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PECULIARITIES OF THE HORSE MEAT AGING

Stefan G. Dragoev[1*], Dessislava B. Vlahova-Vangelova[1], Dessislav K. Balev[1], Kolyo T. Dinkov[2], Aco Kuzelov[3]

[1]University of Food Technologies, Technological Faculty, Department of Meat and Fish Technology, 26 Maritza blvd., Plovdiv, Bulgaria
[2]University of Food Technologies, Technical Faculty, Department of Processes and Apparatus, 26 Maritza blvd., Plovdiv, Bulgaria
[3]Goce Delcev University, Faculty of Agriculture, Department of Food Technology and Processing of Animal Products, 10-A Krste Misirkov, Stip, Macedonia
**Corresponding author: logos2000lt@gmail.com*

Abstract

Over the last decade the horse meat has gone deeper into the field of vision of both consumers and scientists. The objective of this study is to identify the specific features during aging of the horse meat. The changes in microstructure, morphology, protein autolysis, soluble proteins, pH, WHC, drip loss and colour were studied in horse m. Longissimus dorsi during 12 days of wet aging at 0 - 4°C. At 3 d post mortem the A- and I-zones were more difficult to distinguish. Some shortening of the sarcomere was observed. The rigor mortis period in the horse meat occurs between day 3 and day 5. Within this period the muscle fibres were contracted, the red colour component was decreased by 2 - 3 units, the pH and the WHC were minimal - 5.80 and 13 - 14%, respectively, and the drip losses were maximum about 20%. In intra-cellular spaces released free water was found. After 5 d post mortem single cracks and strains were observed - an indicator of the ongoing autolytic processes. The solubility of the proteins is stabilized at about 1.750 mg/ml. An increased share of protein fractions with a molecular weight of 28 - 23 KDa, considered as an indicator of increased meat fragility, was found after 5 days. From 5 to 12 day, higher levels of α-actinin, desmin and light meromyosin were found. After 7 d of post mortem the destructive changes were deepening. Z-lines were very much torn. A- and I-discs were difficult to distinguish. Myofibrils were highly fragmented and I-zones were not distinguishable.

Key words: *m. Longissimus dorsi,* microstructure, morphology, protein autolysis, soluble proteins, drip loss, color

INTRODUCTION

Post mortem changes, leading to the transformation of muscle tissue into meat, have been in the spotlight of scientists since the middle of the twentieth century (Fujimaki & Arakawa, 1958; Hultin, 1984). Longo et al. (2015) confirm the hypothesis that at the centre of this transformation is the process of apoptosis. Post-mortem chemical changes in muscle are associated with the aging of the meat (Davey, 1983) and its tenderness (Lian et al., 2013). Proteolysis processes are referred to as primarily responsible for the aging and maturation of meat (Geay et al., 2001).

In the literature publications discussing the problems of aging meat from 3 to 10 year old horses (Litwinczuk et al., 2008) and foals (Ruiz et al., 2018) and accelerated aging of horse meat by marinating with solutions of calcium chloride can be found (Lourdes Perez et al., 1998). There were no studies on aging of two years old horses bred in the Balkans. This is why we have set ourselves the aim of this study being to identify the specific features in the process of the horse meat aging.

MATERIALS AND METHODS

Horse meat

The objective of the study was horse m. Longissimus dorsi. The meat was supplied by Unitemp Ltd., village of Voyvodinovo, district of Plovdiv. An average sample was taken from 21 horses. The 42 muscles were obtained, packed in plastic containers in air and wet-aged for a period of 12 days at 0 - 4°C. The samples of m. Longissimus dorsi (from the area between 13th and 18th thoracic vertebra about 500 g for each sample) were taken for analysis.

Part of those muscle particles was used for a morphological, microstructural and colour analysis, determination of water holding capacity (WHC) and pH. The meat samples for protein autolysis and pH were mixed and minced in a grinder with a hole diameter 4 mm. The mince meat was homogenized in a homogenizer Knife Mill GRINDOMIX GM 300 (AZoNetwork UK Ltd., Manchester, UK). The homogenized samples were vacuum-packed in table-top vacuum packers LYNX 32 (INTRAMA Group, Dobrich, Bulgaria) and stored for 8h at 0-4°C up to the moment of analysis.

The changes in microstructure, morphology, protein autolysis, soluble proteins, pH, WHC, drip loss and colour were studied.

Light microscopy

For morphological analysis samples of 2 x 1 x 1 cm were used. The fixing and the contrast of the samples were carried out according to the method described by Barbut et al. (2005).

