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RELATION OF MYOFIBRIL FRAGMENTATION TO TEXTURAL AND CHEMICAL PARAMETERS OF AGED PORK *Longissimus dorsi* *

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ABSTRACT

The relation of myofibrilar fragmentation (length of myofibrilar fragments, myofibrilar fragmentation index) to textural (Warner-Bratzler share force) and chemical parameters (non-protein nitrogen changes) of pork *Longisimus dorsi* muscles (acquired 24 h *post mortem*, normal meat quality) were investigated over a 16-day ageing period at 2 °C (± 1 °C). Ageing time significantly affected all parameters at the 1% level or less. The pH value for 16-day aged samples was slightly higher; the average values being 5.61 for nonaged and 5.67 for aged samples. Length of myofibrilar fragments being in average the highest (73 μm) for nonaged samples, and the lowest (15.7 μm and 12.4 μm) for 11 and 16 days aged ones. Myofibrilar fragmentation index increases significantly with storage: for nonaged samples below 30, after a 2-day ageing about 50, and after 5-day ageing 63.7 (determined as described by Olson *et al*., 1976), or after 11-day ageing 56.9 (determined as described by Hopkins *et al.*, 2004) Indexes for Hopkins procedure were about 7% lower ($P \leq 0.001$) compared to those for Olson procedure. Non-protein nitrogen after 11 and 16 days of storage was higher (10.78% and 10.93% of total nitrogen) compared to the nonaged pork (9.39% of total nitrogen). Warner-Bratzler share force was markedly affected by 16-day ageing (nonaged 51.3 N, 16 days 29.2 N). On the basis of instrumentally measured texture differences in thermally treated aged pork we concluded that myofibrilar fragmentation index was a suitable proteolysis rate pointer already from the second day on. The increase in non-protein nitrogen content indicates a release of free amino acids; so, it is a suitable measure of proteolysis after 5 days of ageing.

Key words: pigs / meat / ageing / myofibrilar fragments / length / myofibrilar fragmentation index / non-protein nitrogen / Warner-Bratzler shear force

POVEZAVA MED MIFIBRILARNO FRAGMENTACIJO, TEKSTRURNIMI IN KEMIJSKIMI PARAMETRI ZORENE PRAŠIČJE MIŠICE *Longissimus dorsi* †

IZVLEČEK

Namen raziskave je bil ugotoviti vpliv miofibrilarne fragmentacije (dolžina miofibrilarnih fragmentov, indeks miofibrilarne fragmentacije) na teksturne (Warner-Bratzler strižna trdnost) in

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kemijske parametre (neproteinski dušik) mišice *Longissimus dorsi* (LD) prašiča. V poskus so bile 24 ur *post mortem* vključene leve in desne LD normalne kakovosti šestih prašičev. Mišice smo razdelili na 3 dele, jih vakuumsko embalirali in zoreli 1-, 2-, 3-, 5-, 11- in 16 dni pri temperaturi 2 °C (± 1 °C). Čas zorenja je značilno ($P \leq 0{,}001$) vplival na vse parametre. Vrednost pH se je v 16-ih dneh nekoliko povečala, in sicer je bila povprečno: pri nezorenih mišicah 5,61 in pri zorenih 5,67. Dolžina miofibrilarnih fragmentov je bila v povprečju največja (73 μm) pri nezorenih vzorcih, medtem ko je bila pri 11- in 16 dni zorenih vzorcih značilno manjša (15,7 μm in 12,4 μm). Indeks miofibrilarne fragmentacije se z zorenjem značilno poveča, in sicer je v povprečju: pri nezorenih vzorcih pod 30, pri 2 dni zorenih pod 50 in pri 5 dni zorenih 63,7 (določen po Olsonu in sod., 1976) oziroma pri 11 dni zorenih 56,9 (določen po Hopkinsu s sod.). Indeks določen po Hopkinsu (2004) je okrog 7 % ($P \leq 0{,}001$) nižji glede na indeks določen po Olsonu. Neproteinski dušik je pri 11- in 16 dni zorenih vzorcih večji (10,78 % in 10,93 %) od nezorenih vzorcev (9,39 % od celokupnega dušika). Warner-Bratzler strižna trdnost se v 16-ih dnevih zorenja značilno ($P \leq 0{,}001$) spremeni. Povprečna vrednost nezorenih vzorcev je 51,3 N in pri 16 dni zorenih vzorcih 29,2 N. Na osnovi instrumentalno izmerjene teksture termično obdelanih vzorcev lahko zaključimo, da je indeks miofibrilarne fragmentacije že po drugem dnevu zorenja ustrezni pokazatelj mikrostrukturnih proteolitičnih sprememb, in da je vsebnost neproteinskega dušika ustrezni pokazatelj biokemičnih proteolitičnih sprememb šele po petem dnevu zorenja, ker so povečane vrednosti neproteinskega dušika posledica sproščanja prostih aminokislin.

