

## Effect of Ageing Time on Microstructure, Rate of Desmin Degradation and Meat Quality of Pig *Longissimus Lumborum* and *Adductor* Muscles\*

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This study evaluated the effect of ageing time (45 min, 24 h, 96 h and 168 h) on desmin degradation pattern, meat quality and muscle fibre traits in *longissimus lumborum* (LL) and *adductor* (AD) muscles of 28 Polish Landrace fatteners slaughtered at 105 kg body weight. The results indicate that the rate of desmin degradation depends on pH and type of muscle, and thus on muscle fibre composition. In AD muscle, characterized by significantly higher pH<sub>45</sub>, pH<sub>24</sub> and shear force values, lower drip loss, significantly higher percentage of type I and IIA oxidative fibres, and lower percentage of type IIB glycolytic fibres, desmin degradation is slower than in LL muscle. The rate of desmin degradation is also associated with its localization in muscle fibre. Regardless of muscle type, in all muscle fibres desmin was degraded more rapidly within, rather than at the periphery of the fibres. Additionally, levels of intact desmin and drip loss during *postmortem* meat storage contribute significantly to a reduction in the diameter of three fibre types analysed in AD and LL muscles 24 h *postmortem* and of type IIB fibres in AD muscle 96 h *postmortem*. Furthermore, the decrease in the level of intact desmin is paralleled by a cumulative increase in drip loss and a decrease in shear force at 45 min, 24 h and 96 h *postmortem*.

Key words: Cold storage, muscle fibre, desmin, meat quality, pig.

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Mammalian skeletal muscles consist predominantly of muscle fibres with different morphological, contractile and metabolic properties, namely fast-twitch glycolytic (IIB), fast-twitch oxidative glycolytic (IIA) and slow-twitch oxidative fibres (I). Muscle fibres occupy 75-90% of the muscle volume. Therefore, muscle metabolism is the summation of the activities of the individual muscle fibres which comprise the muscle. Muscle fibre composition varies between anatomical muscles according to functional loads, such that muscles for postural maintenance tend to have fibres with higher oxidative capacity than those muscles solely used for rapid and forceful contractions, such as running (ESSEN-GUSTAVSSON 1993; KARLSSON *et al.* 1999). Muscle fibre composition is one of the most important factors influencing physicochemical parameters of the muscle which

makes it the main determinant of meat quality. During meat formation, *postmortem* changes in muscle tissue determine the subsequent usefulness of meat for production and culinary purposes and they depend, among others, on the ratio of oxidative to glycolytic fibres (ČANDEK-POTOKAR *et al.* 1999; GIL *et al.* 2008), and on the rate of cytoskeletal protein degradation (MORRISON *et al.* 1998; MELODY *et al.* 2004; ZHANG *et al.* 2006; BEE *et al.* 2007). Cytoskeletal proteins are a complex network of fibrous structures that account for over 85% of the cell's proteins. The cytoskeleton is composed of three types of fibres: microtubules, microfilaments, and intermediate filaments. One of the main proteins of intermediate filaments is desmin (HELLIWELL 1999). In skeletal muscle cells, desmin fibres surround myofibrils at the level of the Z-line. This forms a three-dimensional

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complex network interlinking adjacent bands with the cell and nuclear membranes. It also occurs as filaments that connect structural elements of the cell. According to WANG & RAMIREZ-MITCHELL (1983) and LI *et al.* (1997) desmin plays a critical role in the lateral registration of myofibrils. Previous studies have shown that cytoskeletal proteins are degraded during storage of meat, and it has been speculated that their degradation is important to meat quality, especially meat tenderness and water-holding capacity (KOOHMARAIE *et al.* 1994; MORRISON *et al.* 1998; THERKILDSEN *et al.* 2002; WOJTYSIAK *et al.* 2008).

Therefore the aim of this study was to determine the *postmortem* desmin degradation and changes in the microstructure and physicochemical properties of *m. longissimus lumborum* and *m. adductor* in fattening pigs.