Transmission electron microscopy

The preparation of the samples and the transmission electron microscopy were conducted in accordance with the method of Lawrence et al. (2002).

SDS-PAGE electrophoresis of myofibrillar proteins

SDS-PAGE electrophoresis of the myofibrillar extracted proteins was carried out under the Laemmli method (1970).

Free amino nitrogen

The amount of α-free amino groups in the myofibrillar extracted proteins was established as described in Analalytica - EBC (Welten, 2013).

Soluble proteins extracted from myofibrils

Extraction of myofibrillar proteins was performed with PBS buffer (ionic strength 0.55) using Khan describtion (1962). The protein concentration of the extract was determined by Lowry et al. (1951) method.

Water holding capacity of meat

The water holding capacity of meat was determined by the Grau & Hamm method (Modzelewska-Kapitula and Cierach, 2009).

pH value

The pH of the samples was measured electropotentiometrically by the Korceala et al. (1986).

The meat colour characteristics

The colour characteristics of the horse meat samples were determined spectrophotometrically with the CIELab system (Brewer and Wu, 1993).

Statistical analysis

The statistical analysis was made using the method of Draper and Smith (1998). Differences between values below $p \leq 0.05$ were considered statistically significant. All statistical procedures were performed using software Microsoft Excel 5.0.

