

The relationship between protein changes in porcine *longissimus* muscle at different courses of meat tenderisation

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Abstract Among many factors affecting meat tenderness, a special role is attributed to proteins. The aim of this study was to analyse relationships between the tenderness of meat and changes in the abundance of muscle tissue and centrifugal drip proteins as well as in a value of the simple assessment criteria of the glycolysis process in porcine *longissimus thoracis et lumborum* muscles. All of them were used for describing conditions associated with tenderisation process and predicting tenderness. For analysis of protein changes in the muscle tissue (45 min, 48 and 144 h pm) and in its centrifugal drip (48 and 144 h pm), SDS-PAGE technique was applied. The progress of glycolysis was evaluated on the basis of pH value as well as the electrical conductivity (EC) measurements. Warner–Bratzler shear force assessment (48 and 144 h pm) was involved for the evaluation of tenderness. Comparisons concerned four groups of muscles characterised by different courses of tenderisation. The study showed that proteolysis was closely related to the process of glycolysis in muscles which was linked with genotype of pigs. The single correlations between protein changes and meat tenderness varied in the selected groups of muscles. It was possible to make very precise predictions

of the meat tenderness using canonical correlation analysis. Based on the percentage participation of the selected muscle tissue and centrifugal drip proteins and measurements of pH and EC at specified time pm it was possible to predict tenderness with a very high probability, even 89%.

Keywords Pork · Proteins · Tenderness (WBSF) · Electrophoresis (SDS-PAGE) · Pork quality indicators

Introduction

Rapid development of proteomics involves the application of various electrophoretic techniques [12, 14, 52, 55], which allow the identification of many proteins with respect to their properties. It leads to better understanding of the causes and consequences of the phenomena influencing the quality of meat, especially its tenderness [15, 20, 22, 31, 32, 35, 40, 47, 51].

Tenderness belongs to the most important quality parameters of meat, which is especially important by its culinary use. It is a prerequisite of all consumers independent of their age. Usually the tenderisation process is described as the result of protein changes which take place during storage [2, 4, 9, 10, 29, 30, 36, 42]. It is evident from literature data that the rate of muscle protein degradation depends on a number of factors, such as the age of animals [21], their genotype [11, 24, 27, 43, 45] and meatiness [38, 50] as well as the proximate composition of muscles [6–8, 34, 41], being the most important ones. These changes can be associated with breeding and rearing conditions [18, 27, 54]. Usually pork can be tenderised after 5–6 days of cold storage. However, in some cases, pork is already tender after 2 days of storage, but in other situations it is tough even after 1 week of ageing [17].

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The rate and extent of post-mortem (pm) changes in proteins also depends on the character of carbohydrate transformation. They are strictly connected with the conversion of muscles into meat and its ageing. The course of glycolysis determines the final pH value, which, in consequence, affects the culinary and technological quality of meat [28, 56], the scale of protein proteolysis [22, 30, 56] and the ultimate tenderness of meat [20, 25, 51].

Myofibrillar proteins are the most important fraction of proteins. Products of their degradation can be found in the centrifugal drip from meat [13, 37, 48]. Therefore, these two groups of proteins were subjected to analyses in this study.

Taking into account all the above data the aim of this study was to analyse changes occurring in proteins of the *longissimus* muscles and their centrifugal drip and to observe the speed of glycolysis in muscles, all from pigs which were assigned to different genotypes in regard to *RYRI* gene, for more precise description of meat process tenderisation. As a tool in this study it was used canonical data analysis. It allows better explaining relationships between various groups of features related to different courses of meat acidification, proteolysis and meat tenderisation.

Materials and methods

Characteristic of pigs and their meat quality evaluation

The experimental material was the *longissimus thoracis et lumborum* (LTL) muscle from 24 pigs of a known *RYRI* genotype (TT—homozygous negative, CT—heterozygous, CC—homozygous positive) as well as rearing and feeding conditions. Pigs were selected from a total number of 165 fatteners. They were slaughtered at a weight of about 110 kg. Samples were selected on the basis of measurement of the shear force value and they were allocated to four groups (A, B, C, D) differing in the course of the tenderisation process. Muscles assigned to groups A and B originated from fatteners of Line 890 (Line 990 × Pietrain). Samples belonging to group C were hybrids of Landrace × Duroc (L × Dur) descent, and samples from pigs in group D were hybrids of the (Landrace × Yorkshire) × Duroc [(L × Y) × Dur]. Genotypes of analyzed animals in terms of locus *RYRI* were identified using PCR–RFLP (Fujii et al. 1991). All fatteners from group A were CT heterozygotes. Similar genotype expressed half of pigs in the group B, but the second part of pigs in this group was susceptible to stress (TT homozygotes). Pigs belonged to the remaining groups (C and D) were resistant to stress (CC homozygotes). However, similar to the group B the speed of glycolysis in their muscles pm was varied.

