Ann. Anim. Sci., Vol. 16, No. 2 (2016) 585–600 *DOI: 10.1515/aoas-2015-0083*

DE GRUYTER
OPEN

THE EFFECT OF MUSCLE TYPE AND TIME OF STORAGE ON MYOFIBRILLAR PROTEIN PROPORTION IN BEEF*

Marzena Zając[1], Krystyna Palka[2], Beata Mikołajczak[3♦], Edward Pospiech[4]

[1]Department of Animal Products Technology, Faculty of Food Technology, University of Agriculture in Kraków, Balicka 122, 30-149 Kraków, Poland
[2]Department of Animal Products Technology, Faculty of Food Technology, University of Agriculture in Kraków, Balicka 122, 30-149 Kraków, Poland
[3]Institute of Meat Technology, Poznań University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland
[4]Institute of Meat Technology, Poznań University of Life Sciences, Wojska Polskiego 31, 60-624 Poznań, Poland
♦Corresponding author: bemik24@up.poznan.pl

Abstract

Tenderness is usually associated with the proteolysis occurring in muscles. However, most of the studies concentrate on one muscle only. The aim of this study was to describe the changes in myofibrillar protein percentage proportions during the ageing of 8 bovine muscles. Investigations were conducted on the muscles from different parts of the carcass, from the forequarter: *m. pectoralis profundus*, *m. infraspinatus*, *m. triceps brachii*, *m. serratus ventralis*, and from the hindquarter: *m. biceps femoris*, *m. semimembranosus*, *m. semitendinosus* and *m. longissimus dorsi* (*thoracis et lumborum*). The effect of muscle type was significant for all parameters except for percentage proportions of titin (3000÷3700 kDa), MHC (205 kDa) and protein fractions between <205÷42> kDa. Differences between the muscles varied depending on the analysed proteins and the time of storage. A significant effect of ageing time for titin, nebulin (approx. 800 kDa), proteins of molecular weight of 38 kDa, proteins smaller than 42 kDa and in the range of 3000÷205 kDa, 205÷42 kDa and 38÷20 kDa was observed. The decrease of percentage proportions of titin, nebulin and proteins in the range of 3000÷205 kDa and an increase of protein bands in the range of 38÷20 kDa and proteins below 42 kDa was also observed. During the storage period of beef from the 2nd to the 14th day, the progress of myofibrillar proteolysis was different in each muscle. The changes of tenderness were not related to shear force values. It is probable that the changes in other constituents of meat might influence the tenderness more than those in myofibrillar proteins.

Key words: myofibrillar proteins, bovine muscles, ageing, SDS-PAGE

*Source of research financing: The EU Marie Curie Fellowship Scheme.

Various factors generate differences between muscles in regard to tenderness: total and insoluble collagen content, marbling, sarcomere length, fibre diameter etc. depending on the animal's age, breed and type of breeding (Marino et al., 2013; Palka, 2003; Purslow, 2005). It was proved by Marino et al. (2015) that some myofibrillar degradation products appear earlier in muscles which are more tender. Studies of Chen et al. (2015) showed that phosphorylation levels of myofibrillar proteins may vary between muscles of different tenderness, which is also connected with the different pH of meat (Lomiwes et al., 2014; Wu et al., 2014 b). It was proven that the proteolysis of myofibrillar proteins, which comprise also the cytoskeletal fraction, are crucial during the meat tenderisation process (Maltin et al., 2003). It has been analysed in various species and breeds of animals (Claeys et al., 2004; Gil et al., 2006; Sazili et al., 2005). However, the process is complex and there is no unified thesis concerning the influence of individual proteins on tenderness. Myofibrillar and sarcoplasmic proteins or their degradation products have been analysed to determine early toughness predictors in beef or chicken meat. These could be used as a tool for carcasses classification into fast and slow tenderising meat (Gagaoua et al., 2015; Sierra et al., 2012; Tomaszewska-Gras, 2011). There is an indication that the proteolysis proceeds in various ways in selected muscles within the same carcass.

For meat tenderisation, the cytoskeletal protein degradation is the most important, especially in the early stages of ageing. Z-line degradation (α-actinin) and regulatory proteins degradation (troponin T, tropomyosin) are also important (Taylor et al., 1995). Troponin T regulates skeletal muscle contraction and forms small peptide fragments during ageing. It is possible that some degradation products would appear in sarcoplasmic fraction as a consequence of liberation and transforming to an insoluble state (Bjarnadottir et al., 2010). The ca. 30 kDa fragments appearing during the ageing are commonly associated with the tenderisation process and considered to be a part of troponin T (Ho et al., 1994; Olson et al., 1977). Wu et al. (2014 a) reported that also proteins like titin, nebulin and filamin undergo degradation during ageing, but they did not relate it with tenderness. Muroya et al. (2010) proved that the degradation of desmin and fast troponin T isoform is faster in fast twitch glycolytic fibres (type IIb) than in slow twitch oxidative fibres (type I). Muscles composed mainly of type IIb fibres are more susceptible to early proteolytic degradation in comparison to those in which type I fibres are in excess (Xiong et al., 2007). Ouali and Talmant (1990) reported a higher calpain/calpastatin ratio in type IIb than type I muscles in various animals. Maltin et al. (1998) have shown that increasing the proportion of type I fibres improves the tenderness in beef. Therefore, the relationship between muscle fibre type and meat tenderness is still controversial (Chang et al., 2003; Choi and Kim, 2009; Maltin et al., 2003).

Most of the studies either compare muscles originated from animals of various breeds, genotypes, breeding types etc. or concentrate on one muscle which is usually *m. longissimus dorsi* (Choi and Kim, 2009; Muroya et al., 2010; Sierra et al., 2012). Therefore, it was interesting to verify if there is an effect of muscle localisation in the carcass, on the extent of muscle proteolysis during *postmortem* ageing. It could be essential in regard to the meat tenderisation process related to myofibrillar component.

