 1.8, 1.6, and 3.3 %, respectively. The contents of these particular proteins were lower than the values determined by Kołczak et al. [48] for calf, heifer, and cow meat. The amount of these proteins was dependent on their origin and varied between 26.0 and 30.6 % for myosin HC, between 4.0 and 5.9 % for α actinin, and between 18.5 and 21.8 % for actin. The lower amount protein may be due to the fact that the tested samples in the present study contained both myofibrillar proteins and sarcoplasmic proteins, whereas the samples tested by Kołczak et al. [48] contained only purified myofibrillar proteins. Moreover, these differences may be due to the different origin of muscular tissue because the composition of meat protein is dependent on the age of animal, type of muscle, and time of frozen storage [48]. The residual products of the proteolytic changes during

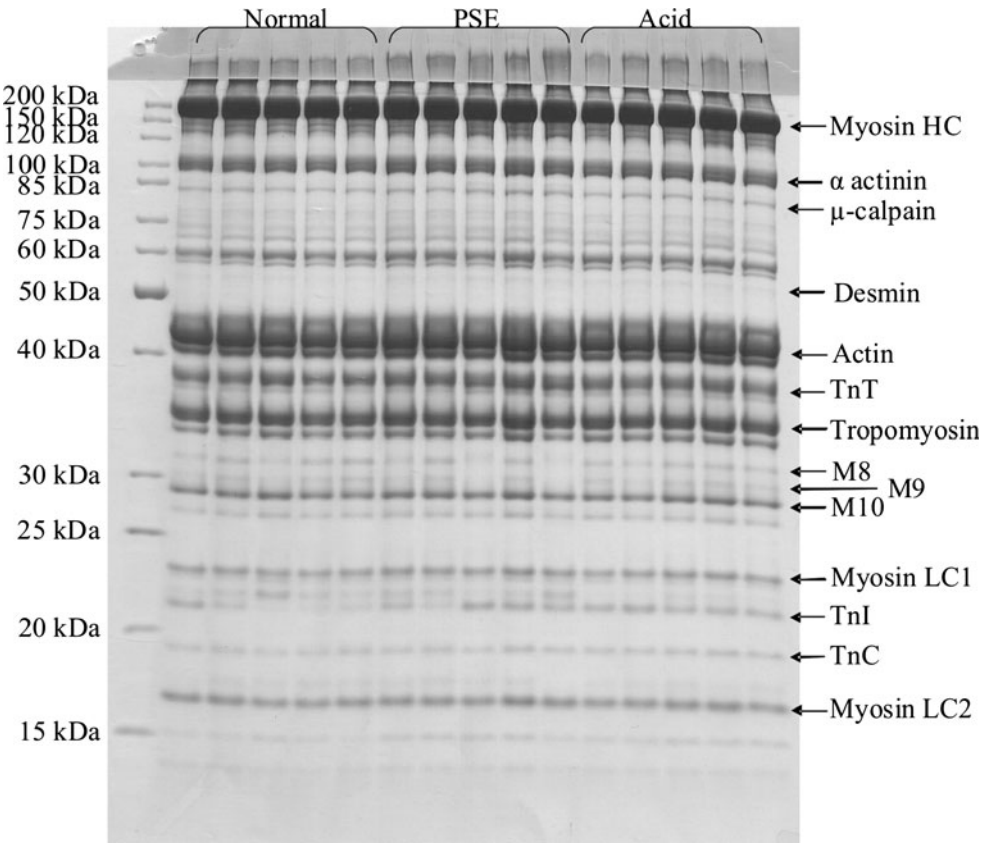

Fig. 1 SDS-PAGE of muscle protein isolated from the *Longissimus thoracis* muscle of normal, PSE, and acid meat. The *left lane* corresponds to the molecular weight scale. The following abbreviations are used: *TnT* troponin T, *TnI* troponin I, and *TnC* troponin C

Table 3 Quantification of muscle proteins from the *Longissimus thoracis* muscle of normal, PSE, and acid meat (Mean value ± SD of five separate samples)