## Material and Methods

The subjects of the study were 28 Polish Landrace fatteners. The animals were acquired from the Agricultural Production Cooperative in Kędzierzyn-Koźle, Poland. Fatteners were reared under the same environmental and production regime. Animals were fattened from 50 to 105 kg body weight using a complete diet containing 13.3 MJ metabolizable energy and 161 g crude protein. This level was calculated based on the diet composition using tabular data for individual components (POLISH FEEDING STANDARDS 1993). When the pigs had attained the appropriate slaughter weight (105 kg), they were electrically stunned and exsanguinated. Feed was withdrawn 12 h before slaughter but water was freely available in lairage.

To determine muscle fibre characteristics, desmin degradation pattern and meat quality traits, samples of *m. longissimus lumborum* (LL) and *m. adductor* (AD) were collected immediately after slaughter, and divided so that all tests could be carried out on the slaughter day (45 min *postmortem*) and repeated at 24 h, 96 h and 168 h *postmortem*. From each muscle, four large samples (about 150 g each) and two small samples (about 50 g each – to determine drip loss) were excised. The first large sample was used to determine pH of meat, to take muscle sections for all immunohistochemical analyses, and to determine Warner-Bratzler shear force 45 min *postmortem*. The other three samples were vacuum packed and cold stored at 4°C to perform analogous analyses within the planned time frame of 24 h, 96 h or 168 h *postmortem*. The samples for the determination of drip loss were cold stored in tightly sealed containers until the end of the experiment at 168 h *postmortem*.

Muscle samples for immunohistochemical analysis were cut into 1 cm<sup>3</sup> pieces (parallel to the muscle fibres) and frozen in isopentane that was cooled using liquid nitrogen and stored at -80°C until subsequent analyses. Samples were mounted on a cryostat chuck with a few drops of tissue-freezing medium (Tissue-Tek; Sakura Finetek Europe, Zoeterwoude, The Netherlands). Transverse serial sections (10-µm thick) were cut at -20°C in a cryostat (Slee MEV, Germany). To distinguish muscle fibre types (I, IIA and IIB), a modified combined method of NADH-tetrazolium reductase activity was used and immunohistochemical determination of the slow myosin heavy chain on the same section with monoclonal antibodies against the skeletal slow myosin heavy chain was performed for 1 h at RT (clone WB-MHCs Leica, Germany, dilution 1:80) (WOJTYSIAK & KACZOR 2011). The reaction was visualized by the NovoLink™ Polymer Detection System (Leica, Germany) according to the manufacturer's instruction. Finally, all sections were dehydrated in a graded series of ethyl alcohol, cleared in xylene and mounted in DPX mounting medium (Fluka, Buchs, Switzerland). A minimum of 300 fibres were counted in each section using a NIKON E600 light microscope. The percentage and diameter of muscle fibre types were quantified with an image analysis system using the Multi Scan v. 14.02 computer program.

To determine desmin activity, frozen sections were fixed with 4% formaldehyde, as paraformaldehyde (PFA), in 0.1 M phosphate buffer (PB) (pH 7.4). Next, sections were incubated for 30 min in 5% normal goat serum (NGS). They were then incubated overnight at 4°C with primary monoclonal antibody (NCL-DES-DERII, Novocastra, UK) at 1:75 dilution. After several washes in 0.01 M sodium phosphate buffer (PBS) containing 0.05% Triton-X, sections were incubated overnight at 4°C with goat anti-mouse secondary antibodies conjugated to Alexa Fluor 555 (Molecular Probes) at 1:250 dilution. After a final washing, preparations were mounted in Vectashield medium (Vector Labs, Burlingame, CA) and examined with a Zeiss Axio Vision A.2 fluorescence microscope. In controls, there was immunostaining observed when primary antisera were omitted.