Material and methods

Materials and sample preparation

Irish Hereford–Friesian (IHF) heifers (n=6) of an average age of 22 months were slaughtered humanely. The conventional procedure was applied. The animals were stunned by captive bolt pistol and exsanguinated. The hot weight of the carcasses was about 250 kg. The carcasses were chilled at 10°C for 10 h and then at 2°C until excision 48 h *postmortem*. On the second day *postmortem* muscles were excised from the left side of the carcass: *m. longissimus dorsi (thoracis et lumborum) (LD)*; *m. biceps femoris (BF)*, *m. semimembranosus (SM)*, *m. semitendinosus (ST) m. pectoralis profundus (PP)*, *m. infraspinatus (IS)*, *m. triceps brachii (TB)* and *m. serratus ventralis (SV)*. The muscles were vacuum packed and stored in a cold room at 2°C until the 14th day *postmortem*. Samples for protein analysis were taken on the 2nd, 7th and 14th day post-slaughter. They were frozen in liquid nitrogen and stored at –80°C until analysis.

Chemical and physical measurements

pH

pH measurements were taken 48 h *postmortem* on freshly excised, intact muscles using a portable pH meter (Orion Research Inc., Boston, MA 02129, USA) and an Amagruss pH electrode (pH/mV Sensors Ltd., Murrisk-Westport, Co. Mayo, Ireland), which was corrected for muscle temperature before being inserted approximately 6 cm into the muscles. Before analysis the pH-meter was calibrated using standard phosphate buffers (pH 4.00 and 7.00) after which the electrode was washed. The electrode was rinsed thoroughly with distilled water between the measurements.

Myofibril preparation

Myofibrills were isolated using the procedure of Etlinger et al. (1976) modified by Murray (1997). On the day of analysis 5 g of the sample was weighed and mixed with 30 mL of LSB buffer (20 mM Tris-Maleinate, 100 mM KCl, 3 mM $MgCl_2$, 3 mM EGTA, 1 mM DTT), 30 µL PMSF solution (10 mg PMSF, 10 mg pepstatin in 20 mL of methanol) and 30 µL E-64 inhibitor of proteinases. It was homogenised for 60 s and filtered through gauze to a test tube. Samples were centrifuged 10 min using 800 g in 4°C. After centrifuging the supernatant was discarded and the precipitate was mixed with 30 µL PMSF, 30 µL E-64 and 30 mL LSB buffer and centrifuged again (repeated 2x). After the final centrifuging the precipitate was mixed with 30 mL of LSB buffer and 30 µL PMSF and centrifuged again (repeated 2x). After the last centrifuging the supernatant was discarded, 30 mL of Triton-X-100 solution was added [LSB with Triton-X-100 (0.5% v/v)] and vortexed for 30 min on ice. It was centrifuged for 10 min using 800 g at 4°C. The precipitate was mixed with 30 mL of LSB buffer and centrifuged again as previously (repeated 3x to remove the detergent). Finally, 4 mg of myofibrils were mixed with buffer (pH 6.8; 8 M urea, 2 M thiourea, 0.05 M Tris-HCl, 0.075 M DTT, 3% (w/v) SDS, 0.05% (w/v) bromophenol blue) (Fritz and Greaser, 1991). Samples were heated for 3 min at 100°C. Protein concentration was determined using a 2-D Quant Kit (GE Healthcare Bio-Sciences).

Resolving gel preparation

Electrophoretic analysis (SDS-PAGE) in the 15% polyacrylamide gel with the addition of 8 M urea using the procedure of Pospiech et al. (2000) was conducted. The gels were produced in triplicate. The separation of myofibrillar proteins was performed using two-phase gels. These phases combine resolving and stacking gel. Their preparation in the cassette began from the resolving layer. The main separation of myofibrillar protein analysis was performed in 15% (8 M urea, 30% (w/v) acrylamide, 75% (v/v) glycerol; 3 M Tris, pH 8.8; 10% (w/v) SDS; 1% (w/v) ammonium persulfate; TEMED 16 µL. A PageRuler Plus Protein Ladder 10 to 250 kDa (Thermo Scientific) containing 9 proteins (250 kDa; 130 kDa; 100 kDa; 70 kDa; 55 kDa; 35 kDa; 25 kDa; 15 kDa; 10 kDa) was used for molecular weight (m.w.) calibration.

Stacking gel preparation

After the resolving gel polymerisation, the stacking gel (10% (w/v) acrylamide, 5% (v/v) glycerol, 0.125 M Tris (pH 6.8), distilled water, 10% (w/v) SDS, 1% (w/v) ammonium persulfate, staining buffer, TEMED) was prepared and poured on the layer of resolving gel.

All gels were prepared using 80 × 100 mm plates with spacers 0.75 mm. 7.5 µl of each sample was loaded into separate wells. The amount of proteins in each sample was 7 µg. The separation was conducted at room temperature in the vertical system using the SE 250 type apparatus (Hoefer Scientific Instruments Company). Electrophoresis was run with a constant current 20 mA per gel. Proteins were visualised by staining in a solution 0.05% (w/v) Coomassie Brilliant Blue R-250, 50% (v/v) methanol, 10% (v/v) acetic acid for 1 hour and destained by diffusion in 10% (v/v) methanol, 4.5% (v/v) acetic acid for several hours.

Image analysis

All the images of the gels were acquired using an imaging system Image Master® VDS Pharmacia Biotech and analysed using the Image Master® 1D Elite v. 4.0 program. Computations were based on the assumption that the area of a single protein band accounts for a percentage ratio in relation to the area of all separated protein bands, which constitutes 100%. Quality identification of selected peaks was conducted on the basis of determinations of molecular weight in kiloDaltons (kDa) using protein standards (Thermo Scientific).

Shear force

The steaks (2.54 cm thick) were cut perpendicular to the muscle fibres and grilled to an internal temperature of 72ºC. They were cooled in the refrigerator and prior to the analysis the cylindrical samples of diameter 14 mm and length 15 mm were cut out longwise to the muscle fibres from the meat slices. The measurements were carried out for a sample cut perpendicular to the direction of the muscle-fibre arrangement using a TA-XT2 apparatus and Warner-Bratzler knife with triangular cut-out. Eight measurements were taken on each steak.

Statistical analysis

All data were subjected to analysis of variance (ANOVA) using STATISTICA 10 software. When significant effects were found ($P<0.05$), the Duncan multiple range test was used to locate significant differences between the means. The results are presented as average values ± standard deviations.