RESULTS AND DISCUSSION

Morphological changes

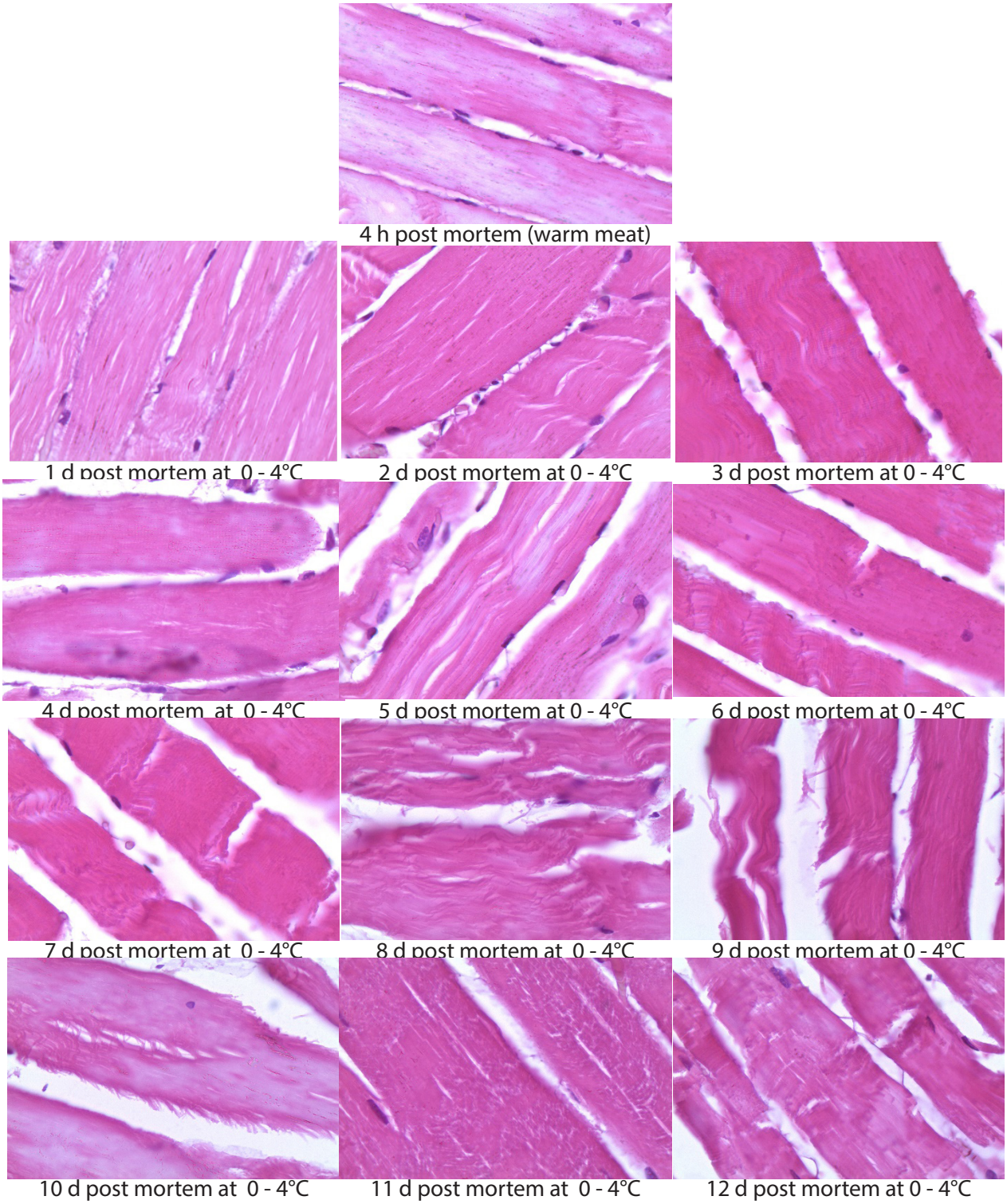

Figure1. Morphological changes of horse *m. Longissimus dorsi* during a period of 12 days of storage at 0 - 4°C. Horse fillets longitudinal cut contrasted with hematoxylin, 1000x.

In Figure 1 are presented changes of the morphology in horse muscle tissue samples during a period of 12 days of storage at 0 - 4°C. Up to the 3 days of cold storage in muscle and connective tissues there were not detected any significant destructive changes. Muscle fibres had a loose structure and preserved their integrity. They were tightly attached to one another. With the onset of rigor mortis the muscle fibres were contrasted. Exudate was excreted in the intercellular spaces. In the initial stages of autolysis (4 days) the muscle fibres recover their loose structure. Some unique cracks and strains appear. This is an indication of the ongoing autolysis changes up to 5 days of refrigeration. Some single cracks and feathering were found. These changes are an indication of ongoing autolytic changes till the 5 days of cold storage. From 6 days to 12 days of the horse meat refrigeration at 0 - 4°C the destructive changes in muscle tissue were getting worse. Larger cross cracks were observed. There was also observed a partial decomposition of protofibrils (Fig. 1).

Changes of electron microscopic determined muscle structure

Figure 2 shows the changes in the microstructure of horse meat samples (m., *Longissimus dorsi*) for a period of 12 days of refrigerated storage at 0 - 4°C.