Intact desmin and degradation products were analysed using Western immunoblotting. To this end, whole muscle extracts were obtained by homogenizing muscle samples with 10 volumes of 50 mM Tris and 10 mM EDTA, pH 8.3. The muscle homogenate was diluted 1:1 with protein denaturing buffer (4% SDS, 20% glycerol, 125 mM Tris, pH 6.8) and heated at 50°C for 20 min. Samples were centrifuged at 16000×g and protein concentration was determined using BCA assay (Sigma Chemical Co, St. Louis USA). Desmin was separated by SDS-PAGE on a 10% separating

acrylamide gel with a 4% stacking acrylamide gel. For electrophoresis, 5  $\mu\text{g}$  protein per lane was loaded. Discontinuous gels were run at 100 V for 1.5 h. Gels were transferred to Immobilon-P transfer membrane (Millipore) overnight at 4°C and 30 mA in buffer containing 25 mM Tris, 193 mM glycine, and 15% methanol. After blocking with 10% non-fat dried milk in Tris-buffered saline, pH 7.4, containing 0.05% Tween 20 for 1 h, the membrane was incubated at room temperature with primary monoclonal antibody (NCL-DES-DERII, Novocastra, UK) at 1:250 for 1 h. Membrane was washed three times (5 min) with TBST after each incubation. Bound primary antibody was visualized with goat anti-mouse alkaline phosphatase conjugate (Pierce Chemical Co., USA) diluted 1:2500, followed by detection of alkaline phosphate activity with bromo-chloroindolyl phosphate and nitroblue tetrazolium. The immunoblots were electronically scanned and then analysed using the densitometric method (ImageJ ver. 1.33U, National Institutes of Health, USA). Peak area values of protein bands were determined and expressed as the percentage of the total band area (major native protein plus degradation products). Immunoblots of each protein were conducted in triplicate.

Muscle pH was measured using a Matthäus (Germany) pH meter with a glass electrode standardized for pH 4.0 and 7.0 according to Polish Standard PN-77/a-82058 with automatic correction for muscle temperature. Drip loss was measured in duplicate samples. After thorough weighing ( $e=0.001$  g), samples of about 50 g were placed in sealed containers. After 24 h of storage, samples were removed from their individual sealed containers, towel dried, and weighed again. The chops were then placed in new sealed containers and stored for additional hours. Following 96 h of storage, samples were again towel dried and weighed. Similarly, the samples of meat were processed after 168 h of storage. For measurements of Warner-Bratzler shear force, meat samples of about 120 g were roasted at 180°C to reach an internal temperature of 76°C and then cooled to room temperature (30 min), and stored for 45 min in a 4°C cooler. Then, four 14 mm in diameter cores were taken from each sample parallel to the muscle fibre orientation. Shear force was measured using a Texture Analyser TA-XT2 (Stable-Micro Systems, UK) with a Warner-Bratzler unit and a triangular blade.

Differences among the muscle types and time of storage were analysed using analysis of variance (General Linear Models procedure), and tested for differences by Tukey's test. A probability of  $P<0.05$  was considered statistically significant. The data were expressed as least squares means (LSM)  $\pm$  standard error (SE).

## Results and Discussion

The present microstructural analysis confirmed that LL muscle is a typical "white" muscle characterized by a significantly greater percentage of type IIB muscle fibres, in contrast to AD muscle which were found to contain a significantly greater percentage of type I and IIA fibres (Table 1, Fig. 1A-B). Such differences in the percentage of individual muscle fibre types result from their location and function (ESSEN-GUSTAVSSON 1993). Meat quality is determined not only by the composition, but also by the size of muscle fibres. RUUSUNEN and PUOLANNE (2004) demonstrated that white muscles are characterized by greater fibres, especially those of type II, which is in agreement with the results of this study, in which significantly greater muscle fibres of all three types, for all the meat storage periods, were noted in LL muscle compared to AD muscle fibres (Table 1). The differences in muscle fibre size between the analysed muscles, shown in the present study, support earlier observations that muscle type determines fibre

Table 1

Least squares means (LSM) and standard error (SE) for muscle fibre percentage and *postmortem* changes in muscle fibre diameter of *m. longissimus lumborum* (LL) and *m. adductor* (AD)

