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Changes in chemical composition and tenderness of selected beef muscles during aging analysed with SDS-PAGE and fluorescence spectroscopy*,**

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The aim of the study was to define the influence of small-molecule proteins originating from the degradation of troponin T and intensity of fluorescence spectra on the tenderness of the *Semitendinosus* (ST) and *Infraspinatus* (IS) muscles, and determining their interdependence with instrumentally measured Warner-Bratzler Shear Force (WBSF). The examined muscles, sampled from Limousin × Holstein-Friesian beef crossbreds differed to a statistically significant extent ($p<0.05$) in the length of the sarcomeres, content of protein, fat, and total collagen. An increase in the activity of proteins originating from the process of degradation of troponin T was highly correlated with the WBSF both in the case of the ST ($r^2=0.851$) and IS ($r^2=0.765$) muscle. The 305÷400 nm emission spectrum, recorded with front-face fluorescence spectroscopy made it possible to calculate the intensity of fluorescence of tryptophan residues. The studies proved a relationship between the WBSF and intensity of tryptophan residues fluorescence on the level of $r^2=0.682$ (ST) and $r^2=0.714$ (IS).

KEY WORDS: beef / fluorescence spectroscopy / muscles / SDS-PAGE / tenderness / tryptophan

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Biological tissue contains naturally present fluorophores, whose emission is known as internal fluorescence (autofluorescence). Meat includes components that contain relatively strong fluorophores, such as i.a. tryptophan, vitamin A, riboflavin, NADH, pyridinoline in collagen, and lipid oxidation products [Horbańczuk *et al.* 1998, Sales and Horbańczuk 1998, Pouzo *et al.* 2016, Horbańczuk and Wierzbicka 2016, 2017, Pogorzelska *et al*. 2018, Zdanowska-Sąsiadek *et al*. 2018], which can be analysed with fluorescence spectroscopy [Durek *et al*. 2016, Kulmyrzaev *et al*. 2012, Oto *et al*. 2013].

Fluorescence spectroscopy is currently considered as an efficient tool for quantitative identification of meat composites, and determining its structural properties. Numerous studies of meat products proved this technique capable of determining the amounts of collagen, elastin, percentage of fatty tissue, and monitoring characteristic changes taking place during aging, such as oxidation of lipids and water holding capacity [Damez and Clerjon 2013, Aït-Kaddour *et al*. 2016, Sahar *et al*. 2009]. As a method, fluorescence spectroscopy scanning has a number of advantages: it is highly sensitive, rapid, non-destructive, and relatively low cost. It can be used both for experimental research and as an on-line sensor for monitoring food products used in commercial production conditions. Tryptophan (symbol Trp) is an organic chemical compound from the group of exogenous protein amino acids, which, thanks to the presence of an aromatic ring, displays natural fluorescence emitted at approximately 350 nm and 290 nm excitation. In line with the results of research conducted by Sahar and Dufour [2015], the fluorescence spectrum of tryptophan allows the identification of the type of muscle and estimation of its physical, chemical, and rheological parameters. In 1987 Swatland initiated a series of studies concerning fluorescence and meat quality aspects [Swatland 1987a]. They focused on measuring the fluorescence of collagen and elastin from connective tissues in meat at 365 nm excitation. The obtained signals of the fluorescence of beef were correlated with qualitative and sensory parameters including gristle content [Swatland 1987b], palatability [Swatland *et al*. 1995], and toughness [Swatland and Findlay 1997]. Moreover, fluorescence techniques were used to measure the amounts of connective tissue [Skjervold *et al*. 2003] and intermuscular fat [Wold *et al*. 1999] in beef. The method also was applicable for identification of polyunsaturated aromatic amines in grilled beef [Sahar *et al*. 2010] and determining oxidative stability of meat and meat products [Veberg *et al*. 2006].

Another tool that allows better understanding development process of the proteins differing with respect to their unit mass during the aging of meat is SDS-PAGE (electrophoretic techniques using polyacrylamide gel with SDS). Changes in the microstructure of muscle fibres occurred during the process of ageing [Lomiwes *et al*. 2014]. Myofibrils are fragmented into smaller structural subunits composed of various numbers of sarcomeres. Degradation of myofibrillar proteins occurs. This reaction is catalysed by the calpain system (including troponin T). The decomposition of troponin T leads to the development of new polypeptides with unit masses of approximately 30 kDa into the muscle tissue extracts; these can prove good indicators of progression of post-slaughter proteolysis that determines meat tenderness [Contreras-Castillo *et al*.