The quality of all muscles was assessed on the basis of pH₁ (45 min pm) and pH₂ (24 h pm) measurements as well as the electrical conductivity (EC) evaluated at 1.5 (EC1) and 24 (EC2) h pm [28, 33].

Samples for electrophoresis

Samples were collected from LTL muscles three times. In order to conduct measurements immediately after slaughter (45' pm), small samples (about 5 g) were cut out of the carcasses at the level of the last rib and wrapped in aluminium foil. Next, they were quickly frozen in liquid nitrogen and stored at −80 °C until analysis. Samples for electrophoresis 48 and 144 h pm were collected in a similar way. However, before the preparation of samples bigger cuts of *longissimus* muscle (around 0.5 kg) were collected from chilled carcasses 24 h pm. Then, they were divided into two equal parts for analysis 48 and 144 h pm. Next, they were vacuum packed and stored at 2–4 °C until the target term of the study. These muscles were used for all other investigations related to the electrophoresis of proteins in meat and centrifugal drip from it, and evaluation of tenderness.

Samples of the muscle tissue for electrophoretic analysis were collected 45 min, 48 and 144 h after slaughter, while centrifugal drip samples—48 and 144 h pm. The centrifugal drip was obtained by centrifugation of 6g of comminuted muscle tissue (for 15 min at 15,000×g at 2 °C) in a Sigma 3K30 centrifuge [19].

SDS-PAGE and western blotting

Electrophoretic separations of the muscle tissue and centrifugal drip proteins were carried out on 15% polyacrylamide gels (199:1 ratio of acrylamide to *N,N'*-methylenebisacrylamide) with the addition of 8 M urea [50]. Separated proteins underwent quantitative evaluation with the Image Master® VCR set of the Pharmacia Company and Image Master® 1D program. The selected muscle tissue and centrifugal drip proteins such as titin, myosin (MHC), troponin T (Tn-T) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were identified by means of the western blotting method described by Fritz and Greaser [14]. Primary monoclonal antibodies used in this study, including mouse anti-titin (clone 9D10), came from the collection of the Meat Science and Muscle Biology Laboratory of the University of Wisconsin (USA). Mouse anti-troponin T (clone JLT-12) and mouse anti-GAPDH (clone GAPDH-71.1) came from Sigma-Aldrich. Mouse anti-myosin (clone MF 20) was purchased from the Developmental Studies Hybridoma Bank at the University of Iowa (USA). Alkaline phosphatase conjugated anti-mouse IgG was a secondary antibody purchased from Sigma-Aldrich. The basis

of proteins selection for the identification of advancement of proteolysis using the western-blotting was the qualitative and quantitative computer assisted image analysis of gels. Special attention was paid on these proteins bands, which changed their intensity and their abundance could be linked with the progress of meat tenderisation.

WBSF measurements

In order to determine the Warner–Bratzler shear force (WBSF) values, 25-mm thick meat slices weighing about 120 g were prepared. They were heated in a Rational Combi convection oven in hot air at 160 °C for about 15 min until the temperature inside them was 72 °C [17]. The WBSF values after 48 and 144 h pm were measured perpendicularly to the direction of muscle fibres, using an Instron Universal Testing Machine, model 1140, equipped with a Warner–Bratzler device [17].

Groups of different courses of tenderisation process

The experimental muscles were divided into four groups of different courses of tenderisation according to shear force values: A—meat remaining tough during the entire 144 h of pm storage; B—meat characterised by typical tenderisation process, i.e. tough after 48 pm and tender after 144 h of pm storage; C—tender or relatively tender meat at both of the aforementioned terms of examination; D—toughest meat 48 and most tender—144 h pm. Meat was considered as tough if the shear force by its cutting exceeded 60 N/cm² and tender when its shear force was lower than 40 N/cm² [3, 32].