Item	LL	AD
Fibre type percentage (%)		
IIB	79.70 $\pm$ 0.60 <sup>x</sup>	52.39 $\pm$ 0.45 <sup>y</sup>
IIA	9.96 $\pm$ 0.16 <sup>x</sup>	19.18 $\pm$ 0.29 <sup>y</sup>
I	10.34 $\pm$ 0.18 <sup>x</sup>	28.43 $\pm$ 0.32 <sup>y</sup>
Fibre diameter ( $\mu\text{m}$ )		
45 min <i>post mortem</i>		
IIB	76.62 $\pm$ 0.40 <sup>xa</sup>	65.62 $\pm$ 0.41 <sup>ya</sup>
IIA	54.68 $\pm$ 0.24 <sup>xa</sup>	49.56 $\pm$ 0.19 <sup>ya</sup>
I	56.42 $\pm$ 0.22 <sup>xa</sup>	48.17 $\pm$ 0.21 <sup>ya</sup>
24 h <i>post mortem</i>		
IIB	67.72 $\pm$ 0.44 <sup>xb</sup>	60.49 $\pm$ 0.36 <sup>yb</sup>
IIA	50.84 $\pm$ 0.39 <sup>xb</sup>	46.14 $\pm$ 0.32 <sup>yb</sup>
I	53.14 $\pm$ 0.35 <sup>xb</sup>	45.23 $\pm$ 0.28 <sup>yb</sup>
96 h <i>post mortem</i>		
IIB	66.85 $\pm$ 0.34 <sup>xb</sup>	55.71 $\pm$ 0.32 <sup>yc</sup>
IIA	49.78 $\pm$ 0.21 <sup>xb</sup>	47.08 $\pm$ 0.24 <sup>yb</sup>
I	52.27 $\pm$ 0.41 <sup>xb</sup>	45.14 $\pm$ 0.33 <sup>yb</sup>
168 h <i>post mortem</i>		
IIB	67.18 $\pm$ 0.37 <sup>xb</sup>	56.28 $\pm$ 0.38 <sup>yc</sup>
IIA	50.64 $\pm$ 0.38 <sup>xb</sup>	47.39 $\pm$ 0.27 <sup>yb</sup>
I	52.98 $\pm$ 0.31 <sup>xb</sup>	46.09 $\pm$ 0.20 <sup>yb</sup>

Values in rows with different subscript are significantly different: x, y ( $P<0.05$ ), values in columns (relative to fibre type) with different subscript are significantly different: a, b, c ( $P<0.05$ ).

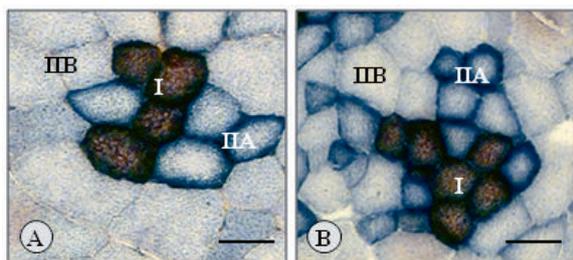


Fig. 1. Exemplary cross-section of *m. longissimus lumborum* (LL) (A) and *m. adductor* (AD) (B) of Polish Landrace pigs: NADH-TR and immunohistochemical MyHC-slow staining: I – red fibres; IIA – intermedia te fibres; IIB – white fibres. Bar = 50  $\mu\text{m}$ .