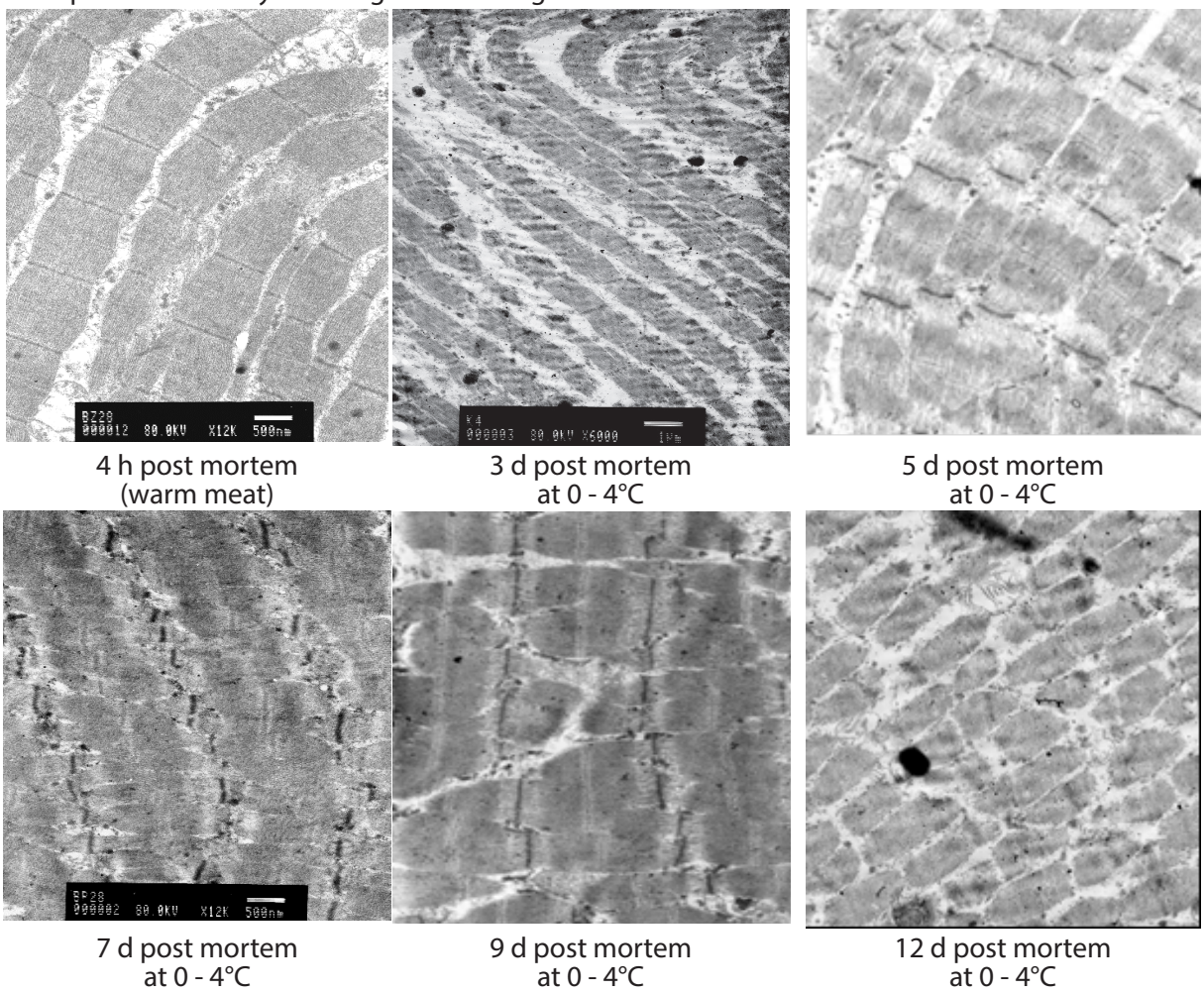

Figure 2. Electron microscopic preparations from longitudinal cuts of horse *m. Longissimus dorsi*, 12000x.

After 4 h post mortem individual sarcomers were clearly identifiable and could retain integrity. A- and I-zones were distinguishable. H-zones and M-lines were clearly visible, no Z-lines were observed. After 3 days post mortem storage A- and I-zones were distinguished with more difficulty. There was established some shortening of the sarcometers. After rigor mortis (6 days) a loosening of myofibrils and a partial recovery of their natural structure was observed. The A- and I-disks were still clearly visible, the Z-lines were preserved. Some changes typical of the meat maturation were noticed. Cross-cracks in the Z-lines and the myofibrils were spotted. After 9 days post mortem the destructive changes in the myofibrils were getting worse. The Z-lines were heavily torn; the A- and I-discs were very hard to distinguish. The myofibrils were highly fragmented, and the I-zones were virtually indistinguishable.

Results from SDS-Page gel electrophoresis

SDS-PAGE electrophoresis during aging of horse meat (0-4 °C) showed that the heavy meromymisin chains(200 KDa) were identified up to 48 hours post mortem. At the 4-day of storage (0-4 °C), the α-actinin content (95 KDa) was minimal. With increasing the aging time (after 5 days of storage at 0-4 °C) due to ongoing proteolytic changes, the protein fractions with a molecular weight of 28-23 KDa, considered as an indicator of tenderness (Huff-Lonergan, 1999) increase. After the 5 day of refrigerated storage at 0 - 4 °C to the end of studied period (12 days) an increase in the α-actinin fraction, desmine and light meromyosin was observed.'