Statistical analysis

Analysis of variance as well as linear and canonical correlation was conducted by means of the Statistica 10.0 PL [49] software to determine differences or relationships between the groups of muscles in terms of their properties. Special attention was paid to the canonical correlation analysis. This method, in relation to linear correlation analysis, allows estimating relationships between two sets of variables. In comparison with multiple regression, in which there is only one dependent variable and multiple independent variables and a linear combination of independent variables is formed, the objective of this method is to find a linear combination for each set of variables. In canonical correlation there is more than one dependent variable and linear combinations are constructed from both dependent and independent variables with the maximum correlation between them. Therefore, canonical correlation analysis produces richer information than multiple regression analysis [26].

Results and discussion

Assessment of meat quality

On the basis of the pH values (pH₁ & pH₂) and EC (EC1 & EC2) measurements it was stated that the samples from all groups of pigs (A, B, C and D) were basically normal quality (RFN). Despite the fact that the quality of the muscles assessed using these criteria was generally accepted, they differed in the rate of glycolysis, as can be seen in Table 1. The highest pH₁ values were observed in muscles of pigs with CC genotype (groups C and D) and the lowest in the group A. Pigs belonging to group A were carriers of *RYR1* gene. It is necessary to mention that the differentiation in

Table 1 Changes of pH and EC values of the muscle tissue during pm storage

Group	Time of post-mortem					
	pH				EC (mS/cm)	
	45'	24 h	48 h	144 h	1.5 h	24 h
A	6.18 ± 0.40	5.62 ^{ab} ± 0.11	5.46 ± 0.05	5.45 ^a ± 0.06	4.36 ^{ab} ± 4.45	5.55 ^b ± 2.95
B	6.45 ± 0.10	5.76 ^{b*} ± 0.16	5.53 ± 0.11	5.76 ^b ± 0.33	3.53 ^{abc} ± 1.21	4.33 ^{ab} ± 1.21
C	6.53 ± 0.18	5.55 ^a ± 0.09	5.47 ± 0.06	5.47 ^{ab} ± 0.04	1.77 ^a ± 0.23	2.57 ^{ac} ± 0.73
D	6.56 ± 0.15	5.80 ^b ± 0.06	5.57 ± 0.14	5.66 ^{ab} ± 0.13	3.91 ^{ab} ± 0.86	4.12 ^{ab} ± 0.69

* , a, b, c Means in the same column with different superscripts are significantly different at $p \leq 0.05$

A—meat remaining tough during the entire 144 h pm changes

B—meat characterised by typical tenderisation process, i.e. tough 48 h pm, and tender after 144 h pm storage

C—tender or relatively tender meat on both aforementioned dates of examination

D—meat toughest 48 h pm, and most tender 144 h pm

regard to muscle acidification at this point of measurement between all groups of pigs were not significant. The fastest and the most deepest progress of muscle pH slope during the first 24 h pm was observed in the group C. These changes occurred further throughout the next 24 h of meat storage. They revealed the lowest values of EC. In spite of some diversity of pH values 48 pm the differences among all groups were again not significant.

In the Table 2 are presented results of the instrumental evaluation of tenderness performed 48 and 144 h pm. The highest hardness 48 pm was noted for the muscles from the group D, a the lowest—in the case of the group C. After 144 h of cold storage of LTL muscles the significant differentiation in tenderness appeared between muscles from the group A and the others. Muscles from the group A were evaluated as the most hard (WBSF >60 N/cm²). It seems that the allocation of muscles to specific groups of tenderness corresponds to their genetic differentiation.

Assessment of post-mortem changes in muscle tissue proteins

We used the western blotting method to identify and assess the percentage proportions of the following changes in the muscle tissue: native titin defined as T1 [16, 50, 53] with a molecular weight (m.w.) of about 3700 kDa as well as one of its degradation products of about 800 kDa. We used the 9D10 anti-titin antibody for immunoblotting against titin. At all terms of analyses the highest proportion of native titin together with its degradation product was found in the muscle tissue from pigs in group A (Table 3). After 48 pm there was a statistically significant difference ($p \leq 0.05$) in the amount of native titin between tough muscles from group A and tender ones from group C. After 144 h pm

Table 2 WBSF values of the *thoracis et lumborum* muscle (N/cm²)