	Meat quality			
	Normal	PSE	Acid	*P*
Myosin	15.86 ± 0.42	15.00 ± 0.93	14.98 ± 0.19	NS
α-actinin	6.56 ± 0.40	6.14 ± 0.28	6.24 ± 0.38	NS
μ-calpain	1.70^{b} ± 0.17	2.36^{a} ± 0.34	2.10^{a} ± 0.28	0.01
Desmin	0.72 ± 0.18	1.00 ± 0.10	1.02 ± 0.26	0.06
Actin	17.46 ± 0.30	17.02 ± 0.45	16.80 ± 0.89	NS
TnT	7.14 ± 0.32	7.28 ± 0.27	6.82 ± 0.24	0.06
Tropomyosin	8.20 ± 0.26	7.92 ± 0.22	7.78 ± 0.45	NS
M8	1.70^{b} ± 0.14	1.52^{a} ± 0.11	1.42^{a} ± 0.08	0.01
M9	0.68^{a,b} ± 0.56	0.48^{b} ± 0.36	1.20^{a} ± 0.16	0.04
M10	3.72^{b} ± 0.38	3.34^{a} ± 0.09	3.20^{a} ± 0.12	0.01
Myosin LC1	3.14^{b} ± 0.18	2.98^{a,b} ± 0.13	2.84^{a} ± 0.15	0.03
TnI	1.30 ± 0.93	1.82 ± 0.33	2.18 ± 0.29	0.10
TnC	1.84^{b} ± 0.21	1.52^{a} ± 0.22	1.54^{a} ± 0.09	0.03
Myosin LC2	3.50 ± 0.31	3.36 ± 0.09	3.18 ± 0.19	0.10

^{a,b} Means with different superscript show significant differences ($P \leq 0.05$). The following abbreviations are used *TnT* troponin T, *TnI* troponin I, *TnC* troponin C

meat maturation were partitioned into polypeptides with molecular weights between 60 and 85 kDa and polypeptides with an approximate molecular weight of 30 kDa. Some of the bands from the first group may be unautolyzed (80 kDa) μ-calpain and its autolysis products (78 and 76 kDa) [7, 8, 10] (Table 3, Fig. 1). The 30-kDa polypeptides are the products of *postmortem* proteolysis of TnT [7, 12]. Similar to results reported by Schäfer et al. [5], several non-identified products of protein degradation with molecular weights of 45, 41, and 39 kDa were observed (Fig. 1).

A significant increase in the μ-calpain band (80 kDa) was observed in the PSE and acid meat samples (Table 3), which was in accordance with a previous study by Bee

et al. [10] who reported a higher abundance of the unautolyzed μ-calpain subunit in meat with lower pH values. In contrast, α-actinin was stable in all three studied meat types, which was confirmed by the studies of Kołczak et al. [48] and Ahn et al. [49].

The M8 and M10 bands (approximately 31 and 29 kDa, respectively) were significantly more abundant in the normal meat than in the acid and PSE meat (Table 3). According to Lametsch et al. [50], proteins with approximate molecular weights of 31 and 29 kDa are the proteolytic products of CK, which is proteolyzed into 3 fragments of 35, 31, and 29 kDa. Therefore, the higher abundance of M8 and M10 indicated a lower content of active CK, which was consistent with the results of Van de Wiel and Zhang [9], who observed a lower content of CK in meat with a normal rate of glycolysis.

In the present study, a significant difference in the amount of the M9 band, which corresponded to a protein with an approximate molecular weight of 30 kDa, was found among the three meat types (Table 3, Fig. 1) with the highest level observed in the acid meat. As stated by many authors, the 30-kDa polypeptide is a degradation product of TnT [12, 48, 51], which was consistent with the present study because a lower abundance of TnT was observed in acid meat ($P_{\alpha} \leq 0.06$) (Table 3). Degradation of TnT may simply be an indicator of overall *postmortem* proteolysis [12] that occurs faster at lower pH levels. Josell et al. [18] reported that higher enzymatic proteolysis activity in meat with low ultimate pH is confirmed by lower levels of nyosin LC1, TnT ($P \leq 0.06$), and TnC. These proteins can be degraded by cathepsin, which is more active at low pH levels. Gil et al. [52] demonstrated that the maximum activity of cathepsin B + L occurs in PSE meat and that the lowest activity of this enzyme occurs in DFD.

SDS-PAGE analysis of protein from drip loss

Figure 2 presents the protein profile of the drip loss from normal, PSE, and acid meat, and Table 4 shows the quantification of each band. Among the identified proteins, the following proteins were the most abundant: glyceraldehyde-3-phosphate dehydrogenase/lactate dehydrogenase (GAPDH/LDH), 14.9 %; enolase (EN), 11.9 %; aldolase (ALD), 11.0 %; creatine kinase/phosphoglycerate kinase (CK/PGAK), 9.9 %; and pyruvate kinase/phosphoglucose isomerase (PK/PGI), 9.1 %. This protein profile was similar to the one obtained by Pérez and Ruiz [53] for muscular protein from raw pork ham.