Results

There was no significant difference between the muscles in pH value (Table 1). The average value for all the muscles was 5.6. These results allowed for excluding the DFD condition. SDS-PAGE was carried out to determine the role of proteolysis during ageing time in selected muscles excised from different locations in the beef carcass. Myofibrillar protein degradation patterns of analysed muscles on the 2nd, 7th and 14th day *postmortem* in 15% gels are presented in Figures 1, 2 and 3 respectively. The percentage content of the titin band, whose molecular weight (m.w.) was assumed to be of approximately 3000÷3700 kDa, nebulin (~800 kDa), myosin heavy chains (MHC) (205 kDa), α-actinin (105 kDa), actin (42 kDa), troponin T (38 kDa), and proteins in the range of: <3000÷205> kDa, <205÷42> kDa, <38÷20> kDa and smaller than 42 kDa was assessed for each muscle. The changes in their percentage proportion as affected by muscle and ageing time are presented in Table 2.

Table 1. pH values of the analysed muscles (means ± SD)

Muscle	LD	BF	SM	ST	IS	PP	TB	SV
pH	5.6±0.1	5.6±0.1	5.6±0.1	5.6±0.1	5.7±0.1	5.6±0.1	5.6±0.1	5.7±0.1

The effect of muscle type on the extent of protein degradation was statistically significant (Table 3). The differences between the muscles varied depending on the analysed fraction and also on the day of analysis, which suggests that the process of proteolysis was different in each muscle. The actin content was significantly higher in TB muscle. The proportion of nebulin (~800 kDa) was the smallest in IS and the largest in LD. There were significant differences between the ST muscle, in which the lowest percentage of 38 kDa protein band was detected, and the TB, in which it was the highest. The percentage of proteins bands in the range of 38÷20 kDa was the highest in IS and BF and the lowest in TB (Table 3). The concentration of the α-actinin band (105 kDa) was significantly higher in IS in comparison to other muscles.

The effect of ageing time was significant ($P<0.05$) for the titin, nebulin, troponin T and proteins in the range of 3000÷205 kDa, 205÷42 kDa, 38÷20 kDa, and also the proteins smaller than 42 kDa (Table 3). A significant increase was noted for 38 kDa protein during ageing. There was only a slight decrease noted in the MHC percentage, but these changes were not statistically significant.

Table 2. Changes (%) in myofibrillar proteins and proteolysis products as affected by muscle and ageing time (means± SD)

Molecular weight (kDa)	Ageing (days)	LD	BF	SM	ST	PP	IS	TB	SV
1	2	3	4	5	6	7	8	9	10
3000–3700	2	6.71 a±0.79	7.42±0.74	7.65±2.82	7.99±2.06	8.92 AB±2.06	9.49 bA±1.30	10.76 bA±2.47	9.61 b±1.68
	7	8.82±0.74	6.78±0.87	7.61±1.00	7.51±1.34	9.38 A±3.66	7.90 A±1.26	8.21 A±2.92	7.46±0.95
	14	8.28 a±1.23	7.26±1.96	7.12±1.40	4.93 b±1.96	6.21 B±1.29	6.62 B±3.08	5.51 B±1.27	7.34±1.35
~ 800	2	2.74±0.46	3.42 A±0.60	3.01 aA±0.31	2.96 a±0.58	2.16±0.18	1.6 b±0.30	2.69 A±0.76	2.07±0.21
	7	2.76 ac±0.75	2.18 cdAB±1.01	1.74 B±1.47	1.99±1.84	1.89 ab±0.47	1.34 b±0.65	1.13 bdB±0.40	1.69±0.33
	14	1.90±0.62	1.49 aB±0.56	1.45 aB±1.66	2.17±1.68	1.23 a±0.92	1.52 a±0.84	1.82 B±0.88	2.95 b±0.81
205	2	22.01±1.10	24.38 aA±0.82	19.79 b±1.44	20.95±1.11	22.81±2.16	22.56±0.86	21.86±0.64	21.37±1.83
	7	21.95±4.4	20.41 B±0.27	21.88±1.11	22.84±2.87	21.68±2.17	20.62±5.26	22.79±2.72	21.71±1.06
	14	21.30±1.40	18.13 aC±0.99	21.72±1.85	23.02 b±1.35	20.28±1.82	23.40 b±1.62	21.60±0.33	20.52±1.94
105	2	2.54 ac±0.60	1.44 a±0.35	1.87±0.23	1.53 ab±0.47	2.24 ac±0.36	1.10 bA±0.34	2.63 cA±0.33	1.94 A±0.35
	7	1.32 a±0.51	1.26 bc±0.27	1.52 a±0.24	1.59 a±0.52	1.24 a±0.56	4.67 bB±1.95	1.21 BC±0.77	1.61 A±0.18
	14	1.29 a±0.75	1.21 a±0.26	1.01 a±0.33	1.42 a±0.43	1.32 a±0.51	1.94 bA±0.94	1.55 aAC±0.36	3.11 bB±2.35
42	2	24.91±2.60	23.35±0.14	26.37±0.54	25.95±1.72	25.28 AB±1.34	24.75±1.67	25.85±0.70	25.70±0.69
	7	23.55±0.32	24.41±1.01	24.71±2.24	25.08±2.91	22.75 aA±1.87	23.21±3.49	26.09 b±3.34	23.66±4.25
	14	23.21 a±0.59	24.88±1.13	24.32±1.84	23.81±0.29	26.42 bB±3.42	25.06±1.37	24.74±2.17	24.50±1.12
38	2	3.52 a±1.33	0.99b A±0.51	3.09 ac±1.46	1.58 bcd±0.48	1.57 bcdA±0.81	2.09±0.77	2.83 adA±0.56	2.45±0.77
	7	3.22±1.73	2.60 B±0.94	3.32±0.50	2.61±1.72	3.29 B±0.17	2.33±0.34	2.57 A±0.94	3.66±2.42
	14	3.38 a±0.87	4.67 bC±0.80	3.04 a±1.26	3.67 a±0.45	4.01 B± 0.91	3.34 a±0.93	4.67 bB±0.37	2.73 a±0.35