Group	WBSF values (N/cm ²)	
	48 h	144 h
A	73.34 ^{b*} ± 14.14	61.31 ^b ± 6.50
B	69.30 ^b ± 8.50	35.19 ^a ± 5.82
C	39.42 ^a ± 3.93	35.82 ^a ± 4.20
D	92.92 ^c ± 9.23	28.70 ^a ± 4.28

*, ^{a, b, c} Means in the same column with different superscripts are significantly different at $p \leq 0.05$

A—meat remaining tough during the entire 144 h pm changes

B—meat characterised by typical tenderisation process, i.e. tough 48 h pm, and tender after 144 h pm storage

C—tender or relatively tender meat on both aforementioned dates of examination

D—meat toughest 48 h pm, and most tender 144 h pm

Table 3 Changes in the relative proportion of muscle tissue proteins during aging of pork differed in its tenderisation course (%)

Muscle tissue proteins/group	Time of storage		
	45'	48 h	144 h
About 3700 kDa (T1)			
A	5.87 ± 1.12	6.80 ^b ± 1.30	7.61 ^c ± 1.61
B	5.36 ± 1.88	5.53 ^{ab} ± 1.58	5.31 ^b ± 1.33
C	4.74 ± 1.24	5.03 ^a ± 1.13	4.96 ^a ± 1.43
D	4.72 ± 1.20	5.45 ^{ab} ± 0.62	5.12 ^b ± 1.20
About 800 kDa			
A	1.60 ^{b*} ± 0.42	1.24 ^b ± 0.33	0.77 ^c ± 0.15
B	1.57 ^b ± 0.58	1.07 ^c ± 0.48	0.58 ^b ± 0.19
C	0.99 ^a ± 0.40	0.62 ^a ± 0.09	0.47 ^{ab} ± 0.16
D	0.87 ^a ± 0.21	0.55 ^a ± 0.15	0.36 ^a ± 0.09
>205 kDa			
A	8.36 ^b ± 0.99	8.99 ^c ± 1.21	9.08 ^b ± 1.43
B	7.55 ^{ab} ± 2.40	7.52 ^{bc} ± 1.78	6.19 ^a ± 1.39
C	5.74 ^a ± 1.26	5.70 ^a ± 1.10	5.77 ^a ± 1.77
D	5.77 ^a ± 1.41	6.46 ^{ab} ± 1.07	5.68 ^a ± 1.34
205 kDa			
A	12.69 ± 0.77	13.21 ^a ± 1.13	13.40 ^a ± 1.74
B	12.59 ± 1.31	13.69 ^{ab} ± 1.48	13.16 ^a ± 0.79
C	13.27 ± 1.25	16.26 ^c ± 1.71	16.18 ^b ± 2.21
D	12.59 ± 0.99	15.35 ^{bc} ± 1.59	14.77 ^{ab} ± 1.05
105 kDa			
A	3.37 ^b ± 0.71	3.62 ^b ± 0.65	3.53 ± 0.24
B	2.67 ^a ± 0.39	2.83 ^a ± 0.47	2.58 ± 0.41
C	2.56 ^a ± 0.19	2.76 ^a ± 0.36	2.72 ± 0.33
D	2.72 ^a ± 0.31	3.05 ^{ab} ± 0.35	2.91 ± 0.45
38–36 kDa			
A	6.21 ^a ± 1.27	5.5 ^a ± 1.05	5.65 ± 1.10
B	6.74 ^a ± 1.09	5.17 ^a ± 1.38	6.19 ± 0.57
C	8.2 ^b ± 0.97	6.86 ^b ± 0.94	6.83 ± 1.04
D	7.31 ^{ab} ± 1.28	6.07 ^{ab} ± 1.00	5.89 ± 1.43

*, ^{a, b, c} Means in the same column with different superscripts are significantly different at $p \leq 0.05$

A—meat remaining tough during the entire 144 h pm changes

B—meat characterised by typical tenderisation process, i.e. tough 48 h pm, and tender after 144 h pm storage

C—tender or relatively tender meat on both aforementioned dates of examination

D—meat toughest 48 h pm, and most tender 144 h pm

the highest ($p \leq 0.05$) amount of T1 was observed in the muscles characterised by the highest shear force value (group A), as compared with the other groups (Table 3). The highest proportion of native titin found in the muscle tissue from group A was probably associated with the genetic background of pigs. They came from experimental fatteners of Line 890, whose muscles exhibited a rapid course of glycolysis pm. Faster glycolysis may limit the