size (FIEDLER *et al.* 1999). The effect of time of meat storage at 4°C on muscle fibre size after 24 h storage showed that the diameters of type I, IIA and IIB fibres decreased significantly in both LL and AD muscle. No effect of meat storage time on the size of LL muscle fibre was observed for the subsequent storage periods. For AD muscle, however, a significant decrease in the diameter of type IIB fibres was found 96 h compared to 24 h *postmortem*. That muscle fibre diameter decreases during storage of meat is evidenced by studies of HEGARTY (1970) and SWATLAND and BELFRY (1985). DIESBOURG *et al.* (1988) suggested that muscle fibre diameter decreases *postmortem* as a result of shrinking lateral connections of myofibrils with the cell membrane. Likewise, HUFF-LONERGAN and LONERGAN (2005) hold that *postmortem* muscle fibre size depends on the extent of degradation of cytoskeletal proteins which connect myofibrils to the sarcoplasm. Desmin is one of these major proteins. It links myofibrils to one another, but also to the cell membrane. These last-mentioned protein connections, known as costameres, have a significant influence on the size of muscle fibres *postmortem* (WANG & RAMIREZ-MITCHELL 1983). The immunohistochemical analysis in the present study showed that desmin degradation is not uniform across muscle fibres (Fig. 2A-H). In both LL and AD muscle, desmin was degraded more rapidly within muscle fibres where desmin forms a fine network between the myofibrils, compared to the periphery of the fibres where desmin was present even after 168 h of meat storage. MORRISON *et al.* (1998) and MUROYA *et al.* (2010) hypothesized that the rate of desmin degradation is also associated with the type of muscle fibre. Accordingly, in type IIB glycolytic muscle fibres, desmin is degraded more rapidly than in type I oxidative fibres. This may be due to the fact that type I fibres contain greater amounts of desmin than type IIB fibres. This supposition is confirmed by the results of the present study, in which both the Western blotting procedure (Fig. 3) and immunohistochemical analysis provided conclusive evidence that in LL muscle, which has a greater

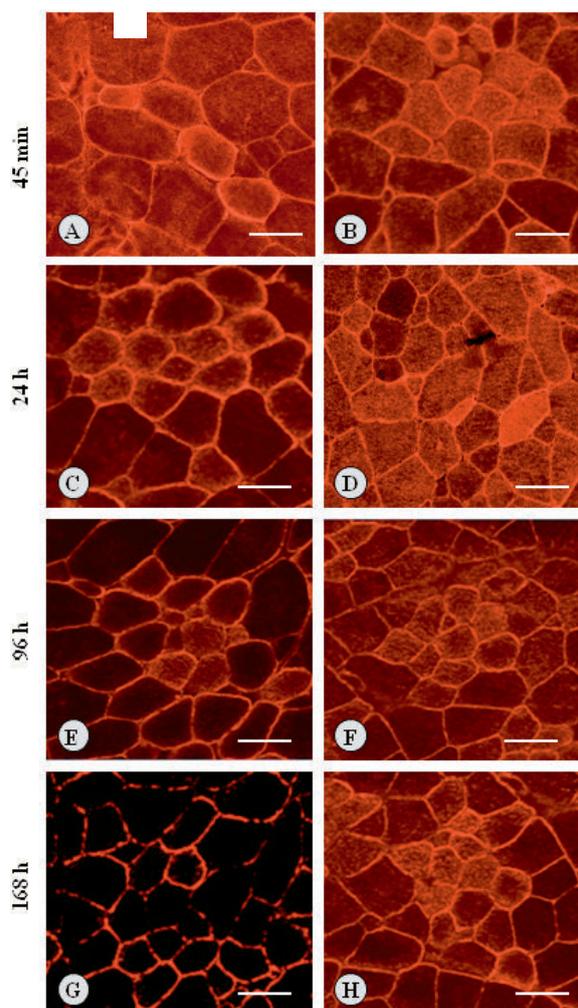
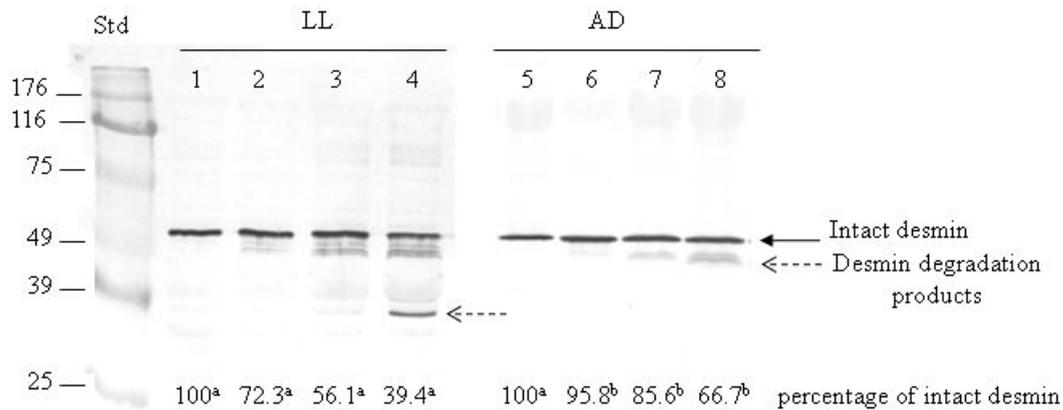