Ključne besede: prašiči / meso / zorenje / miofibrilarni filamenti / dolžina / miofibrilarna fragmentacija / indeks / neproteinski dušik / Warner-Bratzler strižna trdnost

INTRODUCTION

Important changes in chemical composition and structure of muscle tissues take place during ageing. It is known that the process of meat ageing differs for different muscles of the same animal, for different animal species and even for various meat qualities (Devine, 2004; Čandek-Potokar *et al.*, 1999).

The influence of protein proteolysis stages during ageing of beef meat on the increase in tenderness *(*Davey and Gilbert, 1969; Dransfield, 1994; Olson and Parrish, 1977; Olson *et al.*, 1976), and the improvement of its taste and aroma (Nishimura *et al.*, 1988; Smith *et al.*, 1978) have been extensively studied, and recently, interest in different animal species such as pork, is increasing (Okumura *et al.,* 2003). The ageing (conditioning) indicators for tenderness evaluation of aged beef have been reported. The sarcomere length in miofibrils (Strydom *et al.,* 2005), the shear force value, the myofibrillar fragmentation index (MFI) (Olson *et al.*, 1976), the 30 kDa component (Koohmaraie, 1994), phosphorylase b, creatine kinase and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Okumura *et al.,* 2003*)* were correlated with the tenderness of bovine muscles. In the case of pork, stored without as well as with vacuum packaging at low temperature, Okumura *et al.* (2003) have found some useful ageing indicators, such as MFI, the 32-kDa components, peptides P1 and P2 as well as GAPDH.

In the present work, the myofibril fragmentation was examined in the pork *Longisimus dorsi* muscle (LD) stored under vacuum packaging at 2 °C for 16 days *post mortem*, and was compared with the results of texture evaluation. However, we anticipated that the myofibrilar fragment length (MFL) and Warner-Bratzler share force (WBSF) would decrease rapidly, while the MFI, the content of non-protein nitrogen fraction (NPN) would increase with ageing. On the other hand, as a contribution to the methodology of proteolysis evaluation we also wanted to compare two methods for MFI determination.

MATERIAL AND METHODS

Animals and tissue sampling

A total of six crossbred (Swedish Landrace × Large White × Duroc × Hampshire) barrows were included in the study. They were commercially slaughtered; their weight being between 80 and 85 kg (warm carcass weight), they contained 55–60% of lean meat, and had an ultimate pH (24 h *post mortem)* between 5.4 and 5.8. The carcasses were stored for 24 h at 4 °C (± 1 °C). Left and right LD muscles between 4th thoracic and the last lumbar vertebrae were removed from carcasses and used in further study.

Left and right LD muscles were cut into six samples and than vacuum packaged in polyethylene bags. Random sampling provided the part of muscle for a defined time of ageing; thus, the effect of sample location in the muscle was eliminated. After 1, 2, 3, 5, 11 and 16 days of ageing in the refrigerator at 2 °C (± 1 °C) each of 36 samples was divided into three sub-samples. On the first sub-sample (25 mm thick) thermal treatment was performed (grilling at 165 °C to the internal temperature of 70 °C (± 1 °C)). Grilled steaks were prepared for instrumental measuring of the texture (Warner-Bratzler Shear Force − WBSF) after 24 hours of cooling at 4 °C (± 1 °C). The second sub-sample was cut and immediately used for MFI determination. The third sub-sample was homogenized in a blender, repacked into polyethylene bags, frozen at –21 °C (± 1 °C), and was used for myofibrilar length (MFL) and non-protein nitrogen measurements, as well as for determination of water, protein, fat and ash content (carried out as well on nonaged samples − 24 h *post mortem*). Most of analyses were carried out in duplicate; WBSF was measured seven times and MFL 100 times on each sample.

Determination of water, total protein, intramuscular fat and ash content

The water content was determined on samples of 5 g of minced meat. Samples were dried in the oven at 105 °C according to AOAC 950.46 (Official Methods of Analysis, 1997). Total protein content (crude protein, N × 6.25) was assessed by the Kjeldahl method according to AOAC 928.08 (Official Methods of Analysis, 1997). The ash content was determined by mineralization of samples at 550 °C according to AOAC 920.153 (Official Methods of Analysis, 1997). Intramuscular fat content was determined by the method described in AOAC Official Method 991.36. Fat (Crude) in Meat and Meat Products (Official Methods of Analysis, 1997). The total lipids were extracted by hot treatment with petroleum ether as solvent.