The protein profile of the drip loss showed small variations among the three types of meat. Only the W12 band was different between the three meat types because it was found in greater quantity in the PSE and acid meat than in the normal meat (Table 4, Fig. 2). The protein identification by mass spectrometry of W12 revealed a mixture of proteins as follows (Table 5): the B or D chain of hemoglobin and fatty acid-binding protein (FABP). Sayd et al. [54] reported that FABP may be a marker for a low L* value in meat. However, these authors studied soluble proteins extracted from muscles. In the present study, the B chain of hemoglobin was found in the drip loss. Sayd et al. [54] found more hemoglobin in meat classified as belonging to the dark group, which is also characterized by increased oxidative metabolism. These authors also suggested that the higher abundance of hemoglobin may be linked to higher blood flow in muscles, higher hemoglobin content in the blood, and/or degree of bleeding. Thus, we can hypothesize that PSE meat is more prone to release these soluble proteins. The present study showed that the higher abundance of these proteins in the drip loss was related to the lightness of meat (Tables 1, 4). FABP was the second protein identified by mass spectrometry in the W12 band. Functionally, FABP is an intracellular protein that transports fatty acids from the cell membrane to sites of fatty acid oxidation, phospholipid synthesis, or

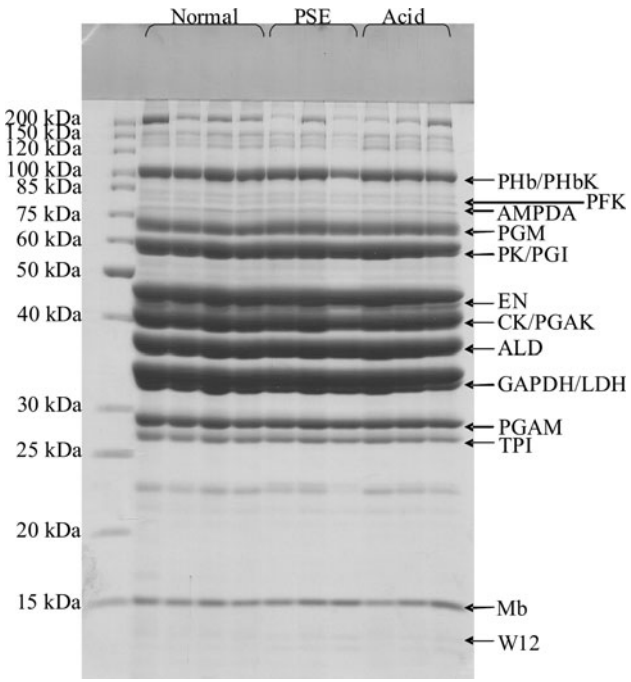

Fig. 2 SDS-PAGE of soluble proteins in the drip loss from the *Longissimus thoracis* muscle of normal, PSE, and acid meat. The *left lane* corresponds to the molecular weight scale. The following abbreviations are used: *PHb/PHbK* phosphorylase b/phosphorylase b kinase, *PFK* phosphofructokinase, *AMPDA* AMP deaminase, *PGM* phosphoglucomutase, *PK/PGI* pyruvate kinase/phosphoglucose isomerase, *EN* enolase, *CK/PGAK* creatine kinase/phosphoglycerate kinase, *ALD* aldolase, *GAPDH/LDH* glyceraldehyde-3-phosphate dehydrogenase/lactate dehydrogenase, *PGAM* phosphoglycerate mutase, *TPI* triosephosphate isomerase and *Mb* myoglobin

Table 4 Quantification of soluble proteins in the drip loss from the *Longissimus thoracis* muscle of normal, PSE, and acid meat (Mean value ± SD of three or four separate samples)