<3000–205>	2	3.31±1.24	3.50±0.88	3.85±0.93	3.38 a±0.55	3.13 A±0.13	2.62b±0.52	3.06±0.80	3.08±0.49
	7	3.27±0.34	2.42±0.60	3.18±0.60	3.38±0.67	2.31 B±0.31	3.32±1.73	2.23±0.56	3.04±1.26
	14	2.10 ad±0.36	2.63 ad±0.57	1.93 acd±1.30	2.18 acd±1.65	1.23 cB±0.92	2.41±0.19	2.69 db±1.22	3.67 b±0.46
<205-42>	2	15.90±2.10	14.32 a±1.84	15.63±2.99	15.69±1.60	15.70±0.68	16.82±1.53	17.66 b±0.64	18.17±0.84
	7	15.57±2.13	17.65±1.58	17.61±2.81	15.99±0.78	17.64±0.78	17.44±2.76	16.40±3.05	17.80±3.77
	14	16.88±0.72	17.02±3.65	15.81±1.75	17.11±1.37	18.56±2.13	16.87±0.98	16.30±1.55	16.91±1.19
<42	2	27.68 a±4.81	16.69 bA±1.18	22.39 cdeA±1.99	18.68 cdA±0.45	22.75 eA±1.47	22.92 eA±0.55	20.47 deA±1.83	22.33 deA±0.88
	7	26.84 ac±4.28	26.43 B±0.33	24.15 A±2.24	24.42 B±1.77	23.54 bA±3.11	26.87 cBC±1.96	23.25 bA±1.11	24.66 A±2.25
	14	27.89±1.70	29.83 aC±2.08	28.38 B±1.47	27.86 B±1.02	27.29 B±2.49	25.64 bAC±3.12	28.11 B±2.50	27.08 B±2.97
<38 – 20>	2	2.98 a±1.03	3.22 a±0.93	2.91 a±0.52	2.69±1.02	3.03 a±1.23	3.72 aAB±0.72	1.48 bA±0.42	2.73 aA±1.15
	7	3.66±1.08	3.93 a±0.15	3.27 a±1.04	3.54 ac±0.59	3.30 ad±1.21	5.14 bcA±2.09	3.28 aB±1.01	2.22 bdA±0.61
	14	3.57 ac±0.32	4.62 a±0.78	3.03 cd±2.16	4.36 ac±1.45	3.95 ac±0.93	3.52 adB±0.68	3.90 acB±0.42	5.41 bB±2.13

a, b – mean values with different letters in the same row indicate significant differences between the muscles (P<0.05).
A, B – mean values with different letters in the same column indicate significant differences between the ageing time within one muscle (P<0.05).

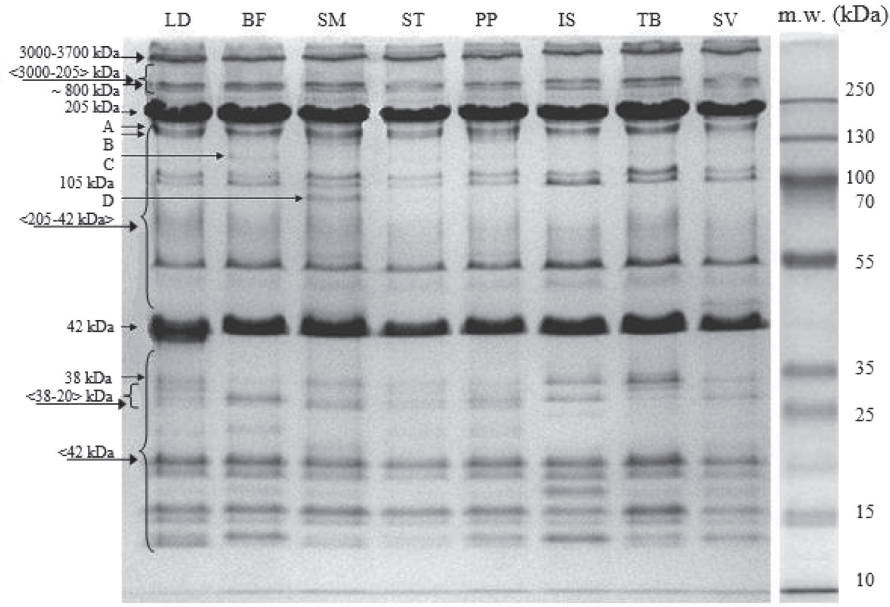

Figure 1. SDS-PAGE separations of myofibrillar extracts from bovine muscle on the 2nd day after slaughter

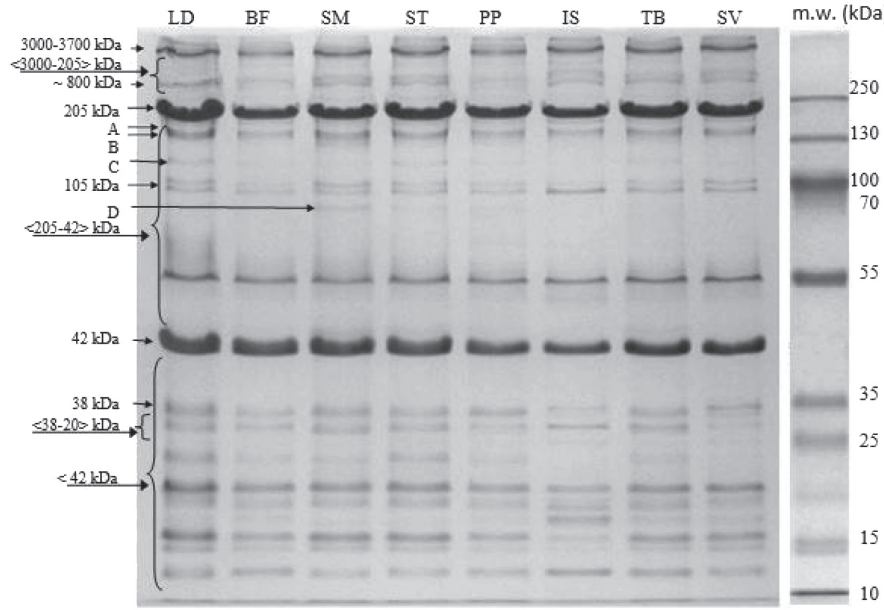

Figure 2. SDS-PAGE separations of myofibrillar extracts from bovine muscle on the 7th day after slaughter