2016, Moczkowska *et al*. 2017a, Onopiuk *et al.* 2018]. Tenderness is the most sensory perceived and measurable using instrumental method feature of meat during the aging [Rios-Mera *et al*. 2017]. It depends on the content, composition, and architecture of the intermuscular connective tissue and the degree of post-slaughter degradation of myofibrillar proteins [Fu *et al*. 2017]. During the post-slaughter ageing, the tenderness of meat increases due to the endogenous proteolysis of muscle proteins [Huff Lonergan *et al*. 2010], which can be monitored with electrophoretic techniques.

The purpose of the study was to ascertain the impact of changes in the amount of tryptophan residues and products of troponin T degradation on the tenderness of *Semitendinosus* (ST) and *Infraspinatus* (IS) muscles sampled from (n=6) Limousin × Holstein-Friesian beef crossbreds, and determine their correlation with the instrumentally measured WBSF (Warner-Bratzler Shear Force). The scope of the study encompassed measurements of pH, sarcomere length, basic meat composition, total collagen, WBSF, and drip and cooking loss, as well as electrophoretic analysis of troponin T decomposition products, and fluorescence analysis of tryptophan residues on the 1st, 7th, 14th, and 21st day post-mortem respectively.

Material and methods

Animals and muscle samples

The experiment was conducted using muscles obtained from Limousin × Holstein-Friesian crossbred bulls (L×HF; n=6) slaughtered at the age of 20±1 months in a local slaughterhouse. The animals were bred in the semi-intensive system on the same farm. Hot carcass weight of bulls ranged from 261.2 to 320 kg. Carcasses were classified using the EUROP classification scale for conformation (S – superior; E – excellent; U – very good; R – good; O – fair; P – poor). The fat cover classification (1 – low; 2 – slight; 3 – average; 4 – high; 5 – very high) was used. The carcasses were graded from R− to R+ with fat cover 2.

The study involved describing characteristics of the muscles by measuring pH values, sarcomere length, basic meat composition, and total collagen content on the first day post-mortem. The ST and IS muscles were wet-aged. The muscles were divided into four parts, vacuum packed into polyethylene bags, and cold stored at 3±1°C. Warner-Bratzler shear force (WBSF), drip and cooking loss, analysis of proteins from troponin T degradation, and fluorescence scan of tryptophan residues were performed on the samples on the 1st, 7th, 14th, and 21st day of wet ageing.

pH determination

The pH value of the muscles was measured in the middle part of ST and IS muscles according to the PN-ISO 2917:2001/Ap1:2002 standard. pH-metric results were obtained with a Testo 205 series pHmeter equipped with an insertion glass electrode (Testo, Inc., NJ, US). Each measurement was performed in three repetitions, using

the mean value as the assay result. The calibration was performed with standardised buffers (pH 4.01 and 7.01) prior to each measurement.

Sarcomere length determination

The measurement of sarcomere length was performed by the laser diffraction, with the use of helium-neon laser, according to the methodology described in Cross *et al*. [1981]. Samples of approximately 8 g of raw meat were immersed with 10 ml of 5% glutaraldehyde solution in 0.1 M $NaHPO_4$ (pH 7.2). After 4 hours, the aldehyde was removed, and meat samples were filled with 0.2 M sucrose solution (the samples were cold stored for 24 h). The scanning involved irradiating the muscle fibres with laser light at 632.8 nm wavelength and observing the diffraction (the bending of light in the slits between filaments). Sarcomere length was measured on the 1st day post-mortem, using the distance between the 0th and 1st beam of diffracted light [Cross *et al*. 1981].

Spectrometric quantification of basic meat composition

Basic composition of the samples was determined with the use of near-infrared (NIR) spectroscopy. In order to obtain homogeneous mixtures for each cut, 100 g of representative meat samples were collected and homogenised with a blender. Samples were placed on a petri dish in the NIR spectroscopy (NIRFlex N-500, Büchi Labortechnik AG, Flawil, Switzerland) with a spectral range of 12500-400 cm^{-1} in reflectance mode. Spectral analysis software NIR Ware 1.1 and NIR Cal 5.1 (Büchi, Flawil, Switzerland) was used to determine protein, fat, water, and ash content (%) [Wyrwisz *et al.* 2016].