Determination of pH value

pH value was measured directly using a spear combined glass-gel electrode type 03 (Testo pH electrode) with thermometer (type T, Testo penetration temperature probe) connected to pH meter (Testo 230, Testo). The pH meter was calibrated using pH = 4 and pH = 7 buffers and recalibrated after every 20 readings. Accuracy of reading was ± 0.01 pH unit. pH was measured six times per muscle; at day 1 (nonaged sample) and at 2, 3, 5, 11 and 16 days *post mortem* (aged samples).

Determination of MFL

Myofibrilar fragment suspension was prepared as outlined by Hopkins *et al.* (2000). A minced 0.5 g sample was placed in ice-cold vessels for homogenisation in 30 mL of ice-cold buffer. Homogenisation (Ultra-turrax T 25 with dispersing element S 25 N – 18 G at 15,000 rpm) was performed by two bursts of 30 s with a 30-s break on ice between them. The buffer

was 0.1 M KCl (Kemika, 112097), 1 mM EDTA (Merck, 1.08418), 1 mM NaN_3 (Merck, 1.06688), 7 mM KH_2PO_4 (Merck, 1.05108) and 18 mM K_2HPO_4 (Kemika, 1116108). Myofibril suspensions were filtered (1.0 mm mesh strainers) to remove connective tissue.

An aliquot (a drop) of the myofibril suspension was placed on a microscopic slide and was examined microscopically (Nikon Microphot-FXA, 20×10 magnify) using a camera (Sony DXC-930P) and a picture analysing programme (LUCIA_MTM). For each sample 6 different areas were chosen and 100 fragments were measured. MFL was expressed in μm.

Determination of MFI

MFI was determined according to the methods described by Olson *et al.* (MFI-Olson) (Olson *et al.,* 1976) and Hopkins *et al.* (MFI-Hopkins) *(*2000) using a UV/VIS spectrophotometer (Ultrospec 2000, Pharmacia Biotech). It was expressed as absorbance of a myofibril protein solution (concentration 0.5 mg mL^{-1}) at 540 nm multiplied by 100.

Determination of total nitrogen and non-protein nitrogen

All nitrogen contents were measured using Kjeldahl's method according to AOAC 928.08 (Official Methods of Analysis, 1997). Total nitrogen (TN) was measured on minced 1 g samples. Method for determination the non-protein nitrogen (NPN) was as fallows: a minced 5 g sample was homogenized with 40 ml of 3% (w/w) trichloroacetic acid (Ultra-turax T 25 with dispersing element S 25 N – 18 G, 120 s at 20,000 rpm). Then, the mixture was passed through a Sartorius no. 388 filter paper. Filtrate was analysed by BÜCHI Kjeldahl Line acording to AOAC 928.08 Kjeldahl method (Official Methods of Analysis, 1997). NPN was expressed as percent of TN.

Determination of WBSF

Steak (25 mm thick) was grilled at 165 ºC to 70 °C (± 1 °C) internal temperature and cooled for 24 h at 4 °C (± 1 °C). Seven cylinders (diameter 12.7 mm) were removed parallel to the longitudinal orientation of the muscle axis. Each cylinder was shared at the centre with a Warner-Bratzler shear 'V' slot blade (thickness of 3.0 mm and a triangular aperture of 60°) using a TA.XT plus texture analyser (Stable Micro Systems). The crosshead speed was 3.3 10^{-3} m s^{-1}. Newtons (N) being the units of the measurement.

Data analysis

For statistical evaluation of experimental data, the computer program SAS/STAT (SAS Software, 1999) was used. Basic statistical parameters were calculated by the MEANS procedure. Data were tested for normal distribution and analysed by the GLM (General Linear Model) and TTEST paired procedures. For data analyses two statistical models were used. For analysing the data for pH value, WBSF, MFL, MFI and NPN the statistical model [1] was used. The statistical model [2] for MFI included the effect of analytical method. The models were described by the following equations:

$$y_{ijk} = \mu + AT_i + A_j + e_{ijk} \quad [1]$$

where y = the observation parameter, μ = general mean, AT_i = effect of i^{th} ageing time (i = 1, 2, 3, 5, 11 and 16 days *post mortem*), A_j = effect of j^{th} animal (j = l to 6), and e = residual random term with variance σ^2_e.

$$y_{ijk} = \mu + AT_i + M_j + e_{ijk} \quad [2]$$

where y = the observation parameter, μ = general mean, AT_i = effect of i^{th} ageing time (i = 1, 2, 3, 5, 11 and 16 days *post mortem*), M_j = effect of j^{th} analytical method (j = Olson *et al*. (1976), Hopkins *et al.* (2000)) and e = residual random term with variance σ^2_e.