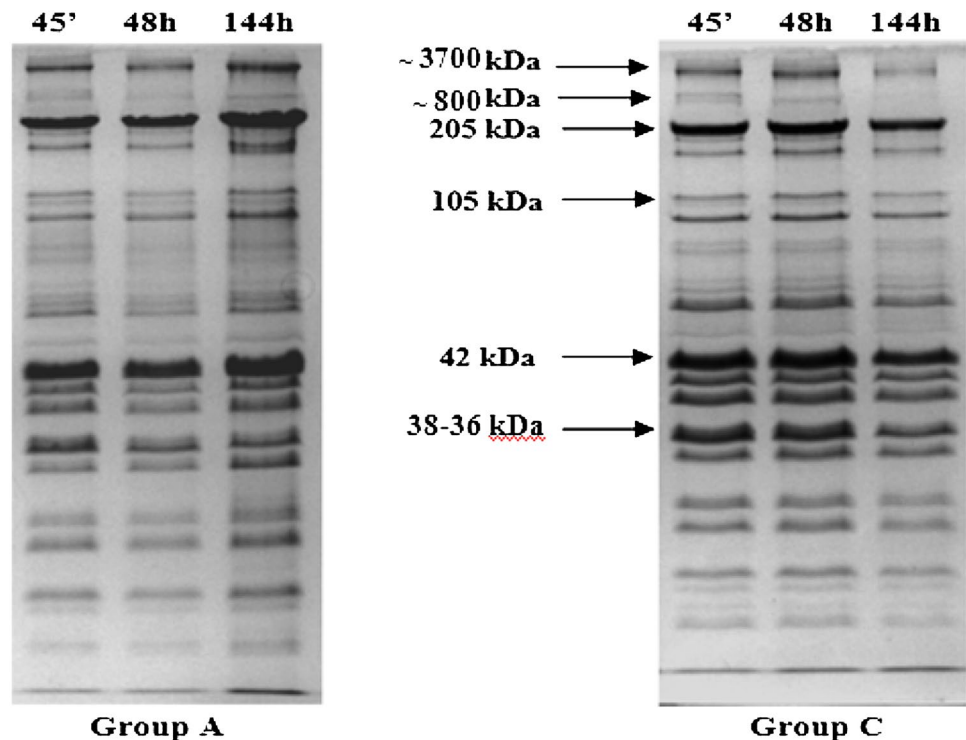
process of native titin degradation and its liberation from the cytoskeletal structures and it may exert negative influence on tenderness, as indicated in experiments conducted by other researchers [1, 44]. The analysis of correlation in group A showed a significant negative relationship between the pH value measured 24 h pm and the proportion of T1 in the tissue 48 and 144 h pm ($r = -0.94$ and -0.82 ; $p \leq 0.05$, respectively). As far as group C is concerned, the value of the correlation coefficient between the percentage of T1 in the muscle tissue 48 pm and the pH value 24 and 48 pm amounted to $r = 0.83$ and 0.82 , respectively ($p \leq 0.05$). These data indicate close correlation between native titin degradation and the rate of glycolysis. In all the groups there were statistically significant correlations between tenderness of the muscle tissue and the intensity of the T1 band 48 and 144 h pm ($r = 0.57$ and 0.65 ; $p \leq 0.05$, respectively). However, the values of correlation were not very high. This indicates that the process of meat tenderisation is relatively significantly associated with the degradation of cytoskeletal proteins. They are a scaffolding for myofibrils, in which titin participates to a considerable extent.

The next band visible on polyacrylamide gel (Fig. 1) was a product of titin (T1) degradation of about 800 kDa. Its derivation from titin was confirmed by immunoblotting using the 9D10 anti-titin antibody. Its proportion was the highest ($p \leq 0.05$) in the tough meat of groups A and B 45 min and 48 pm, amounting to: 1.60 and 1.57% or 1.24 and 1.07%, respectively. On the other hand, the smallest

quantities of this protein were found in tender muscles of groups C and D after 144 h of cold storage (Table 3).