Collagen content

The total collagen content in meat was determined according to Reich [1970]. Meat samples of approx. 2 g were immersed in deionised water, with addition of 375 mg $ZnCl_2$ and 5 ml of concentrated HCl. The sample was then hydrolysed at 105°C for 12 h in an incubator (Binder GmbH B28, Merazet, Poland). After chilling 6M NaOH solution was added until pH of 8.0 was achieved. The mixture was filtered (Whatman qualitative filter paper, Grade 1, Sigma Aldrich Inc., US) to obtain clear light yellow filtrate. Thus obtained 2.0 ml hydrolysate was mixed with 1.0 ml of 0.05 M $CuSO_4$, 1.0 ml of 2 M NaOH, and 1.0 ml of 6% H_2O_2. The flasks were placed in a water bath of 70°C for 10 min. Then 2.0 ml of 6.5 M H_2SO_4 and 5.0 ml of 3% dimethylaminobenzaldehyde were successively added. The flasks were placed in a water bath of 70°C for 50 min, and filled up to 25 ml with 10% water solution of n-propanol. Absorbance was measured for 560 nm wavelength with a multifunctional Tecan device (TecanSpark™ 10M, Männedorf, Switzerland). The amount of hydroxyproline was determined based on comparison with a standard curve. The collagen content (mg of collagen per g of meat) was calculated from the hydroxyproline content using the coefficient 7.25 [Modzelewska-Kapituła *et al.* 2015].

Warner-Bratzler Shear Force (WBSF)

Meat samples of 100±10 g were heated in a water bath at 75°C until the temperature in their geometrical centre reached 72°C. Cylinder-shaped samples (1.27 cm in diameter and 2.5±0.2 cm in length) were cut out along the fibres from the thermally treated pieces of meat. The measurement of shear force was performed with Instron 5965 device (Instron, Norwood, MA, US) with 500 N head and Warner-Bratzler attachment [Wyrwisz *et al*. 2016].

Drip loss

The extent of drip loss was defined by weight, and expressed as % compared to the initial mass of sample (before vacuum packaging). The following formula was used for the calculation: %Drip loss=((sample mass before storage-sample mass after storage)/sample mass before storage) × 100%.

Cooking loss

The extent of cooking loss was determined through measurement of sample mass before (M_i) and after (M_f) heat treatment, following cooling to ambient temperature. Heat treatment was performed as in section 2.6.

SDS-PAGE and Western Blotting analysis for protein degradation

Electrophoretic separation of meat proteins was run on any-kD gradient polyacrylamide gel (Bio-Rad Laboratories, Hercules, US) in the presence of sodium dodecyl sulphate (SDS-PAGE) and 8 M of urea with a Mini-PROTEAN Tetra Cell (Bio-Rad Laboratories, Hercules, US). The image of the electrophoretic separation of proteins in polyacrylamide gel was scanned with a Molecular Imager Gel Doc™ XR+ visualisation system running Image Lab™ Software 5.2 (Bio-Rad Laboratories, Hercules, US). For calculation purposes it was assumed that the surface of a single strand of protein has its percentage share in the total surface of all the separated proteins in a given gel sample, which amounts to 100%. The SDS-PAGE Molecular Weight Broad Range Standard (Bio-Rad Laboratories, CA) was used as the standard. [Moczkowska *et al*. 2017b].

Immunoblot analysis used the Western blot technique for transferring the proteins from the polyacrylamide gel to Polyvinylidene (PVDF) membranes (voltage: 150 V, time: 90 min). The membrane with proteins was incubated with troponin T primary antibody (Monoclonal Anti-Troponin T antibody produced in mouse, dilution 1:200, Sigma Aldrich Inc., US), and alkaline phosphatase was used as a secondary antibody (Anti-Mouse IgG Alkaline Phosphatase antibody produced in goat affinity isolated antibody, dilution 1:30000, Sigma Aldrich Inc., US). After the rinsing of the secondary antibodies, the proteins were detected by triggering colour reaction in NBT/BCIP. The identified proteins were subjected to electrophoretic analysis.