Least square means for experimental groups were obtained using the LSM procedure and were compared at the 5% probability level. Relations between instrumental and chemical parameters were assessed by Pearson correlation coefficients using the CORR procedure.

RESULTS AND DISCUSSION

Proving the homogeneity of the samples

Basic statistical parameters for chemical composition of raw pork LD muscle are shown in Table 1, the data clearly show the homogeneity of the samples.

Table 1. Basic statistical parameters for chemical composition of pork LD day 1 *post mortem* (N = 6)

Preglednica 1. Osnovni statistični parametri za kemijsko sestavo nezorene (prvi dan *post mortem*) prašičje LD mišice (N = 6)

Parameter/(g/100 g)	$\bar{x}$	Min.	Max.	SD	CV/%
Water Voda	74.2	73.2	75.6	0.83	1.11
IMF IMM	1.47	0.80	1.80	0.32	21.75
Protein Beljakovine	23.1	21.5	24.1	0.69	2.30
Ash Minerali	1.12	1.05	1.22	0.04	3.95

N – number of observations / število vzorcev, $\bar{x}$ – mean / povprečje, Min. – minimal value / minimalna vrednost, Max. – maximal value / maksimalna vrednost, SD – standard deviation / standardni odklon, CV (%) – coefficient of variation / koeficient variabilnosti, IMF – intramuscular fat / IMM – intramuskularna maščoba.

The average pH value of all our measurements 24 h *post mortem* was 5.61 ± 0.19; colour of muscles was appropriate for normal muscle quality. pH value as physicochemical criteria showed pork meat quality to be normal. pH_{24} values were comparable to pH_{24} from pigs of different age at slaughter and different feed restriction investigated by Čandek-Potokar *et al.* (1998) or those from pigs being slaughtered without or under minimal stress investigated by Henckel *et al.* (2000).

It is also known that pH increases for some tenths of a pH-unit due to ageing. In this study, an approximately 0.06-unit increase of pH value was determined after 16-days of ageing (Table 2).

Myofibril fragmentation

Meat tenderness is related also to structural (and biochemical) properties of skeletal muscle fibres, especially those of myofibrils and intermediate filaments. Histological studies dealt with

myofibrils breaking into shorter segments during *post mortem* storage of muscle, this phenomenon is called myofibril fragmentation. It is considered a useful ageing indicator of aged meat (Veiseth *et al.*, 2001). Myofibril fragmentation can be estimated by different methods: by homogenization of muscle, determination of protein content and measurement of the turbidity of samples adjusted to a standard protein concentration, by examination of myofibrils under a light microscope (Takahashi *et al.*, 1967; Moller *et al.,* 1973), by passing homogenized muscle through a filter system and recording the weight of the sample product removed (Reagan *et al.*,1975; Purchas *et al.*,1997) or by measuring of myofibrilar fragment length (Fernandez and Tornberg, 1994). Positive correlation between the rate of myofibrilar fragmentation and the tenderness of the meat is well known.

Table 2. Effect of ageing (1, 2, 3, 5, 11 and 16 days at 2 °C (± 1 °C)) on pH value, myofibrilar length, myofibril fragmentation index determined according to the methods described by Olson *et al.* (1976) and by Hopkins *et al.* (2000), non-protein nitrogen content, and Warner-Bratzler Shear Force of pork LD muscle (Model [1], N = 36)

Preglednica 2. Vpliv časa zorenja na vrednost pH, dolžino miofibrilarnih fragmentov, indeks miofibrilarne fragmentacije (določen po Olsonu s sod., 1976 in Hopkinsu s sod., 2000), neproteinski dušik in Warner-Bratzler strižno trdnost prašičje LD mišice (Model [1], N = 36)

Effect of: Vpliv:	Ageing/days Zorenje/dnevi						SE	P value
	1	2	3	5	11	16		
pH value Vrednost pH	5.62^{b}	5.61^{b}	5.52^{c}	5.62^{b}	5.64^{ab}	5.68^{a}	0.02	<0.001
MFL/μm DMF/μm	73.0^{a}	53.1^{b}	31.8^{c}	24.7^{d}	15.7^{e}	12.4^{e}	1.62	<0.001
MFI-Olson IMF-Olson	29.4^{c}	52.8^{b}	56.7^{b}	63.7^{a}	62.5^{a}	63.2^{a}	1.92	<0.001
MFI-Hopkins IMF-Hopkins	28.5^{d}	44.8^{c}	45.3^{c}	50.5^{b}	56.9^{a}	60.0^{a}	1.60	<0.001
NPN/(% of TN)	9.39^{d}	9.57^{cd}	9.73^{c}	10.12^{b}	10.78^{a}	10.93^{a}	0.10	<0.001
WBSF/N WBSS/N	51.3^{a}	37.9^{b}	35.8^{b}	32.7^{c}	31.5^{cd}	29.2^{d}	0.98	<0.001