Fig. 2. Exemplary cross-section of *m. longissimus lumborum* (LL) (A, C, E, G) and *m. adductor* (AD) (B, D, F, H) of Polish Landrace pigs: immunohistochemical detection of desmin (NCL-DES-DERII). Samples were stored for 45 min (A, B), 24 h (C, D), 96 h (E, F) and 168 h (G, H). Bar = 50  $\mu\text{m}$ .

proportion of type IIB fibres, desmin was degraded more rapidly in all periods of meat storage compared to AD muscle, which has a significantly greater proportion of type I fibres. MELODY *et al.* (2004) note that *postmortem* degradation of cytoskeletal proteins is considerably influenced by the activity of proteolytic enzymes. The proteolytic enzymes involved in *postmortem* proteolysis are  $\mu$ -calpain and m-calpain, as well as their specific inhibitor calpastatin, which form the calpain system (NOWAK 2005). Calpains are cysteine proteases activated by  $\text{Ca}^{2+}$  ions. They are non-lysosomal neutral proteases found in the sarcoplasm. In muscle fibres these enzymes do not occur freely in the sarcoplasm but are connected to intracellular structures – in the case of muscles, these are myofibril Z lines, where desmin is also localized. This location of the calpain system in the sarcoplasm is



a, b - means (between muscles) with different subscript differ significantly at  $P < 0.05$

Fig. 3. Western blot analysis of desmin in *m. longissimus lumborum* (LL) and *m. adductor* (AD). Samples were stored for 45 min (line 1, 5), 24 h (line 2, 6), 96 h (line 3, 7) and 168 h (line 4, 8). The molecular weights (in kDa) of standard proteins (Std) are indicated on the left.

probably one of the principal reasons for the more rapid degradation of desmin within muscle fibres, observed in the present study. Earlier research also showed that type I fibres are the main site of calpains. OUALI (1990) in pigs show that slow-twitch red muscles, which exhibit the lowest ageing rate and the smallest extent of proteolysis, have the highest calpain content and show greatest calpain to calpastatin ratio. Similarly, O'HALLORAN *et al.* (1997) noted that fast glycolytic muscles in cattle have a lower calpastatin activity and are rated more tender than slow muscles, both by sensory and shear force analysis. This observation may also explain the slower degradation of desmin in AD compared to LL muscle in the present study (Fig. 2, 3). In addition, HUFF-LONERGAN and LONERGAN (2005) reported that muscle fibre size is determined by both the *postmortem* rate of desmin degradation and drip loss. The authors hypothesize that in fibre characterized by a rapid *postmortem* degradation of desmin connected to costameres, no decrease in fibre diameter is observed even despite high drip loss. Fibres shrink when desmin degradation is slow, and this may explain the decreasing diameter of type IIB muscle fibres in AD muscles even after 96 h of meat storage at 4°C. As indicated by an earlier study of RYU and KIM (2005), the magnitude of drip loss is also determined by the composition of muscle fibres. The same authors showed that muscles with a greater proportion of type IIB muscle fibres are characterized by higher drip loss. Drip loss value is also influenced by the size of muscle fibres. RYU and KIM (2005) and ČANDEK-POTOKAR *et al.* (1999) noted negative correlations between muscle fibre diameter and drip loss. Thus, the lower drip loss from AD muscle in all the meat storage periods analysed

(Table 2) may be associated not only with the smaller proportion of type IIB fibres in the composition of muscle fibres, but also with the smaller diameters of the three muscle fibre types under analysis. It is obvious that cumulative drip loss increases with increasing period of meat storage (ZHANG *et al.* 2006; BEE *et al.* 2007; WOJTYSIK *et al.* 2008). In the present study, analogous results were found for both AD and LL muscle (Table 2).