Front face fluorescence measurements

Emission spectrums of 305-400 nm were recorded with front-face fluorescence spectroscopy by setting excitation wavelength at 290 nm. The range of emission spectrum and excitation for the identification of tryptophan residues in proteins was chosen on the grounds of the studies conducted by Sahar et al. (2009). Fluorescence was measured with multifunctional Tecan device (TecanSpark™ 10M, Männedorf, Switzerland) equipped with a xenon lamp. The incidence angle of the excitation radiation was set at 56° to ensure that reflected light, scattered radiation, and depolarisation were minimised. To stabilise the sample temperature before fluorescence measurement, the meat was stored for 30 min at ambient temperature (18±1°C) [Pouzo *et al*. 2016]. Fluorescence spectres were recorded 3 times for each raw meat sample.

Statistical analysis

Statistical analysis was performed using the Statistica 10.0 software package (StatSoft Inc., Tulsa, OK, US). The response variables measured during the experiments were analyzed by ANOVA using Fisher's LSD test with the least significant difference at significance level of $p < 0.05$ within the same muscle. The effects: type of muscle and aging time were tested using MANOVA for a significance level of $p<0.05$, $p<0.01$, and $p<0.001$. The results in the tables are presented as mean values and standard deviations (SD).

Results and discussion

pH, sarcomere length, basic composition, total collagen

The study included the measurements of pH, sarcomere length, basic meat composition, and total collagen at 24 h post-slaughter. The results are presented in Table 1.

Table 1 Mean ± standard deviation values of pH_{24}, sarcomere length, basic meat composition, and total collagen of *Semitendinosus* (ST) and *Infraspinatus* (IS) muscles at 24 h post mortem

Parameter	Muscles	
	Semitendinosus (ST)	*Infraspinatus* (IS)
pH_{24}	5.48^{a}±0.06	5.63^{b}±0.04
sarcomere length (μm)	1.97^{a}±0.28	2.61^{b}±0.30
Moisture (%)	75.45^{a}±0.48	74.28^{a}±1.35
Protein (%)	22.84^{b}±0.47	20.86^{a}±0.23
Fat (%)	1.48^{a}±0.28	3.77^{b}±0.36
Ash (%)	1.25^{a}±0.07	1.37^{a}±0.08
Total collagen (g/100g)	0.49^{a}±0.12	1.64^{b}±0.38

^{ab}In rows means bearing different superscripts differ significantly at $p<0.05$.

The investigated muscles differed significantly ($p<0.05$) in sarcomere length, and protein, fat and total collagen content. No differences were found, however, in moisture and ash content. The IS muscle had much longer sarcomeres (2.61±0.30 μm), twice as much fat (3.77±0.36%), and approximately three times as much total collagen (1.64±0.38 g/100 g meat) as compared to ST muscle.

As stated by Ertbjerg and Puolanne [2017] the main reasons behind differences in meat tenderness include sarcomere length, content of connective tissue (mostly collagen), and intensity of post-slaughter proteolysis of myofibrillar proteins. This was also corroborated by studies conducted on the meat of sheep described by Starkey *et al*. [2016]. Moreover, the course of post-slaughter reactions that influence changes in tenderness depends on the rate at which available energy drops and aerobic metabolism shifts to anaerobic, which results in the generation of lactic acid and in consequence in the lowering of meat pH [Polati *et al*. 2012].

During anaerobic glycolysis, lactic acid acidifies the medium in a process that continues until glycolytic enzymes have been inactivated due to the low pH or depletion of glycogen supplies. The formation of lactic acid and production of H^+ ions decreased the muscle pH to around 5.4-5.7 during the first 24-48 hours post-mortem [Maltin *et al*. 2003, Onopiuk *et al*. 2016]. The pH values measured 24 h post-slaughter pointed towards the appropriate course of post-slaughter changes in the assessed beef muscles. The difference in pH_{24h} in the two analysed muscles (ST and IS) was caused by the different pace of energy transformations, which in turn was closely dependent on the physical activity and metabolic transformations in vivo. The fast-glycolytic *Semitendinosus* muscle of the fast-twitch type featured a lower pH_{24h}, which reached 5.48±0.06. The pH_{24h} value for the *Infraspinatus* muscle was statistically significantly higher and reached 5.63±0.04. The muscles showed no dark (DFD: dry, film, dark) or exudative (PSE: pale, soft, exudative) meat symptoms [Immonen *et al*. 2000].

Analysing the level of total collagen, it can be stated that both of the studied muscles had high collagen content. Muscle collagen concentration depends on a number of factors, including the location of the muscle, animal age, scope of activity, and animal breed [Chriki *et al*. 2013, Purslow 2005]. Frequently activated muscle types, such as the *Semitendinosus* and *Infraspinatus*, have fairly high collagen concentration compared to muscles that are less frequently activated, such as the *Longissimus lumborum* and *Psoas major* [Starkey *et al*. 2016].