N – Number of observations. SE – standard error. Least squares means with a different superscript within rows differ significantly ($P \leq 0.05$). Levels of significance: statistically significant: $P \leq 0.05$; highly statistically significant: $P \leq 0.001$. MFL – myofibrilar fragment length. MFI-Olson – myofibril fragmentation index (Olson *et al.,* 1976). MFI-Hopkins – myofibril fragmentation index (Hopkins *et al.*,2000). NPN – non-protein nitrogen. TN – total nitrogen. WBSF – Warner-Bratzler Shear Force.

N – število obravnavanj. SE – standardna napaka ocene. Pričakovane srednje vrednosti z različnimi nadpisanimi črkami ^{a,b,c,d,e} se statistično značilno ($P \leq 0.05$) razlikujejo. Stopnja značilnosti: statistično značilna: $P \leq 0.05$, statistično visoko značilna: $P \leq 0.001$. DMF – dolžina miofibrilarnih fragmentov. IMF-Olson – indeks miofibrilarne fragmentacije (1976). IMF-Hopkins – indeks miofibrilarne fragmentacije (2000). NPN – neproteinski dušik. TN – celokupni dušik. WBSS – Warner-Bratzler strižna trdnost

Generally, ageing can and does affect the MFL of pork meat (Table 2). The average MFL was 73 μm for day 1 *post mortem* and decreased significantly with the time of ageing. After 11 days

(15.7 μm) and 16 days (12.4 μm) of ageing the lowest MFL was measured, however the difference (between 15.7 μm and 12.4 μm) was not statistically significant. Therefore we assume that a great part of fragmentation measured occurred within day 3 and day 5 of ageing. The coefficient of variation for MFL was above 130%. This high variation in myofibril length was probably due to different activity of enzymes as well as due to nonenzyme process of decomposition (e.g. by calcium ions).

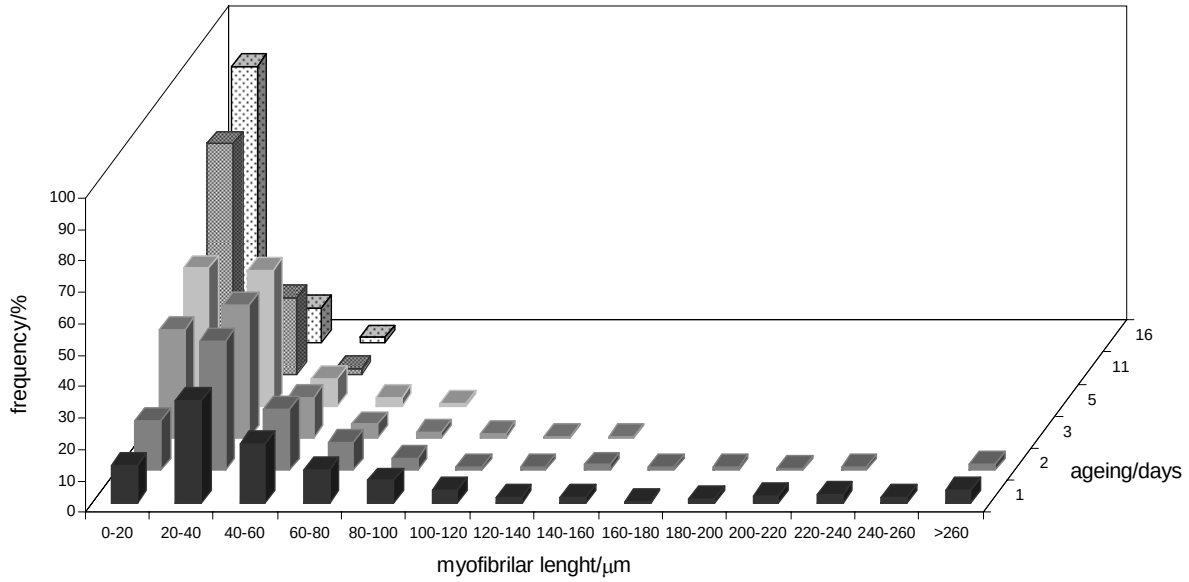

Figure 1. Relative frequency distribution of myofibrilar length of pork LD muscles as a function of ageing time.