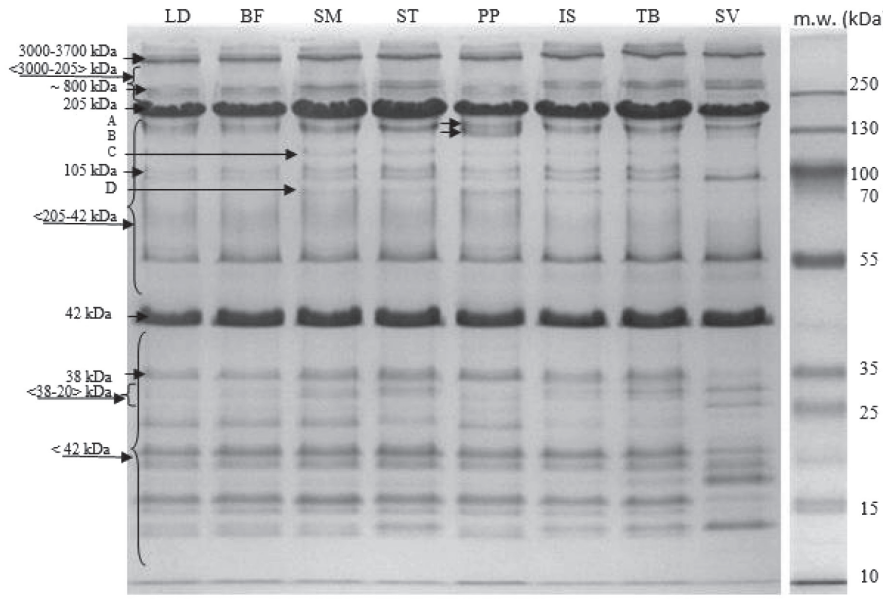

Figure 3. SDS-PAGE separations of myofibrillar extracts from bovine muscle on the 14th day after slaughter

A slight decrease in the percentage concentration of α-actinin (m.w. 105 kDa) was also noted during ageing time, but it was not statistically significant. No changes were observed in the proportion of actin and protein bands with m.w. in the range 205÷42 kDa during ageing. The amount of titin (3000÷3700 kDa) was decreasing during cold storage. A significant difference was observed between its amount on the 2nd and the 14th day of ageing and between the 7th and 14th day, but not between the 2nd and the 7th day (Table 3). The proportions of this protein band in the PP, IS and TB muscles was almost at the same level until the 7th day, and on the 14th day it was slightly lower (Table 2). In the SM muscle it did not change during the storage period, while in the ST its degradation was visible only on the 14th day (Table 2).

Comparing muscles individually, there were differences in the proportion of titin on the 2nd day of storage between the LD muscle, in which it was the lowest, and the IS, SV and TB muscles, where it was the highest (Table 2). After 14 days significant differences were noted only for the LD and ST muscles. The end effect of titin degradation in all muscles was comparable.

The content of nebulin (m.w. approx. 800 kDa) was decreasing during cold storage, although the pattern of changes varied between the muscles. Significant changes were observed only in the BF, SM and TB muscles. In the BF muscle its degradation was slow, whereas in the SM and TB muscles the most distinct changes took place during the first 7 days of *postmortem* storage and, from then onwards, only slight differences were noted (Table 2). There were significant differences among the muscles in the percentage of proteins in the range 3000÷205 kDa, especially on the 14th day

of storage. On that day the amount of these proteins was the highest in the SV and the lowest in the PP muscle (Table 2). The proportions of these proteins decreased during ageing (Table 3) although individual muscle analyses indicate significant differences only for the PP muscles. Statistically significant correlations ($P<0.05$) between percentage proportion of these proteins and shear force values for the PP ($r=0.71$) and BF ($r=0.66$) muscles were observed. The PP and BF were among the toughest cuts and the degradation of the proteins of high m.w. was still observable on the 14th day of ageing. It is possible that these muscles would need longer ageing periods to gain an acceptable tenderness.

Table 3. Least square means (LS) based on 2-factor analysis of variance referring to the effect of muscle type and ageing time

Traits of variability (kDa)	Muscle								Ageing time (days)		
	LD	BF	SM	ST	PP	IS	TB	SV	2	7	14
3000–3700	7,94 a	7,11 a	7,55 a	6,90 a	8,09 a	7,93 a	7,91 a	8,25 a	8,47 A	7,96 A	6,70 B
~ 800	2,44 Aa	2,33 a	2,08 abc	2,35 ac	1,73 bc	1,50 Bb	1,85 abc	2,28 a	2,56 A	1,84 B	1,81 B
205	22,00 a	21,00 a	20,95 a	22,27 a	21,58 a	21,94 a	22,09 a	21,30 a	21,94 a	21,74 a	21,24 a
105	1,62 Ca	1,27 B	1,44 B	1,42 B	1,56 BC	2,54 Aa	1,75 B	2,17 Cb	1,76 a	1,80 a	1,61 a
42	23,48 B	24,63 B	25,01 B	24,82 B	24,80 B	24,17 B	25,49 A	24,66 B	24,96 a	24,31 a	24,63 a
38	3,32 AC	2,79 ABC	3,11 ABC	2,52 B	2,98 ABC	2,60 ABC	3,38 C	2,94 ABC	2,28 A	2,95 B	3,64 C
<3000–205>	2,80 ab	2,89 ab	2,99 ab	3,28 a	2,45 b	2,79 ab	2,70 ab	3,21 ab	3,41 A	2,89 B	2,36 C
<205–42>	16,10 a	16,34 a	16,32 a	16,35 a	17,5 a	16,86 a	17,00 a	16,89 a	16,04 A	17,01 B	16,96 B
<42	27,21 A	24,33 B	25,05 B	23,98 B	24,63 B	25,28 B	23,93 B	24,60 B	21,92 A	25,02 B	27,72 C
<38–20>	3,35 abcdef	3,88 acf	3,18 ce	3,35 abcdef	3,46 abcdef	4,09 df	2,86 be	3,53 abcdef	2,86 A	3,54 B	3,99 B

a, b – mean values with different letters in the same row indicate significant differences ($P<0.05$) separately for each experimental factor.

A, B – mean values with different letters in the same row indicate significant differences ($P<0.01$) separately for each experimental factor.

Discussion

The differences in tenderness and general eating quality between muscles are well known (Grześkowiak et al., 2002; Shackelford et al., 1995). What is actually affecting them has been a subject of numerous studies (Herrera-Mendez et al., 2006;

Lepetit, 2007; Ngapo et al., 2002; Sawdy et al., 2004). The results obtained in this study show that the proteolysis effect is also muscle dependent. Every muscle should be analysed separately when it comes to ageing results and the whole carcass condition cannot be based on a single muscle analysis.