The results concerning collagen levels are coherent with the conclusions obtained by Von Seggern *et al*. [2005] and Sahar *et al*. [2009], who corroborated that chuck muscles (*Infraspinatus*) contained more collagen than round muscles (*Semitendinosus*). The total collagen content in raw muscles ranged from 0.32 to 2.57 g/100 g meat (detailed data not included). The *Infraspinatus* featured high intramuscular connective tissue content [Hildrum *et al*. 2009, Purslow 2002] and was chosen as a material for the present study precisely for its high collagen content [Modzelewska-Kapituła *et al*. 2015], since the collagen present in connective tissue is an important structural component of muscles which is responsible for muscle tenderness. Many authors,

including Jeremiah *et al*. [2003] and Chriki *et al*. [2013] have demonstrated a negative correlation between collagen content and meat tenderness. However, it is not only the collagen content but also the degree of cross-linkage between collagen molecules that influences beef tenderness. As Jeremiah *et al*. [2003] proved, there are significant negative correlations between tenderness on the one hand and total and insoluble collagen fractions (r= -0.380 and r= -0.510, respectively) on the other. Impact of collagen on tenderness depends on the type of muscle, its location in the carcass, composition and structure, and the method and temperature heating.

The results of measurements of the shear force, drip loss, and cooking loss are presented in Table 2. A beneficial impact of cold ageing at the temperature of 3±1°C on tenderness profile of the studied muscles was observed. On the 1st day post-mortem, the ST and IS muscles characterized high shear force, which amounted to 73.45±3.05N and 46.83±3.54N respectively. Cold ageing contributed to a statistically significant decrease of the shear force value on all the days of ageing (D7, D14, D21) included in the analysis. The value of the shear force throughout the 21-day cold ageing period dropped by 49.88% and 40.21% for the ST and IS muscles respectively. The results obtained demonstrated a similar tendency to the conclusions of the studies conducted by Moczkowska *et al*. [2017b] investigating among others the *Semitendinosus* and *Infraspinatus* muscles in Charolaise × Holstein Friesian cattle. Similarly, the studies conducted by Zając *et al*. [2016] corroborated that a drop in WBSF is directly dependent on the duration of ageing and the type of investigated muscle. During 14-day ageing of muscles obtained from Irish Hereford-Friesian heifers the shear force in ST and IS muscles dropped by approximately 24%. In turn, a study analysing Belgian Blue and Norwegian Red cattle conducted by Van Wezemael *et al*. [2014] demonstrated that the WBSF of *Semitendinosus* and *Infraspinatus* muscles is strictly dependent on the breed.

An increase in drip loss and cooking loss parallel to the meat ageing time was observed. Compared to the ST muscle, the IS muscle had a largest drip loss and lower cooking loss on all the days of testing. The

Table 2. Changes in myofibril shear force, drip loss, and cooking loss in 1st, 7th, 14th, and 21st day *post mortem*

Parameter	Muscle	Aging (days)				Effects		
		1D	7D	14D	21D	muscle	aging	muscle x aging
Shear force (N)	ST	73.45^{Ba}±3.05	60.60^{Bb}±2.03	41.19^{Ac}±2.54	36.81^{Bd}±2.20	***	***	***
	IS	46.83^{Aa}±3.54	37.70^{Ab}±3.41	33.10^{Ac}±2.35	28.04^{Ad}±2.43			
Drip loss (%)	ST	-	3.08^{Aa}±0.21	3.51^{Aa}±0.48	4.09^{Ab}±0.31	**	**	**
	IS	-	3.23^{Aa}±0.30	3.90^{Ab}±0.32	4.82^{Bc}±0.45			
Cooking loss (%)	ST	32.54^{Ba}±0.35	33.03^{Ba}±0.58	34.37^{Bb}±0.83	35.34^{Bb}±0.74	***	**	**
	IS	24.34^{Aa}±1.02	25.58^{Aa}±1.32	25.83^{Aa}±1.48	29.36^{Ab}±1.31			

[a,A]... In rows means bearing different superscripts differ significantly at: small letters – $p<0.05$; capitals – $p<0.01$.
*$P<0.05$; **$P<0.01$; ***$P<0.001$.