	Meat quality			
	Normal	PSE	Acid	*P*
PHb/PHbK	7.27 ± 0.57	6.60 ± 0.96	6.80 ± 0.35	NS
PFK	0.97 ± 0.39	1.16 ± 0.12	1.23 ± 0.06	NS
AMPDA	1.10 ± 0.18	1.13 ± 0.15	1.13 ± 0.06	NS
PGM	6.75 ± 0.39	7.30 ± 0.17	7.03 ± 0.15	0.09
PK/PGI	8.90 ± 0.27	9.40 ± 0.36	9.13 ± 1.17	NS
EN	11.90 ± 0.32	11.80 ± 0.10	12.13 ± 0.91	NS
CK/PGAK	9.75 ± 0.54	10.07 ± 0.55	7.83 ± 3.49	NS
ALD	10.67 ± 0.31	11.10 ± 0.78	11.43 ± 1.14	NS
GAPDH/LDH	15.05 ± 0.39	14.80 ± 1.06	14.77 ± 0.90	NS
PGAM	7.20 ± 0.41	6.70 ± 0.17	6.80 ± 0.30	NS
TPI	3.90 ± 0.14	3.73 ± 0.06	4.03 ± 0.58	NS
Mb	3.25 ± 0.17	3.30 ± 0.10	3.33 ± 0.51	NS
W12	0.10^{b} ± 0.00	0.87^{a} ± 0.31	$0.63^{a,b}$ ± 0.50	0.03

[a,b] Means with different superscript show significant differences ($P \leq 0.05$). The following abbreviations are used *PHb/PHbK* phosphorylase b/phosphorylase b kinase, *PFK* phosphofructokinase, *AMPDA* AMP deaminase *PGM* phosphoglucomutase, *PK/PGI* pyruvate kinase/phosphoglucose isomerase, *EN* enolase, *CK/PGAK* creatine kinase/phosphoglycerate kinase, *ALD* aldolase, *GAPDH/LDH* glyceraldehyde-3-phosphate dehydrogenase/lactate dehydrogenase, *PGAM* phosphoglycerate mutase, *TPI* triosephosphate isomerase, *Mb* myoglobin

Table 5 LC-MS/MS identification of W12 spots in the drip loss from the *Longissimus thoracis* muscle

Sequence reference[a]	Protein name[a]	Mascot score[b]	Sequence coverage (%)[c]	Nb of peptide matches[d]	Th. MW Da[e]
gil809283 gil809285	B or D chain, porcine hemoglobin	348	78	14	16,025
Gil2143386	Fatty acid-binding protein [Sus scrofa]	86	9	1	14,740

[a] Protein names and accession numbers were derived from the Swiss-Prot database

[b] The MASCOT baseline significant score is 67

[c] Percent of coverage of the entire amino acid sequence

[d] Number of matched peptides in the database search

[e] Theoretical MW recorded in the mammalian taxonomy NCBI database and observed MN (calculated from the spot position on the gel)

triacylglycerol synthesis. Heart FABP (H-FABP) is expressed predominantly in muscle cells, and adipocyte FABP (A-FABP) is expressed almost exclusively in adipocytes. H-FABP expression is clearly higher in an oxidative muscle than in a glycolytic muscle. Significant associations between genetic variation at the A-FABP and H-FABP gene loci (FABP4 and FABP3) and intramuscular fat content (IMF) have been identified [55, 56]. Other studies, however, do not show clear associations between the genetic variance of FABP3 and IMF [57–60].

Correlation between muscle protein and quality traits

The analysis of correlation between muscle protein and meat quality traits showed that the μ-calpain (80 kDa) band was negatively correlated with pH_1 ($r = -0.70$ and $P_\alpha \leq 0.05$), pH_{24} ($r = -0.54$ and $P_\alpha \leq 0.05$), and ultimate pH ($r = -0.56$ and $P_\alpha \leq 0.05$). These results were consistent with those reported by Bee et al. [10] who showed that μ-calpain is less abundant in meat with higher pH values. In the present study, the ultimate pH was also negatively correlated with desmin (53 kDa) ($r = -0.60$ and $P_\alpha \leq 0.05$), which was in accordance with the study reported by Bee et al. [10], who reported lower degradation of desmin in meat with lower pH values. These authors suggested that the autolysis of μ-calpain occurs earlier in meat with faster rates of pH decline, which may explain the lower degradation of desmin in meat with low pH values. Meat with low pH values is characterized by a pale color, thus resulting a higher L* value (Table 1), which explains

the positive correlation between μ-calpain and L* ($r = 0.62$ and $P_\alpha \leq 0.05$). A significant relationship between α-actinin and pH_1 ($r = 0.63$ and $P_\alpha \leq 0.05$) was also observed. Pospiech et al. [61] mentioned that the release of α-actinin from the Z disk may contribute to the increased meat tenderness and water-holding capacity.