Table 2

*Postmortem* changes in pH, drip loss and shear force of *m. longissimus* (LL) and *m. adductor* (AD). Values are expressed as Least squares means (LSM)  $\pm$  standard error (SE)

Item	LL	AD
pH		
45 min	6.29 $\pm$ 0.02 <sup>xa</sup>	6.48 $\pm$ 0.02 <sup>ya</sup>
24 h	5.71 $\pm$ 0.01 <sup>xb</sup>	5.97 $\pm$ 0.02 <sup>yb</sup>
96 h	5.64 $\pm$ 0.02 <sup>xb</sup>	5.76 $\pm$ 0.03 <sup>xb</sup>
168 h	5.66 $\pm$ 0.04 <sup>xb</sup>	5.73 $\pm$ 0.05 <sup>xb</sup>
Drip loss [%]		
45 min	-	-
24 h	1.94 $\pm$ 0.23 <sup>xa</sup>	0.61 $\pm$ 0.02 <sup>ya</sup>
96 h	4.03 $\pm$ 0.05 <sup>xb</sup>	1.92 $\pm$ 0.03 <sup>yb</sup>
168 h	6.59 $\pm$ 0.06 <sup>xc</sup>	3.08 $\pm$ 0.05 <sup>yc</sup>
Shear force (N)		
45 min	75.4 $\pm$ 1.18 <sup>xa</sup>	106.2 $\pm$ 1.793 <sup>ya</sup>
24 h	59.3 $\pm$ 0.90 <sup>xb</sup>	84.7 $\pm$ 1.56 <sup>yb</sup>
96 h	45.8 $\pm$ 0.93 <sup>xc</sup>	63.4 $\pm$ 1.403 <sup>yc</sup>
168 h	47.2 $\pm$ 1.01 <sup>xc</sup>	59.3 $\pm$ 1.37 <sup>yc</sup>

Values in rows with different subscript are significantly different: x, y ( $P < 0.05$ ), values in columns with different subscript are significantly different: a, b, c ( $P < 0.05$ ).

In the case of 24-h drip loss, the highest juice loss was found to occur 24 h *postmortem*. Such high drip loss during the first 24 h after slaughter is associated with muscle fibre structure, more specifically with myofibril arrangement and sarcomere length. Myofibrils which comprise muscle fibres, are composed of two myofilament types: thin actin filaments and thick myosin filaments. BERTRAM *et al.* (2002) put forward the hypothesis that actin filaments have a higher proportion of water compared to myosin filaments. Hence, during *postmortem* contraction due to the sliding of thin filaments into thick filaments leading to the shortening of sarcomeres, water loss from the cell is higher. In addition, drip loss value is considerably affected by the denaturation of cytoskeletal proteins. The degradation level of desmin, notably that located at costameres, has a significant effect on the degree of opening of drip channels, which determines the extent of drip loss (MORRISON *et al.* 1998; KRISTENSEN & PURSLOW 2001; MELODY *et al.* 2004). Different results were obtained by SCHAFER *et al.* (2002), who found that desmin degradation is not correlated to drip loss. It should be mentioned, however, that these authors only analysed the rate of desmin degradation between 3 and 24 h *postmortem*.