largest drip loss was observed on the 21st day of ageing in the IS muscle (4.82±0.45%), and the highest cooking loss in the ST muscle (35.34±0.74). The reason for it was mainly the diverse presence of collagen in the examined muscles. Large content of collagen present in connective tissue, which is a protein known for its incomplete structure, has a negative impact on meat water holding capacity (WHC) and lowers its quality by increasing the drip loss. Yielding to thermal hydrolysis, collagen has a capacity to retain water during thermal processing, which results among others from its emulsifying and gelling properties [Lepetit 2008]. That is why the IS muscle, rich in collagen, presented a higher drip loss and lower cooking loss when compared to the ST muscle.

SDS-PAGE and Western Blotting analysis for protein degradation

Meat tenderness improves during the endogenous proteolysis of muscle proteins in post-slaughter aging of meat. The quicker the metabolism of muscle fibres in vivo, the quicker the changes observed in proteins responsible for tenderising the muscle tissue after slaughter [Mohrhauser *et al*. 2013].

Observed differences in troponin T activity and the activity of small-molecule (32÷27 kDa) proteins originating from its breakup were revealed in SDS-PAGE electrophoretic protein separation and identification with monoclonal antibodies targeting against TnT (Western blot technique) (Fig. 1).

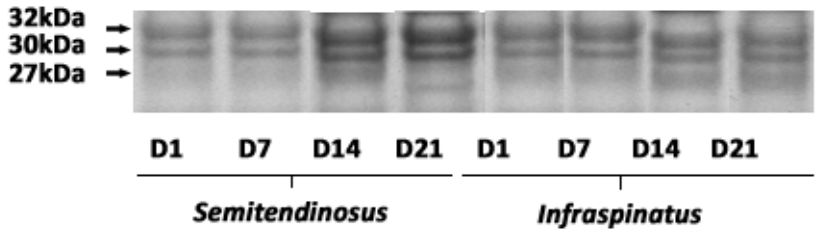

Fig. 1. Presence of degraded forms of troponin T corroborated in Western blotting using monoclonal antibodies targeting against specific proteins on 1st, 7th, 14th, and 21st day post mortem in *Semitendinosus* and *Infraspinatus* muscles.

Examination of the course of changes in troponin T activity produced the conclusion that its activity decreased during aging. The ST muscle had statistically significant ($p<0.05$) higher levels of troponin T as compared to the IS muscle on D1 (3.89±0.24% and 3.05±0.16%, respectively). The results are presented in Figure 2. With the duration of storage, the level of troponin T decreased to the parallel development of proteins with lower mass unit (32-27 kDa). An increase in the activity of the proteins originating in the process of degradation of troponin T is highly correlated with the shear force both for the ST ($r^2=0.851$) and IS ($r^2=0.765$) muscle. The results are presented in Figure 3.

A comparison of the profiles of myofibrillar proteins from ST and IS muscles resulted in a conclusion that the proteins from TnT degradation are present in larger

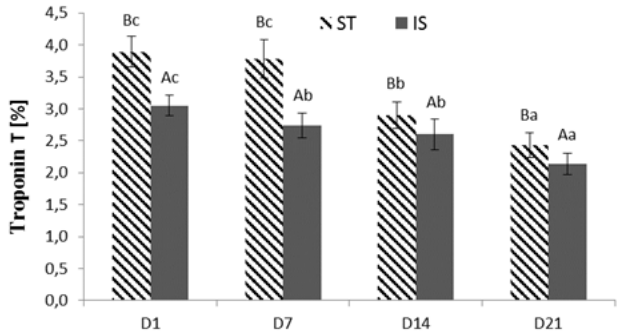

Fig. 2. Amount of troponin T measured on the 1st, 7th, 14th, and 21st day post mortem in *Semitendinosus* (ST) and *Infraspinatus* (IS) muscles.