Slika 1. Relativna frekvenca porazdelitve dolžine miofibrilarnih fragmentov prašičje mišice LD v odvisnosti od zorenja.

Myofibril breaking into shorter fragments during *post mortem* ageing of muscle is presented in Figure 1. In nonaged samples the size of fragments being between 0 and 20 μm (12%), 20 and 40 μm (33%), 40 and 60 μm (19%), 60 and 80 μm (10%), some of them being longer (28%). Generally, fragments isolated from samples 2, 3 and 5 days *post mortem* have the size of 0 to 20 μm, 20 to 40 μm and 40 to 60 μm. In samples at 11 and 16 days of ageing the average myofibrilar fragment length is in the range between 0 and 20 μm (73% and 88% of all measured fragments, respectively). The relative frequency in the first class (between 0 and 20 μm) progressively increases with *post mortem* time (Figure 1).

Direct comparison of these data with those from literature is difficult, since the myofibril fragment length depends on ageing condition, species and process of homogenising. Early as well as recent studies dealt with the changes in sarcomere length (Herring *et al.,* 1965; Rees *et al.,* 2002) or myofibril fragment length for beef, lamb or chicken muscles. Myofibril fragmentation of beef and pork LD, as Strydom *et al.* (2005) and Čandek-Potokar *et al.* (1998) emphasize, was significantly influenced by ageing (beef: 2 days 34.2 μm, 14 days 24.7 μm; pork: 1 day 19.4 μm, 4 days 9.77 μm). Our results agree with their data in spite of the fact that measured lengths acquired on pork LD after 2 days of ageing are noticeably longer (53.1 μm). These differences are probably due to the use of different extraction procedure and different homogenisers (Olson *et al.,* 1976; Hopkins *et al.,* 2000; Culler *et al.,* 1978).

The fragmentation of myofibrils has been observed during post-mortem ageing for 16 days and its index increased ($P < 0.001$) until day 16 (Table 2). The present study shows MFI values

ranging from less than 30 to more than 60. The MFI values of nonaged samples were below 30, values about 50 have been reached at day 2 of ageing. Increase after day 5 of ageing in MFI-Olson was hardly found. MFI-Hopkins after day 11 of ageing remained unchanged.

Absolute MFI values for 3-day aged pork LD acquired in this study are somewhat lower compared to those of Veiseth *et al*. (2001) for pork as well as those of Bruas-Reignier and Brun-Bellut (1996) for bulls reported for at the same *post mortem* time, but on the other hand are comparable to MFI for 2 to 20-day aged pork loins as investigated by Okumura *et al.* (2003). These differences are probably due to the use of different myofibril preparation, such as speed/time of homogenization and type of homogenizer particularly the blade type, used scaling factor 200, 150 or 100 and the state of the sample (fresh or frozen and thawed) (Hopkins *et al.,* 2000; Hopkins *et al.,* 2004), anatomical parts of the pigs taken as samples, days *post mortem*, etc. It should be emphasized that the difference between MFI determined on fresh and frozen muscles was not significant (Veiseth *et al.*, 2001; Hopkins *et al.*, 2000). Comparisons of the data in Table 2 with other published reports indicate that at day 1 *post mortem* ovine muscle exhibits 2 times higher MFI values (Hopkins *et al.,* 2000) compared to pork LD muscle in this study.

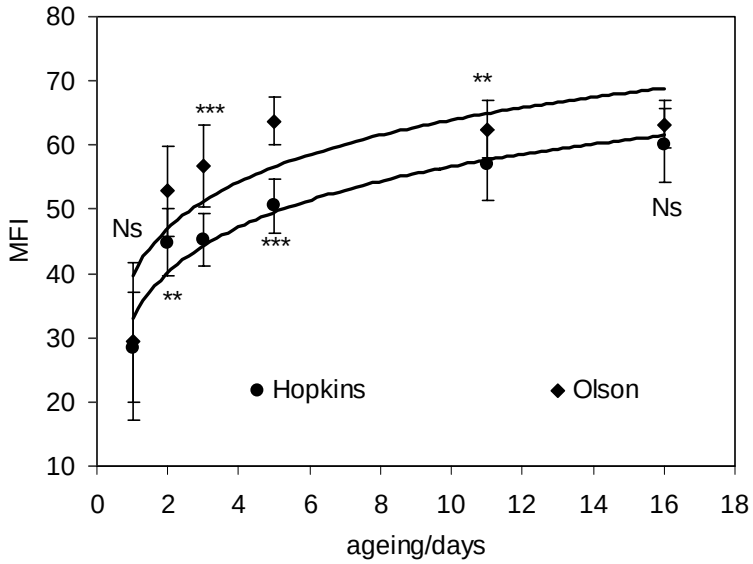

Figure 2. Comparison of MFI (myofibril fragmentation index) examined as described by Olson *et al.* (1976) and Hopkins *et al.* (2000), from aged pork LD muscles (mean ± standard deviation) (Model [2], Levels of significance: not significant: Ns – $P > 0.05$, statistically significant: ** $P \leq 0.01$, statistically highly significant: *** $P \leq 0.001$).