We observed an increasing tendency in the proportion of the 205 kDa band on electropherogram during meat storage. Investigations conducted by Huff-Lonergan et al. [22] and Taylor et al. [51] on bovine skeletal muscles proved that some titin degradation products may have the same molecular weight as myosin heavy chains (MHC) and this might explain the increased proportion of this band in this study. After 48 pm, the 205 kDa protein band proportion was the highest in the muscles of group C, as compared with the tough meat of groups A and B. Following 144 h pm, the highest amount of the 205 kDa protein was recorded in the muscles of group C, as compared with groups A and B. Grześ et al. [18] showed that the lesser tenderness of pork from *RYR1^T* carriers (with the CT and TT genotypes) was related to the greater percentage of MHC isoform type IIb. There was also better tenderness of the bovine *longissimus dorsi* muscle of four cattle breeds (Polish Holstein–Friesian, Polish Red, Hereford and Limousine). It was characterised by the largest percentage of MHC isoform type IIa as well as the effect of the μ -calpain (*CAPN1S*) gene as a factor determining meat tenderness [24]. Differences in MHC isoforms may also be related to changes in the water-holding capacity of meat [18]. In this case, a larger amount of centrifugal and thermal drip of meat collected from pigs exposed to stress (TT) 144 h post mortem could have been related, among other things, with an increased content of MHC isoform type IIb [18].

Fig. 1 SDS-PAGE profiles of muscle tissue proteins from pork. Group A—samples from tough meat during the entire 144 h pm, Group C—samples from tender or relatively tender meat after 48 and 144 h pm



The protein band of 105 kDa, was most probably α -actinin. It is a factor which cements the Z disc structure and which is responsible for the sarcomere stability [5]. It is believed that this protein does not undergo degradation but is released from cytoskeletal structures during pm proteolysis [10, 11]. This gradual process of liberation was observed during the course of tenderisation of the four groups of muscles examined. At all terms of study the percentage of this protein was relatively high in tough muscles in group A: 3.37% (45'), 3.62% (48), and 3.53% (144 h) as well as in group D (3.05%) 48 pm (Table 3). In most cases the differences between this (group A) and other (B, C and D) groups were statistically significant ($p \leq 0.05$). Investigations carried out 48 pm showed that the content of this protein was the lowest in group C (2.76%) and B (2.83%). The correlation analysis for all the groups under study showed a significant relationship between the intensity of the 105 kDa band (48 and 144 h) and shear force assessed 144 h pm ($r = 0.50$ and 0.54 ; $p \leq 0.05$, respectively).

For a long time the rate of troponin T (Tn-T—m.w. of about 38 kDa) degradation has been considered as an indicator of meat tenderisation processes [42]. The statistical assessment revealed a decrease of about 38 kDa protein band abundance practically in all muscles of the groups under study during their cold storage (Table 3). At the first term of examination the percentage of the Tn-T band was statistically significantly higher in the meat of group C (8.21%) than in groups A and B. After 48 pm the proportion of this protein in the muscles of group C decreased to 6.86%. In comparison with group C, 48 pm, the lowest levels of this protein were recorded in groups A and B (5.51 and 5.17%, respectively) ($p \leq 0.05$) (Table 3).

Assessment of changes in the proportion of centrifugal drip proteins

Several studies point out [13, 37, 43] that changes in myofibrillar proteins can find their reflection in separations of centrifugal drip proteins obtained from the muscle tissue at the same terms. The electrophoretic analysis of centrifugal drip proteins showed increased proportions of bands whose m.w. reached 3700–2400 kDa in all the groups during 144 h pm storage. At the first term of investigations (48 pm), the mean proportion values of this protein ranged from 0.83 to 2.00%, while after 144 h pm—from 2.05 to 2.71% (Table 4). The presence of titin T1 and titin T2 (m.w. about 2400 kDa) in the fraction of centrifugal drip was confirmed by western blotting using the anti-titin monoclonal antibody 9D10. The increase of its percentage proportion between 48 and 144 h pm might indicate that beside probably degradation of this protein its release from muscle cytoskeletal structure took place. As a result of this reaction followed an increase of

Table 4 Changes in the relative proportion of the centrifugal drip proteins from pork differed in its tenderisation (%)