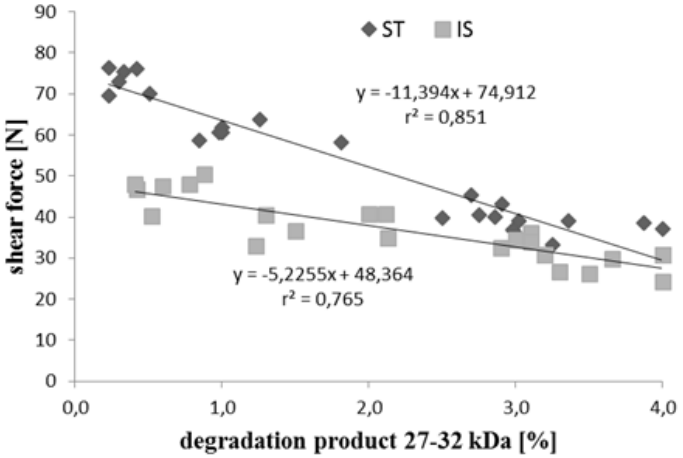

Fig. 3. Dependence between shear force (N) and 27-32 kDa products of TnT degradation (%) in ST and IS muscles measured on the 1st, 7th, 14th, and 21st day of aging.

quantities in the IS than in the ST muscle, and that on the 1st day post-mortem already. The low amount of TnT degradation products in ST on the 1st day post-mortem correlates with the highest shear force value, which is explained by the slower proteolysis of proteins of this fast-glycolytic muscle. During muscle ageing, the troponin T proteins building myofilaments and sarcomere structure were gradually decomposing with a parallel increase in the number of small-particle proteins, which was also confirmed in the studies by Sun *et al*. [2014], Cruzen *et al*. [2014], Moczkowska *et al*. [2015], and Onopiuk *et al*. [2018]. In turn, Marino *et al*. [2013] demonstrated significant quantitative changes dependent on the studied cattle breed, where the drop in TnT during the 21-day ageing amounted to by 29.77%, 15.79%, and 16.11% in Podolian, Friesian, and Romagnola × Podolian respectively. Results obtained in this study demonstrated that both the type of muscle and the duration of

ageing are statistically significant factors that affect the activity of TnT and proteins with unit mass of 32-27 kDA.

Characteristics of spectra obtained from fluorescence spectroscopy

Emission spectra recorded with 290 nm excitation for ST and IS muscles demonstrated different intensity and shapes at maximum wavelength of approximately 330-340 nm (Fig. 4). These spectra could be attributed to tryptophan residues in proteins, as corroborated i.e. in the studies of Aït-Kaddour *et al*. [2016], Sahar and Dufour [2015], and Skjervold *et al*. [2003].

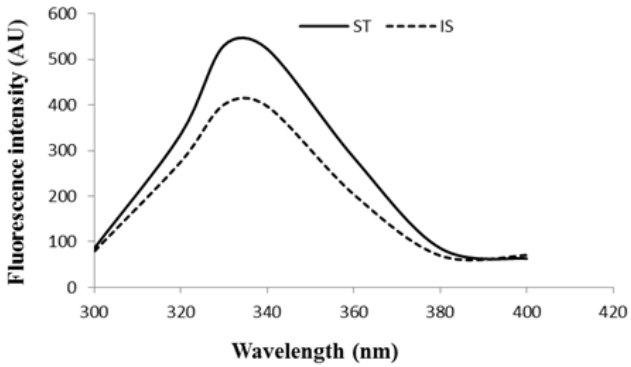

Fig. 4. Emission spectra of beef samples on the 1st day post mortem recorded between 305 and 400 nm with excitation wavelength set at 290 nm.

Tryptophan fluorescence provides information about the structure of protein [Vivian and Callis 2001]. Changes in proteolysis and pH value during ageing may result in the change of protein and tryptophan particle conformation, which has an impact on the fluorescent spectra. The factors that change the conformation of proteins are also the oxidation reactions continuing during ageing, which may contribute to changes in intensity of tryptophan fluorescence [Pouzo *et al*. 2016]. As it was suggested by Andersen and Mortensen [2008] the fluorescence of proteins depends on how tryptophan is exposed in the three-dimensional configuration of the proteins. The spectra obtained demonstrate slightly higher fluorescence as compared to the spectra obtained by Pouzo *et al*. [2016]. The reason behind the differences could be continuing oxidation processes and endogenous proteolysis of muscle proteins. The changes in proteolysis and even slight pH changes could result in changes in the conformation of myofibrillar proteins and tryptophan particles, which in result/ consequence resulted in changes in the intensity of fluorescence. Additionally, there was an important role of the particular type of muscle. The studies of Sahar *et al*. [2009] confirm that fluorescence spectra may show slightly different intensities depending on the type of muscle examined. Importantly, the intensity of the spectra allows to anticipate chemical and rheological parameters of the muscles.