M8 (31 kDa) and M10 (29 kDa) were negatively correlated with glycogen (M8, $r = -0.69$ and $P_\alpha \leq 0.05$; M10 $r = -0.52$ and $P_\alpha \leq 0.05$) and GP (M8, $r = -0.68$ and $P_\alpha \leq 0.05$; M10, $r = -0.52$, and $P_\alpha \leq 0.05$), and positively correlated with ultimate pH (M8, $r = 0.80$ and $P_\alpha \leq 0.05$; M10, $r = 0.58$ and $P_\alpha \leq 0.05$). Moreover, M8 (31 kDa) was negatively correlated with L* ($r = -0.64$ and $P_\alpha \leq 0.05$) and b* ($r = -0.53$ and $P_\alpha \leq 0.05$), and positively correlated with juiciness ($r = 0.67$ and $P_\alpha \leq 0.05$). As mentioned earlier, the M8 and M10 polypeptides are products of CK degradation and the lower abundance of M8 and M10 in meat may indicate a higher content of active CK. Van de Wiel and Zhang [9] observed a higher content of CK in meat with lower pH levels and they hypothesized that high CK levels cause rapid degradation of creatine phosphate (CP) and an increased rate of glycolysis, which, in turn, may cause a more rapid pH decline and muscle contraction, thus resulting in a high drip loss. High glycogen and PG are indicators of a faster rates of glycolysis and, in turn, a rapid decline of pH and pale color [39, 40], which was verified with the present results (Table 1).

TnC was less abundant in PSE and acid meat than in normal meat, and the opposite result was observed for TnI. Moreover, TnC was negatively correlated with drip loss ($r = -0.52$ and $P_\alpha \leq 0.05$). TnC and TnI are involved in the regulation of muscle contraction, with antagonist effects. Thus, TnC and TnI were inversely correlated with meat quality traits such as pH_1 (TnI, $r = -0.18$ and $P_\alpha \leq 0.05$; TnC, $r = 0.52$ and $P_\alpha \leq 0.05$), ultimate pH (TnI, $r = -0.56$ and $P_\alpha \leq 0.05$; TnC, $r = 0.76$ and $P_\alpha \leq 0.05$), color parameters L* (TnI, $r = 0.40$ and $P_\alpha \leq 0.05$; TnC, $r = -0.78$ and $P_\alpha \leq 0.05$), b* (TnI, $r = 0.53$ and $P_\alpha \leq 0.05$; TnC, $r = -0.57$ and $P_\alpha \leq 0.05$), glycogen (TnI, $r = 0.55$ and $P_\alpha \leq 0.05$; TnC, $r = -0.71$ and $P_\alpha \leq 0.05$), and GP (TnI, $r = 0.64$ and $P_\alpha \leq 0.05$; TnC, $r = -0.60$ and $P_\alpha \leq 0.05$). The average TnC/TnI ratio was 1.41 for normal meat, 0.83 for PSE meat, and 0.70 for acid meat. These results suggested some regulation changes in the PSE and acid meat. Lin et al. [62] and Pospiech et al. [61] reported that TnI competes with TnT for the same binding sites on TnC and that the variation in binding affinity between TnC and TnT, as modulated by Ca^{2+}, may have an important role in a Ca^{+2}-regulated mechanism of muscle contraction. Josell et al. [20] reported that the increased level of proteolysis is initiated by an increased rate of ATP degradation and rapid pH decline.

The significant correlation between tropomyosin and tenderness ($r = -0.69$ and $P_\alpha \leq 0.05$) indicated an increase in meat tenderness together with an increased degree of tropomyosin degradation. Tropomyosin is also proteolyzed by cathepsin, which is more active at low pH levels, and confirms the aforementioned results of Josell et al. [18] and Miller et al. [45] who reported that meat with low pH levels is characterized by higher tenderness.

Myosin LC1 was positively correlated with ultimate pH ($r = 0.55$ and $P_\alpha \leq 0.05$) and negatively correlated with the b* color parameter ($r = -0.53$ and $P_\alpha \leq 0.05$). These data were in agreement with the results presented by Choi et al. [16] who reported that meat with faster glycolysis rates contain less myosin LC1 and they demonstrated that myosin LC isoforms can influence muscle glycolytic rate during the early *postmortem* period.