*Postmortem* changes that occur during meat storage also determine another quality parameter, namely tenderness. Meat tenderness is one of the most important organoleptic attributes that affect its palatability, and is a major factor contributing to the consumer acceptance of the raw material. The results of mechanical measurement of tenderness, performed by instrumental analysis of LL and AD shear force, are presented in Table 2. In AD and LL muscles, this parameter was significantly the highest at 45 min *postmortem*, and, with increasing meat storage period, i.e. from 45 min to 96 h *postmortem* a gradual, significant decrease in this parameter was found. When comparing shear force values between the analysed muscles, significantly higher values of this parameter for all the meat storage periods were observed for AD compared to LL muscle, which may be related to the composition of muscle fibres. As demonstrated by RYU and KIM (2005), muscle with a higher proportion of type I muscle fibres, i.e. AD muscle in the present study, is characterized by higher shear force values. Analogous relationships were reported by RENAND *et al.* (2001) in their analysis of beef tenderness. Different results were noted by CAMERON *et al.* (1998), who showed a positive effect of type IIB fibre percentage and a negative effect of type I fibres on shear force values. On the other hand, ORZECOWSKA *et al.* (2008) did not show any significant correlations between percentage of different muscle fibre types and shear

force. As regards the relationships between muscle fibre size and meat tenderness, the literature also provides many discordant data (RYU & KIM 2005; CAMERON *et al.* 1998; ORZECOWSKA *et al.* 2008). This is probably because this trait is the outcome of many factors, both *ante-* and *postmortem*. Much evidence shows that tenderization of meat is above all the result of enzymatic proteolysis of cytoskeletal proteins. MELODY *et al.* (2004) also point to significant relationships between the level of undegraded desmin and meat tenderness. In contrast, THERKILDSEN *et al.* (2002) observed that differences in tenderness are unrelated to the degradation of desmin. The presence or absence of significant correlations between meat tenderness and the level of undegraded desmin is probably associated not only with the degradation of muscle proteins, but also with many other factors such as intramuscular fat content, collagen content, or sarcomere length. O'HALLORAN *et al.* (1997) point to the interaction of muscle water and protein structures as an important factor of meat tenderness. OFFER and COUSINS (1992) consider that changes in water holding and binding capacity of muscle fibre depend largely on the degradation rate of myofibril cytoskeletal proteins *postmortem*. In turn, the degradation of these proteins, as noted before in this discussion, is influenced mainly by the calpain system. By degrading the Z line, these enzymes cause the fragmentation of myofibrils, i.e. degrade proteins such as desmin (DOLATOWSKI *et al.* 2004), troponin T and I (OFFER & COUSINS 1992), tropomyosin, protein C, vinculin, titin, and nebulin (GOLL *et al.* 1990; KOOHMARAI 1994), which is one of the important elements of meat tenderization. The m- and  $\mu$ -calpains differ in the concentration of calcium ions necessary for their activation. The amount of calcium ions in the muscle cell is only sufficient to activate  $\mu$ -calpain. When the concentration of calcium ions increases, m-calpain is activated. According to CLAEYS *et al.* (2001), a more rapid pH decline, accompanied with a higher  $\text{Ca}^{2+}$  concentration, resulted in increased  $\mu$ -calpain autolysis. Such high sensitivity of calpains to the pH of meat is probably the main reason for the differences in the level of desmin degradation observed in the present study between the analysed muscles, where significantly higher pH<sub>45</sub> and pH<sub>24</sub> values were noted in AD compared to LL muscle (Table 2). Such significant relationships between pH of meat and the level of desmin degradation were also reported by OFFER (1991) and WOJTYSIAK *et al.* (2008). Moreover, the differences in pH values observed in the present study between the analysed muscles may be due to differences in muscle fibre composition. RYU and KIM (2005) showed that unlike type IIA and I fibre content, an increase in type IIB fibre content is paralleled by a decrease in meat pH 45 min *postmor-*

tem. This relationship probably arises from the slower rate of ATP hydrolysis by type I fibres compared to type IIB fibres.

In summary, it should be stated that meat storage time has a significant effect on muscle fibre size and level of desmin degradation. In turn, the rate of desmin degradation depends on its localization in the cell, meat pH, as well as the type of muscle and the associated composition of muscle fibres. In addition, the degradation level of this protein directly influences physicochemical parameters such as drip loss and shear force. Therefore, both biochemical and histochemical properties of muscle fibres, as well as desmin activity, can be considered as factors modulating the *postmortem* quality of pork.

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