Slika 2. Primerjava IMF (indeks miofibrilarne fragmentacije) prašičje mišice LD, določenega po Olsonu s sod. (1976) in Hopkinsu s sod. (2000) (srednja vrednost ± standardni odklon) (Model [2], stopnja značilnosti: neznačilna: Ns – $P > 0{,}05$, statistično značilna: ** $P \leq 0{,}01$, statistično visoko značilna: *** $P \leq 0{,}001$).

Figure 2 shows the changes in MFI examined as described by Olson *et al.* (1976) and Hopkins *et al.* (2000), and prepared from pork LD muscles stored at 2 °C for 1–16 days after slaughter. Main differences between these two methods are in the amount of meat sample, volume of isolating medium (buffer) per mass unit of meat sample, the sequence of centrifugations and filtrations, time of homogenization and number of washings. Increases in MFI-Olson and MFI-Hopkins were continuously observed during storage for 16 days; on the average, the values ($P < 0.001$) for MFI-Hopkins are about 7% lower compared to those for

MFI-Olson, with the exceptions for the nonaged and 16-day aged samples, similar values were obtained.

Repeatability of both MFI methods was established by analysing the same sample in six replicates, the coefficient of variation for MFI-Olson being 4.6% and that for MFI-Hopkins 9.0%.

Textural and chemical parameters

Table 2 shows also the effect of ageing time on textural properties of roasted pork meat. The WBSF values were significantly different for different ageing times. Significantly the highest values (51.3 N) across the fibres were determined 1 day *post mortem*. After 2 or 3 days samples were significantly tenderer, the lowest values (31.5 N and 29.2 N) were observed after 11 and 16 days of ageing. According to the statement of Van Oeckel *et al*. (1999), pork meat (stored for 48 h at 4 °C, frozen stored during several months at –18 °C and grilled until an internal temperature of 74 °C, followed by cooling in tap water for 40 min) had a WBSF value of 35.5 N. Results of our study (37.9 N) are in slight discrepancies with their data, since lower values were determined, very probably due to different genotypes, different cooling and different parts of LD (*thoracis vs. lumborum*) muscles used. On the other hand, Fortin *et al.* (2005) determined higher values (62.9 N) on grilled loins (2 days *post mortem*, internal temperature of 72 °C, cooled in an ice/water bath, chilled for 24 h). Significantly lower absolute values in our study could be explained mainly due to smaller diameter (12.7 mm *vs.* 19 mm) of cylinders.

The non-protein nitrogen fraction (NPN) corresponds to the muscle's soluble non-protein compounds containing nitrogen (creatine, creatine phosphate, nucleotides, urea etc., amino acids and small peptides derived from the protein metabolism *post mortem*) (Bruas-Reignier and Brun-Bellut, 1996; Mikami *et al.*, 1991). Significant increase in NPN content during days 3–11 of ageing (Table 2, Figure 3) is in agreement with conclusions of Bruas-Reignier and Brun-Bellut (1996), who claimed that the increase in NPN can be considered an indicator of beef meat proteolysis. NPN content reached the highest value at about day 11 *post mortem*; by this time the maximum release of amino acids and small peptides has probably occurred.

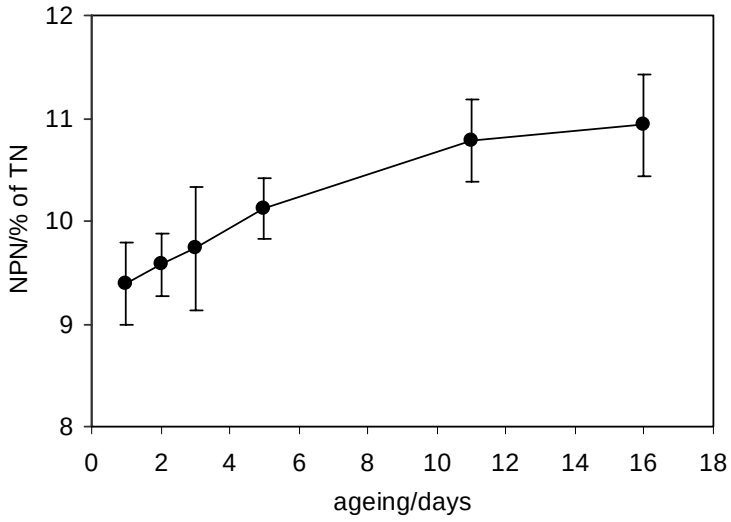

Figure 3. Effect of ageing time on non-protein nitrogen content (NPN) (% of total nitrogen − % of TN) in aged pork LD muscles (mean ± standard deviation).