Correlation between drip loss proteins and quality traits

The analysis of correlation between proteins from drip loss and myofibrils highlighted specific associations. The soluble proteins from the drip loss showed less significant correlation with meat quality traits. In PSE meat, which is characterized by a rapid pH decline (pH_1), phosphoglucomutase (PGM), the enzyme of the glycolytic pathway involved in the interconversion between glucose-1-P and glucose-6-P, was more abundant (Table 4). Moreover, PGM was negatively correlated with pH_1 ($r = -0.68$ and $P_\alpha \leq 0.05$) and positively correlated with L* ($r = 0.68$ and $P_\alpha \leq 0.05$) and drip loss ($r = 0.80$ and $P_\alpha \leq 0.05$). This higher quantity of PGM may suggest a potential increase in the mobilization of carbohydrates in *postmortem* degradation. Xu et al. [63] showed that PGM expression increased in the Large White breed, which is characterized by increased glycolytic metabolism, increased carbohydrate usage, and less lipid usage than the Meishan breed. These authors hypothesized that the intensive selection for lean muscle growth in Western pig breeds induces a shift in muscle metabolism toward a more glycolytic and less oxidative fiber type [64]. This phenomenon may be partially explained by the presence of pigs with higher PG and faulty meat in the studied breed of pig, which were intensively selected for lean muscle growth.

GAPDH/LDH was positively correlated with the b* color parameter ($r = 0.65$ and $P_\alpha \leq 0.05$). In some studies, GAPDH is considered as an indicator of the process of crushing the meat in cured and fermented products [61]. LDH is involved in the regeneration of *postmortem* NADH, which reduces metmyoglobin to deoxymyoglobin and maintains the color stability of muscle [13, 14]. The redox state of myoglobin is influenced by the b* color parameter [13]. Moreover, ALD was positively correlated with the b* color parameter ($r = 0.75$ and $P_\alpha \leq 0.05$), but

phosphorylase b/phosphorylase b kinase (PHb/PHbK) was negatively correlated with ALD ($r = 0.76$ and $P_\alpha \leq 0.05$). ALD and PHb/PHbK are enzymes in the glycolytic pathway and their activity influences the rate of *postmortem* glycolysis. The ALD enzyme is applied in rabbit meat as an indicator of glycolytic metabolism [65]. A higher significant activity of these enzymes was observed by Ramirez et al. [65] in rabbits with higher glycolytic characteristics and higher b* values, which was consistent with the results of the present study (Tables 1, 4). Phosphorylase kinase (PH) exists in two forms as follows: active a and non-active b. In PSE meat, the activity of PHa is higher than normal meat [66]. PSE meat is characterized by a pale color, which is also connected with the activity of the aforementioned enzymes.

Additionally, CK/PGAK was positively correlated with juiciness ($r = 0.67$ and $P_\alpha \leq 0.05$). CK is responsible for the conversion of creatine phosphate (CP) into creatine and ATP. ATP production is necessary to keep the muscle in a relaxed state. When 70 % of CP is degraded, then ATP is replenished by the degradation of glycogen. Glycolysis also produces lactate, H^+, and heat, thus resulting in decreased pH levels and protein denaturation by approximately 20 %. Thus, the decline in pH depends on initial concentration of CP and glycogen [9, 67]. Van de Wiel and Zhang [9] suggested that higher CK levels cause a rapid degradation of CP, which may, in turn, promote glycolysis, a more rapid pH decline, and muscle contraction, thereby, resulting in a high drip loss. Therefore, we hypothesize that a higher abundance of CK in drip loss indicates a lower content of CK in meat, which causes a slower degradation of CP and, consequently, the normal course of maturation. It is well known that such meat is juicier, which was verified by the present study (Table 2).

Conclusions

The results of the present study confirm a lower technological quality of PSE and acid meat. From a sensory point of view, PSE meat was characterized by lower color intensity and acid meat was characterized by the lowest levels of juiciness. The muscle protein profile showed a significantly a higher quantity of μ-calpain in PSE and acid meat compared to normal meat. Additionally, lower quantity of polypeptides with molecular sizes 31 and 29 kDa, which were products of CK degradation, was observed in faulty meat (meat with higher rates of glycolysis). The lower quantity of myofibrillar proteins (myosin LC1, TnT, and TnC) in meat with higher glycogen levels and lower pH levels showed higher rates of proteolysis due to the higher enzymatic proteolysis activity in these meats. TnC and TnI were associated with meat quality. Moreover, these results showed that TnC/TnI ratio can be a pertinent marker of *postmortem* muscle metabolism and is related to the textural properties of meat. Furthermore, several soluble proteins from the drip loss were related to meat quality, especially PGM and the B chain of hemoglobin in PSE meat.

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