Examination of Figure 4 showed that ST and IS muscles differed in the intensity of fluorescence. Maximum fluorescence for the ST muscle was recorded at intensity of approx. 530.17±8.86 AU, and for the IS muscle at approx. 400.67±11.84 AU. Examining Figure 5 presenting fluorescence intensity during ageing, it can be maintained that fluorescence spectra do not produce statistically significant changes during the 21-day ageing in an individual muscle. A slight increase in the intensity of the spectra in the 7th, 14th, and 21st days as compared to the 1st day was caused by the changes in the amount of water. The meat samples used in the study were vacuum packed in polyethylene bags, which protected them from direct access of oxygen. Thanks to this, the process of oxidation had no negative bearing on tryptophan degradation. As the studies conducted by Estévez *et al*. [2008] demonstrated, tryptophan is an amino acid sensitive to oxidation stress. The correlation between shear force and fluorescence

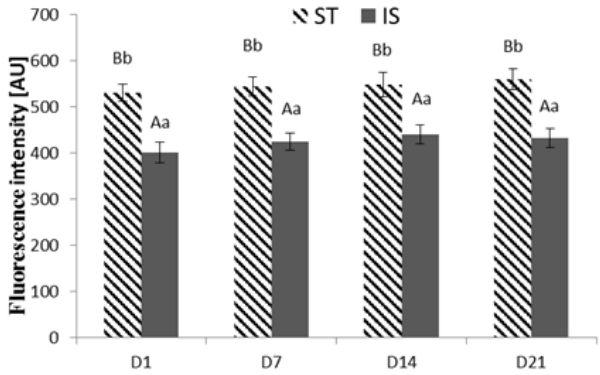

Fig. 5. Fluorescence intensity of tryptophan residues (maximum intensity reached by the peak; arbitrary units, AU) measured on the 1st, 7th, 14th, and 21st day post mortem in *Semitendinosus* (ST) and *Infraspinatus* (IS) muscles.

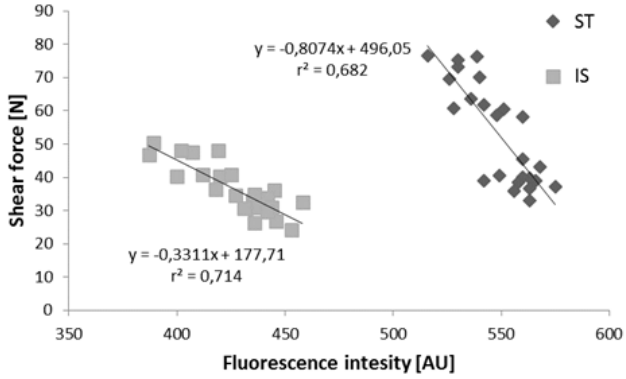

Fig. 6. Correspondence between the shear force (N) and emission spectra fluorescence intensity in the range of 305-400 nm with excitation wavelength 290 nm (AU) in ST and IS muscles measured on the 1st, 7th, 14th, and 21st day of aging.

intensity of tryptophan residues is presented in Figure 6. The correlation coefficients obtained were at the level of r^2=0.682 (ST) and r^2=0.714 (IS).

Conclusion

Current study demonstrated that beef tenderness depends on basic meat composition, sarcomere length, collagen content, and the degree of post-slaughter degradation of proteins. An increase in the activity of proteins that originate from the process of troponin T degradation was highly correlated with the shear force, in the case of both the ST and the IS muscles with r^2=0.851 and r^2=0.765, respectively. The measurement of the amount of these proteins may be an efficient indicator of changes in beef tenderness during ageing.

Emission spectra in the range of 305-400 nm with excitation wavelength of 290 nm recorded with front-face fluorescence spectroscopy made it possible to determine fluorescence intensity of tryptophan residues. The correlation coefficients obtained at the level of r^2=0.682 and r^2=0.714 for *Semitendinosus* and *Infraspinatus* muscles resulted from the fact that tryptophan does not undergo major changes during the ageing of meat. The factors that may modify the amount of tryptophan are processes of its oxidation and changes caused by the changing amount of water in meat. Further research on the use of fluorescence spectroscopy for predicting beef tenderness is worth conducting since the method has a large number of advantages: it is highly sensitive, rapid, non-destructive, and relatively cheap.

Conflict of interest

The authors declare no conflicts of interest.

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