Slika 3. Vpliv časa zorenja na vsebnost neproteinskega dušika (NPN) (% od celokupnega dušika − % od TN) v prašičjih mišicah LD (srednja vrednost ± standardni odklon).

Correlations

Correlation coefficients between instrumental values and physicochemical proteolysis parameters of pork LD muscles are shown in Table 3.

WBSF is associated with the MFL, strong positive correlation between the parameters was observed ($R = 0.82^{***}$). Furthermore, the WBSF was negatively correlated with variations in MFI values, MFI-Olson ($R = –0.82^{***}$) and MFI–Hopkins ($R = –0.77^{***}$), which is in agreement with the results of Culler *et al*. (1978). These results further on substantiate the fact that the usual term for the state of tenderness, namely, the expression myofibril fragmentation tenderness, is an appropriate one to describe tenderness of a conventionally aged pork LD muscle.

Table 3 further on shows that the increase in the NPN content is not related to the extent of the myofibril fragmentation. The increase in MFI or decrease in MFL and WBSF, which occurred mainly during the first 5 days *post mortem*, are not directly related to the increase in amino acid content and small peptide content, which increased from day 5 to day 11 of pork ageing.

Table 3. Pearson correlation coefficients between textural, chemical and myofibril fragmentation parameters of pork LD muscles (N = 36)

Preglednica 3. Pearsonovi korelacijski koeficienti med teksturnimi, kemijskimi in fragmentacijskimi parametri prašičje mišice LD (N = 36)

Parameters Parametri	WBSF WBSS	MFI-Olson IMF-Olson	MFI-Hopkins IMF-Hopkins	NPN
MFL DMF	0.82^{***}	$–0.84^{***}$	$–0.87^{***}$	$–0.65^{***}$
WBSF WBSS	1.00	$–0.82^{***}$	$–0.77^{***}$	$–0.58^{***}$
MFI-Olson IMF-Olson		1.00	0.86^{***}	0.58^{***}
MFI-Hopkins IMF-Hopkins			1.00	0.67^{***}

Levels of significance: statistically significant: * $P \leq 0.05$ and ** $P \leq 0.01$; highly statistically significant: *** $P \leq 0.001$. WBSF – Warner-Bratzler Shear Force. MFL – Myofibrilar length. MFI-Olson – myofibril fragmentation index (1976). MFI-Hopkins – myofibril fragmentation index (2000). NPN – non-protein nitrogen.
Stopnja značilnosti: statistično značilna: * $P \leq 0.05$ in ** $P \leq 0.01$, statistično visoko značilna: *** $P \leq 0.001$. WBSS – Warner-Bratzler strižna trdnost. DMF – dolžina miofibrilarnih fragmentov. IMF-Olson – indeks miofibrilarne fragmentacije (1976). IMF-Hopkins – indeks miofibrilarne fragmentacije (2000). NPN – neproteinski dušik.

CONCLUSIONS

Generally, 16-day ageing of pork meat does affect the microstructure, texture and non-protein nitrogen content. Myofibrilar fragmentation increases significantly from the first day *post mortem* during the entire ageing period. Up to 60% decrease of MFL occurs within the first 3 days and up to 50% increase of MFI within the first 2 days of ageing. We assume that a great part of fragmentation occurred between day 2 and day 5 of ageing. It can be concluded that under the conditions of this experiment, 60% of tenderisation of pork LD occurs within 2 days *post mortem* and 84% within 5 days. WBSF is associated with the MFL ($R = 0.82^{***}$), MFI-Olson ($R = –0.82^{***}$) and MFI-Hopkins ($R = –0.77^{***}$). The increase in the NPN content was not related to the extent of myofibril fragmentation. Myofibrilar fragmentation, which occurred

mainly during the first 5 days *post mortem*, was not directly related to the increase in amino acid content and small peptide content, which both increased also after 5 days of ageing.

As far as methods for MFI determination are concerned, it can be concluded that on the average the values for MFI-Hopkins are about 7% lower compared to those for MFI-Olson.

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