Centrifugal drip proteins/group	Time of storage	
	48 h	144 h
3700–2400 kDa		
A	0.91 ^{a*} \pm 0.17	2.23 \pm 0.97
B	1.56 ^{ab} \pm 0.78	2.05 \pm 0.76
C	0.83 ^a \pm 0.22	2.63 \pm 0.73
D	2.00 ^b \pm 1.10	2.71 \pm 0.87
153 kDa		
A	2.93 \pm 0.76	3.45 ^a \pm 0.68
B	2.76 \pm 0.58	2.84 ^a \pm 0.42
C	2.86 \pm 0.90	3.09 ^a \pm 0.55
D	3.01 \pm 0.52	4.24 ^b \pm 0.29
38–36 kDa		
A	20.55 ^c \pm 1.88	20.05 ^b \pm 1.18
B	11.70 ^a \pm 1.05	11.89 ^a \pm 0.79
C	12.76 ^a \pm 1.99	10.86 ^a \pm 1.18
D	15.90 ^b \pm 3.87	11.62 ^a \pm 1.18

*, ^a, ^b, ^c Means in the same column with different superscripts are significantly different at $p \leq 0.05$

A—meat remaining tough during the entire 144 h pm changes

B—meat characterised by typical tenderisation process, i.e. tough 48 h pm, and tender after 144 h pm storage

C—tender or relatively tender meat on both aforementioned dates of examination

D—meat toughest 48 h pm, and most tender 144 h pm

weakening the external cytoskeletal structure, what could foster an improvement of meat tenderness. This phenomenon was probably reflected also by the measurements of the muscle shear force. Its lower values in groups B, C and D in comparison to the group A were noted 144 h pm (Table 2). The T1 degradation with progressing meat aging was observed also in earlier studies [16, 23, 45].

The statistical analysis of the proportion of proteins with m.w. of about 153 kDa revealed an increase in their proportion in the course of storage, although sometimes it was relatively small. At the first term of examination (48 pm), their mean percentage ranged from 2.76 to 3.01%, while after 144 h pm—from 2.84 to 4.24%. In comparison with the other groups the highest proportion (statistically significant difference) was determined in group D, where the tenderness of muscles was the highest 144 h pm. The increased proportion of this protein band observed in the drip may have been caused by the liberation of MHC degradation products. This assumption comes from comparisons of our results with the experiments by Sawdy et al. [46]. They used SDS-PAGE and mass spectrometry and identified fragments of similar size. It shows that MHC degradation products can be

identified in the drip and their presence could be connected with the process of meat tenderisation.

We paid special attention to changes in the proportion of protein in a weight range of 38–36 kDa, which was identified as the glyceraldehyde-3-phosphate dehydrogenase (GAPDH with an m.w. of about 37 kDa) by means of western blotting, using the specific monoclonal antibody (clone GAPDH 71.1). The highest ($p \leq 0.05$) proportion of this protein band was observed in tough muscles, i.e. in group A—20.55% (48) and 20.05% (144 h). The smallest proportion of the protein band at the first term of examination was recorded in drips derived from groups B and C (Table 4). In other studies researchers observed that this band disappeared during cold storage of packed meat [39] and simultaneously, the quantity of this protein decreased noticeably in tender muscles [48]. In our study we noted that the amounts of this protein in drips derived from tender muscles in groups B, C and D 144 h pm were statistically significantly ($p \leq 0.05$) lower than in group A (tough meat at both terms). Moreover, we found statistically significant ($p \leq 0.05$) differences in the proportion of this band in the drip from groups A and D between 48 and 144 h pm. The correlation analysis for all the experimental groups revealed a significant correlation between the proportion of the 38–36 kDa band on gel from both terms of examination and tenderness determined 144 h pm (respectively: $r = 0.67$ and 0.87 ; $p \leq 0.05$). It confirms that the decreased proportion of this protein observed in the drip was associated with the tenderisation process.

Canonical analysis of results

The obtained results revealed that in some cases, in our study—groups, changes of single proteins did not fully or

with a similar probability described the process of meat tenderisation. Its pattern varied depending on the muscles acidification process and their diverse proteolysis. Because these changes to some extent might be linked to the pigs genotypes we mentioned them. Now, to get deeper or more precise relationships it can be used various methods of the statistical evaluations [33, 38, 51]. Our special attention was turned to the canonical correlation analysis which offers the possibility to observe relationships between sets of variables (Table 5).

In order to make a canonical correlation analysis the traits under study were divided into two sets of variables. The first set comprised determined (dependent) variables, i.e. meat tenderness assessed by means of the Warner–Bratzler shear force at each or at one of the terms under analysis. The other set included data determining the proportion of protein in the muscle tissue and the centrifugal drip on the gel. In some cases the other data set was extended by the values of pH_1 and EC1 (Table 5).