Table 4. Shear force (N) values of grilled muscles after 2, 7 and 14 days of ageing at 4°C (means ± SD)

Muscle	2 days	7 days	14 days
LD	61.8 bcx±7.8	46.1 cdy±4.9	36.3 cdy±4.9
BF	52.0 cdx±4.9	40.2 dxy±2.9	37.3 cdy±1.9
SM	103.0 ax±9.8	92.2 ax±15.7	66.7 ay±5.7
ST	73.6 bx±4.9	56.9 bcy±2.0	55.9 aby±6.9
IS	41.2 dx±5.9	37.3 dx±3.9	31.4 dx±2.9
PP	67.7 bx±7.8	54.9 bcxy±2.9	53.0 by±5.9
TB	70.6 bx±7.8	63.8 bxy±7.8	52.0 by±2.9
SV	72.6 bx±7.8	56.9 bcy±4.9	48.1 bcy±3.9

a, b – different letters in the same column indicate significant differences between muscles on the same day of *postmortem* ageing ($P<0.05$).

x, y – different letters in the same row indicate differences between ageing time of the same muscle ($P<0.05$).

The muscles LD and IS belong to the group of the most tender muscles in the carcass. Proteins smaller than 42 kDa appeared in these muscles in the highest proportions. Sierra et al. (2012), analysing animals of various genotypes, showed that the proteolysis pattern during ageing (2, 14 and 21 days *postmortem*) differs, but is correlated with the tenderisation scheme. In our study a significant increase of proteins smaller than 42 kDa was observed in almost all analysed muscles except the LD (Table 2). A similar rise in the size of this fraction was observed in meat from 12-month-old cattle in comparison with 6-month-old cattle (Iwanowska et al., 2010).

The changes observed in almost all the analysed fractions of proteins confirm previous results indicating that there are proteolytic changes occurring during cold storage of meat (Fritz et al., 1993; Pospiech et al., 2003; Iwanowska et al., 2010). The band of 205 kDa fraction, which was assumed to be MHC, decreased slightly. This is in agreement with the general opinion that its proportion does not change during ageing time (Gil et al., 2006; Kołczak et al., 2003). However, there are results indicating that the extent of MHC degradation can be related to meat tenderness. Sawdy et al. (2004) observed two protein bands with molecular weight of about 150 kDa appearing in acrylamide gels, which were identified as the products of MHC degradation. In earlier studies (Porzio et al., 1979) there were also 90–170 kDa proteins identified, and their appearance was linked with myosin and M-line degradation. Therefore, it is possible that the protein bands A and B presented in Figures 1–3 might be MHC degradation products. Experiments conducted by Grześ et al. (2010) revealed that

a higher amount of myosin heavy chains type IIb may be connected with lower tenderness values in meat originating from pigs of variable genotypes. It was also stated that significant differences in MHC polymorphism of bovine muscles result from calpastatin gene CAPN1S effect, which may also determine tenderness (Iwanowska et al., 2011). Further analysis is necessary to detect the origin of bands A and B. Analysing muscles individually, there was a significant decrease of the proportion of the MHC band in the BF muscle. Its amount was the highest on the 2nd day and the lowest on the 14th day between all the muscles. Bands A and B in that muscle seem to be almost invisible at the beginning of ageing and very distinct after 14 days of storage. This may prove that those bands are of MHC origin.

A slight decrease in 105 kDa proportion was also noted during ageing time, but it was not significant. Similarly, Iwanowska et al. (2010), analysing four breeds of bulls, did not observe changes in 105 kDa content, which is usually associated with α-actinin. It is a protein connected with Z-line. That slight reduction in its amount may indicate that the tenderisation process is connected with Z-line degradation. Although, as Taylor et al. (1995) have claimed, the degradation of I-line and not Z-line occurs during the *postmortem* tenderisation process. Almost no changes during ageing were observed in the percentage of bands with m.w. between 205÷42 kDa, nor in 42 kDa protein, which we assumed could be actin. The size of the band of titin decreased during cold storage – it is a protein that undergoes degradation during ageing time, resulting in the formation of the titin 1 (T1) and the titin 2 (T2) bands. Watanabe and Devine (1996) revealed that the titin 1 band disappears 48 h after slaughter and there is only the titin 2 band that is visible.

Comparing muscles individually there were differences in the percentage content of titin on the 2nd day of storage between the LD muscle which was the lowest and the IS, SV and TB muscles which was the highest. After 14 days significant differences were noted only for the LD and ST muscles (Table 2). Probably the end effect of the degradation of this protein in all muscles was similar. In the case of these muscles, a significantly higher tenderness of the LD muscles compared to the ST was noticed.

We observed a decrease of 800 kDa (probably nebulin) during cold storage but it was variable in the muscles. Huff-Lonergan et al. (1995) noticed the complete disappearance of nebulin by the 7th day *postmortem*. At the same time Fritz and Greaser (1991) described rapid nebulin degradation until 48 h *postmortem*. We started the analyses on the 2nd day after slaughter, therefore if those rapid changes had occurred they would not have been visible in our study.

The percentage of 38 kDa protein, which could be identified as troponin T, did not change significantly in most of the analysed muscles during ageing time; however, there were exceptions. In the BF, PP, ST and TB muscles its proportion increased, which was rather surprising (Table 2). It is possible that some other degradation products from larger proteins having the same molecular weight appeared along with troponin T as one band. Such a situation was also described by Szalata (2003). We expected that the amount of troponin T degradation products will increase during storage. Muroya et al. (2006), who analysed the *longissimus*, diaphragm and masseter muscles and observed differences between muscles in *postmortem* protein deg-

radation, suggested that each TnT isoform degrades into a specific fragment in each type of muscle with regard to their metabolism.

The differences between the muscles in the amount of proteins smaller than 42 kDa were significant only on the 2nd day *postmortem*. The BF muscle revealed the lowest and the LD the highest proportion of these proteins. It may indicate that the proteolysis in all the analysed muscles reached the same level at the end of cold storage of the meat.

The results showing the influence of muscle type and ageing time on shear force values are presented in Table 4. The shear force values were decreasing in all the muscles during ageing time, as was expected. In this study we were looking for a correlation between myofibrillar protein proportion and the results from instrumental tenderness analysis. There was a positive correlation between shear force values and nebulin (~800 kDa) and MHC content (r=0.79 in both cases). With the decrease of the shear force values there was an increase of protein fractions smaller than 42 kDa, and proteins in the range 42÷205 kDa noted. The correlation coefficients between them and tenderness changes were estimated at r=–0.83 and r=–0.54 respectively. For six of the eight analysed muscles there were negative correlations ($P<0.05$) noted between shear force values and protein fractions smaller than 42 kDa: BF (r=–0.83), SM (r=–0.64), ST (r=–0.75), PP (r=–0.56), TB (r=–0.79) and SV (r=–0.62). This dependency was not observed in the LD and IS. As these are very tender parts of the carcass it could indicate that the proteolysis influencing tenderness occurred earlier, or there are other factors affecting that quality trait.