The analysis of the canonical correlation between tenderness (48 and 144 h pm) and the percentage of titin T1 and its degradation products in the muscle tissue (about 800 kDa) and centrifugal drip (3700–2400 kDa), and 38–36 kDa proteins (in the centrifugal drip and muscle tissue 48 and 144 h pm) revealed the highest correlation and determination coefficients ($C_R = 0.94^{**}$; $R_C^2 = 0.89$) (comparison I). This suggests that the degradation of these proteins significantly affects the meat tenderisation process.

A highly significant canonical correlation ($C_R = 0.94^{**}$) was also observed between shear force values after 48 and 144 h pm and the proportion of centrifugal drip proteins with m.w. of 3700–2400 kDa, 153 kDa and 38–36 kDa, pH_1 and EC1 values (comparison IV). A high value of the canonical correlation coefficient ($C_R = 0.88^{**}$) was also obtained by correlating the proportion of 3700, 800,

Table 5 Values of selected canonical correlations and determinant coefficients between sets of analysed muscle traits

Comparisons	Characteristics		Coefficients	
	Dependent (being explained)	Independent (explaining)	C_R —canonical correlation value	R_C^2 —determinant coefficient value
I	Shear force values 48 and 144 h pm	Muscle tissue proteins: 3700 kDa, 800 kDa and centrifugal drip proteins: 3700–2400 kDa, 38–36 kDa (48 and 144 h pm)	0.94 ^{**}	0.89
II	Shear force value 144 h pm	Centrifugal drip proteins: 3700–2400 kDa, 153 and 38–36 kDa (144 h pm)	0.90 ^{**}	0.79
III	Shear force value 144 h	Muscle tissue proteins: 3700, 800 and 105 kDa (144 h pm)	0.80 ^{**}	0.64
IV	Shear force values 48 and 144 h pm	Centrifugal drip proteins: 3700–2400, 153 and 38–36 kDa (48 and 144 h pm) and pH_1 and EC1 values	0.94 ^{**}	0.88
V	Shear force values 48 and 144 h pm	Muscle tissue proteins: 3700, 800 and 105 kDa (48 and 144 h pm) and pH_1 and EC1 values	0.88 ^{**}	0.79

^{**} Coefficients statistically significant at $p \leq 0.01$

105 kDa muscle tissue proteins marked 48 and 144 h pm with both pH₁ and EC1 values as well as shear force values 48 and 144 h pm (comparison V).

These results let us conclude that meat tenderness can be very accurately estimated 48 and 144 h pm on the basis of protein changes analysed using 1D electrophoresis and glycolysis process assessment criteria (pH₁ and EC1). The extended applicability of canonical variable analysis was also revealed by the assessment of relationships between the proportion of centrifugal drip proteins with an m.w. of 200 kDa and 42 kDa detected by using 2DE and the pH and EC values [38]. In this case the most significant ($p \leq 0.01$) relationships were noted between percentage proportion of 200 kDa protein (3 h pm) and pH₁ with pH₂ ($C_R = 0.98$). Similar relationship was ascertained between the proportion of 42 kDa protein (48 pm) and pH₂ with EC₂₄ ($C_R = 0.96$) values. High canonical correlations ($C_R = 0.69$ – 0.98) were also stated between proportion of above-mentioned centrifugal drip proteins and the shear force and cooking loss values 144 h pm; however, they were not statistically significant [38].

Conclusions

1. The process of meat tenderization in the porcine *longissimus* muscle is associated with degradation or release of muscle tissue proteins especially with m.w. of about 3700, 105 and 38 kDa as well as the changes of centrifugal drip proteins in m.w. ranges of 3700–2400 kDa and 38–36 kDa.
2. The high values of correlation coefficients ($r = 0.82 \div 0.94$, $p \leq 0.05$) between the percentage of native titin (T1) in the muscle tissue 48 and 144 h pm and the pH 24 and 48 pm indicate close correlation between T1 degradation and the rate of glycolysis in muscles which was also linked with genotype of pigs.
3. The canonical correlation results point out that it is possible to predict meat tenderness with high probability (even 89%) on the basis of selected protein changes analysed by means of electrophoresis and additionally, in some cases—by assessment of criteria of the glycolysis process (pH₁ and EC1).

Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

Compliance with ethics requirements This article does not contain any studies with human or animal subjects.

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