Special attention was paid to a band marked as D in Figures 1–3. It was smaller in regard to m.w. than in the case of α-actinin and was observed on the 2nd day *postmortem* only in the SM. It was also visible in other muscles (ST, PP, IS and TB), but only after 14 days of storage. The appearance of this protein was not related to meat tenderness. The sensory analysis carried out on the same muscles showed that the SM was one of the toughest muscles; it was also the least juicy and flavoursome (Nowak et al., 2004). Shear force measurements conducted on grilled muscles confirmed the results of sensory analysis. SM and ST were the least tender muscles even after the 14-day ageing time. Another band marked C in Figures 1–3 appeared only in the BF and PP on the 2nd day *postmortem* and on the 14th day it was visible in all muscles except for the SV. Further analysis is necessary to determine the origin of these proteins.

The proteolysis occurring during ageing was visible in all the analysed muscles and its progress was variable for each muscle. The abundance of proteins varied individually for each muscle and also the proteolysis of selected protein fractions proceeded faster or slower depending on the muscle. Analysis of the correlations between shear force values and proportion of proteins content for each muscle suggests that the variability depended on the muscle. The appearance of certain non-identified proteins, specific to selected muscles, could not be related to meat tenderness. Therefore, further studies are necessary to establish the origin of the appearing proteins or fragments of proteins and also to analyse the other factors influencing the differences in tenderness between muscles.

References

Bjarnadottir S.G., Hollung K., Faergestad E.M., Veiseth-Kent E. (2010). Proteome changes in bovine *Longissimus thoracis* muscle during the first 48 h postmortem: shifts in energy status and myofibrillar stability. J. Agr. Food Chem., 58: 7408–7414.

Chang K.C., da Costa N., Blackley R., Southwood O., Evans G., Plastow G., Wood J.D., Richardson R.I. (2003). Relationships of myosin heavy chain fibre types to meat quality traits in traditional and modern pigs. Meat Sci., 64: 93–103.

Chen L., Li X., Ni N., Liu Y., Chen L., Wang Z., Shena O.W., Zhang D. (2015). Phosphorylation of myofibrillar proteins in post-mortem ovine muscle with different tenderness. J. Sci. Food Agric., DOI 10.1002/jsfa.7244.

Choi Y.M., Kim B.C. (2009). Muscle fiber characteristics, myofibrillar protein isoforms, and meat quality. Livest. Sci., 122: 105–118.

Claeys E., De Smet S., Balcaen A., Raes K., Demeyer D. (2004). Quantification of fresh meat peptides by SDS-PAGE in relation to ageing time and taste intensity. Meat Sci., 67: 281–288.

Etlinger J.D., Zak R., Fischman D.A. (1976). Myofibrillar composition. J. Cell Biol., 68: 123–141.

Fritz J.D., Greaser M.L. (1991). Changes in titin and nebulin in postmortem bovine muscle revealed by gel electrophoresis, western blotting and immunofluorescence microscopy. J. Food Sci., 56: 607–610, 615.

Fritz J.D., Mitchell M.C., Marsh B.B., Greaser M.L. (1993). Titin content of beef in relation to tenderness. Meat Sci., 33: 41–50.

Gagaoua M., Terlouw E.M., C., Boudjellal A., Picard B. (2015). Coherent correlation networks among protein biomarkers of beef tenderness: What they reveal. J. Proteomics, 128: 365–374.

Gil M., Ramírez J.A., Pla M., Ariño B., Hernández P., Pascual M., Blasco A., Guerrero L., Hajós G., Szerdahelyi E.I., Oliver M.A. (2006). Effect of selection for growth rate on the ageing of myofibrils, meat texture properties and the muscle proteolytic potential of *m. longissimus* in rabbits. Meat Sci., 72: 121–129.

Grześ B., Pospiech E., Koćwin-Podsiadła M., Łyczyński A., Krzęcio E., Mikołajczak B., Iwańska E., (2010). Relationship between the polymorphism of myosin heavy chains and selected meat quality traits of pigs with different susceptibility to stress. Arch. Tierzucht, 53: 65–72.

Grześkowiak E., Borzuta K., Wichłacz H., Strzelecki J. (2002). Sensory traits of 13 culinary cuts obtained from carcasses of young Black-and-White slaughter cattle. Anim. Sci. Pap. Rep., 20, suppl.: 179–186.

Herrera-Mendez C.H., Becila S., Boudjellal A., Ouali A. (2006). Meat ageing: Reconsideration of the current concept. Trends Food Sci. Tech., 17: 392–405.

Ho C.Y., Stromer M.H., Robson R.M. (1994). Identification of the 30kDa polypeptide in postmortem skeletal muscle as a degradation product of troponin-T. Biochimie, 76: 369–375.

Huff-Lonergan E., Parrish F.C. Jr, Robson R.M. (1995). Effects of postmortem aging time, animal age, and sex on degradation of titin and nebulin in bovine *longissimus* muscle. J. Anim. Sci., 73: 1064–1073.

Iwanowska A., Iwańska E., Grześ B., Mikołajczak B., Pospiech E., Rosochacki S., Juszczuk-Kubiak E., Łyczyński A. (2010). Changes in proteins and tenderness of meat from young bulls of four breeds at three ages over 10 days of cold storage. Anim. Sci. Pap. Rep., 28: 13–25.

Iwanowska A., Grześ B., Mikołajczak B., Iwańska E., Rosochacki S., Juszczuk-Kubiak E., Pospiech E., (2011). Impact of polymorphism of the regulatory subunit of the μ-calpain (CAPN1S) on the proteolysis process and meat tenderness of young cattle. Mol. Biol. Rep., 38: 1295–1300.

Kołczak T., Pospiech E., Palka K., Łącki J. (2003). Changes of myofibrillar and centrifugal drip proteins and shear force of *psoas major* and *minor* and *semitendinosus* muscles from calves, heifers and cows during post-mortem ageing. Meat Sci., 64: 69–75.

Lepetit J.A. (2007). Theoretical approach of the relationships between collagen content, collagen cross-links and meat tenderness. Meat Sci., 76: 147–159.

Lomiwes D., Farouk M.M., Wu G.,. Young O.A. (2014). The development of meat tenderness is likely to be compartmentalized by ultimate pH. Meat Sci., 96: 646-651.
Maltin C., Balcerzak D., Tilley R., Delday M. (2003). Determinants of meat quality: tenderness. P. Nutr. Soc., 62: 337–347.
Maltin C.A., Sinclair K.D., Warriss P.D., Grant C.M., Porter A.D., Delday M.I., Warkup C.C., (1998). The effects of age at slaughter, genotype and finishing system on the biochemical properties, muscle fibre type characteristics and eating quality of bull beef from suckled calves. Anim. Sci., 66: 341–348.
Marino R., Albenzio M., Della Malva A., Santillo A., Loizzo P., Sev A. (2013). Proteolytic pattern of myofibrillar protein and meat tenderness as affected by breed and aging time. Meat Sci., 95: 281–287.
Marino R., Della Malva A., Albenzio M. (2015). Proteolytic changes of myofibrillar proteins in Podolian meat during aging: focusing on tenderness. J. Anim. Sci., 93:1376–1387. DOI: 10.2527/jas.2014-8351.
Muroya S., Nakajima I., Oe M., Chikuni K. (2006). Difference in postmortem degradation pattern among troponin T isoforms expressed in bovine longissimus diaphragm, and masseter muscles. Meat Sci., 72: 245–251.
Muroya S., Ertbjerg P., Pomponio L., Christensen M. (2010). Desmin and troponin T are degraded faster in type IIb muscle fibers than in type I fibers during postmortem aging of porcine muscle. Meat Sci., 86: 764–769.
Murray B.E. (1997). A biochemical and structural study of easily releasable myofilaments in skeletal muscle. PhD Thesis, University College Cork.
Ngapo T.M., Bergea P., Culioli J., Dransfield E., de Smet S., Claeys E. (2002). Perimysial collagen crosslinking and meat tenderness in Belgian Blue double-muscled cattle. Meat Sci., 61: 91–102.
Nowak M., O'Sullivan A., Maher S., Troy D.J. (2004). Effect of muscle type and cooking method on eating quality of beef. Proceedings of the International Food Conference "Thinking beyond tomorrow", Dublin, p. 168.
Olson D.G., Parrish F.C., Dayton W.R., Goll D.E. (1977). Effect of postmortem storage and calcium activated factor on the myofibrillar proteins of bovine skeletal muscle. J. Food Sci., 42: 117–124.
Ouali A., Talmant A. (1990). Calpains and calpastatin distribution in bovine, porcine and ovine skeletal muscles. Meat Sci., 28: 331–348.
Palka K. (2003). The influence of post-mortem ageing and roasting on the microstructure, texture and collagen solubility of bovine *semitendinosus* muscle. Meat Sci., 64: 191–198.
Porzio M.A., Pearson A.M., Cornforth D.P., (1979). M-line protein: presence of two non-equivalent high molecular weight components. Meat Sci., 3: 31–41.
Pospiech E., Greaser M., Mikołajczak B., Szalata M., Łyczyński A. (2000). Degradation and release of titin in pork muscles. Proc. 46th ICoMST, Buenos Aires, Argentine, 4.I-P1: pp. 426–427.
Pospiech E., Grześ B., Łyczyński A., Borzuta K., Szalata M., Mikołajczak B. (2003). Muscle proteins and their changes in the process of meat tenderization. Anim. Sci. Pap. Rep., 21, Supl. 1: 133–151.
Purslow P.P. (2005). Intramuscular connective tissue and its role in meat quality. Meat Sci., 70: 435–447.
Sawdy J.C., Kaiser S.A., St-Pierre N.R., Wick M.P. (2004). Myofibrillar 1-D fingerprints and myosin heavy chain MS analyses of beef loin at 36 h postmortem correlate with tenderness at 7 days. Meat Sci., 67: 421–426.
Sazili A.Q., Parr T., Sensky P.L., Jones S.W., Bardsley R.G., Buttery P.J. (2005). The relationship between slow and fast myosin heavy chain content, calpastatin and meat tenderness in different ovine skeletal muscles. Meat Sci., 69: 17–25.
Shackelford S.D., Weeler T.L., Koomaraie M. (1995). Relationship between shear force and trained sensory panel tenderness ratings of 10 major muscles from *Bos indicus* and *Bos taurus* cattle. J. Anim. Sci., 73: 3333–3340.
Sierra V., Fernandez-Suarez V., Castro P., Osoro K., Vega-Naredo I., Garcıa-

-Macıa M., Rodrıguez-Colunga P., Coto-Montes A., Olivan M. (2012). Identification of biomarkers of meat tenderisation and its use for early classification of Asturian beef into fast and late tenderising meat. J. Sci. Food Agr., 92: 2727–2740.

Szalata M. (2003). Determination of relationship between proteolytic enzyme activity of pork from pigs of various meatiness (in Polish). (PhD dissertation). Wydział Technologii Żywności Akademii Rolniczej w Poznaniu.

Taylor R.G., Geesink G.H., Thompson V.F., Koohmaraie M., Goll D.E. (1995). Is Z-disk degradation responsible for postmortem tenderization? J. Anim. Sci., 73: 1351–1367.

Tomaszewska-Gras J., Schreurs F.J.G., Kijowski J. (2011). Post mortem development of meat quality as related to changes in cytoskeletal proteins of chicken muscles. Brit. Poultry Sci., 52: 189–201.

Watanabe A., Devine C. (1996). Effect of meat ultimate pH on rate of titin and nebulin degradation. Meat Sci., 42: 407–413.

Wu G., Clerens S., Farouk M.M. (2014 a). LC MS/MS identification of large structural proteins from bull muscle and their degradation products during post mortem storage. Food Chem., 150: 137–144.

Wu G., Farouk M.M., Clerens S., Rosenvold K. (2014 b). Effect of beef ultimate pH and large structural protein changes with aging on meat tenderness. Meat Sci., 98: 637–645.

Xiong Y.L., Mullins O.E., Stika J.F., Chen J., Blanchard S.P., Moody W.G. (2007). Tenderness and oxidative stability of post-mortem muscles from mature cows of various ages. Meat Sci., 77: 105−113.

Received: 23 VII 2015
Accepted: 1 XII 2015