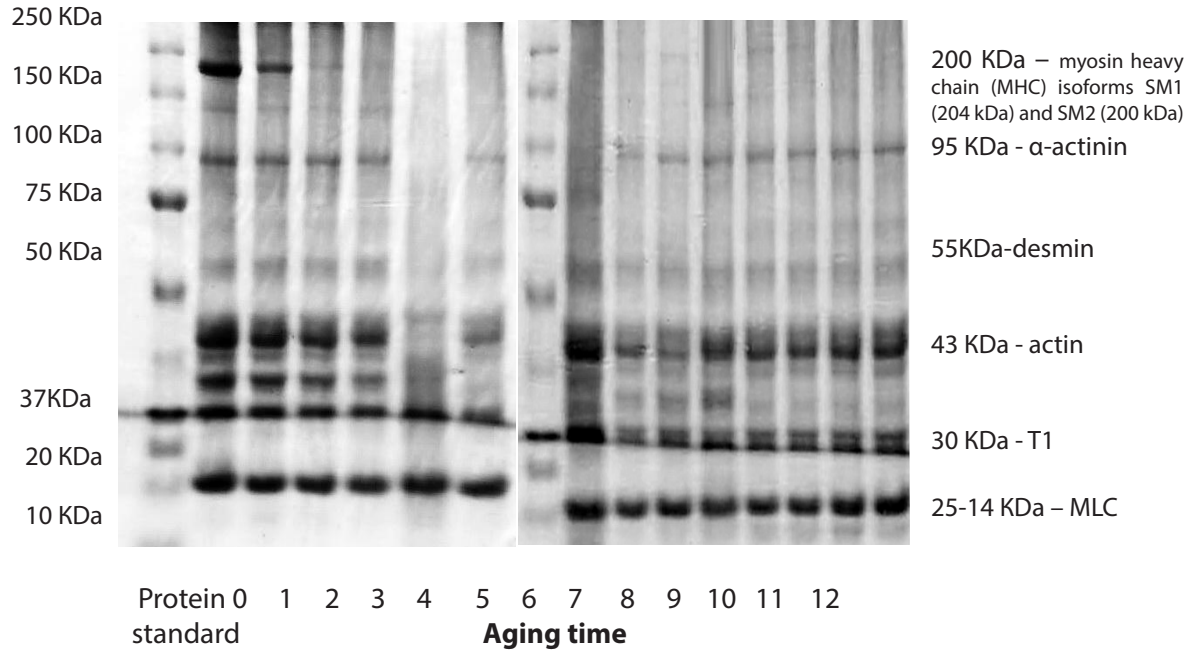

Figure 3. SDS-Page gel electrophoresis of horse *m. Longissimus dorsi* during 12 days of aging at 0 – 4°C

Free amino nitrogen

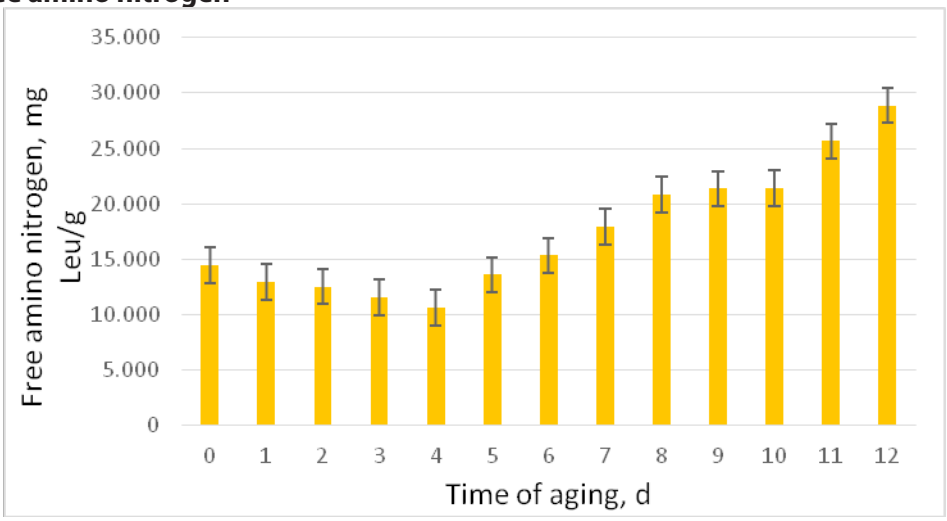

Figure 4. Changes of free amino nitrogen in horse *m. Longissimus dorsi* during 12 days of aging at 0 – 4°C.

During the first 5 days of the horse meat storage at 0 - 4°C (Fig. 4) there were no statistically significant ($p > 0.05$) changes in the content of free amine nitrogen (FAN). From 6 day to 8 day of the experiment due to proteolysis processes (Geay et al., 2001) the amount of free amine nitrogen in the meat increased by 46.7% ($p \leq 0.05$).

From 8 day to the end of the study period - 12 days (0 - 4°C) a reverse trend was found, namely: the FAN content of the horse meat was reduced by 35% (Fig. 4, $p < 0.05$). The decrease in FAN in the final stages of sample storage (0 - 4°C) corresponds well with the reduction of the solubility of proteins during the same study period (Fig. 4).

Soluble proteins

Immediately after obtaining (4 h post mortem) horse meat was characterized by a relatively high pH (pH = 6.75, Tab. 1) and water holding capacity (WHC) (Fig. 6). For the next 3 d (0 - 4°C) the solubility of the protein fraction was decreased by 5.7% ($p \leq 0.05$, Fig. 5). The results obtained correspond to the pH and WHC data (Tab. 1 and Fig. 6) and are indirect evidence of a rigor mortis. After 3 days autolysis was detected under the effect of endogenous tissue enzymes and a number of biochemical transformations (Hultin, 1984). As a result, after 6 days the solubility of the meat proteins increased significantly ($p \leq 0.05$). From 6 day to the end of the study period (12 d, 0 - 4°C) a reverse trend was established.

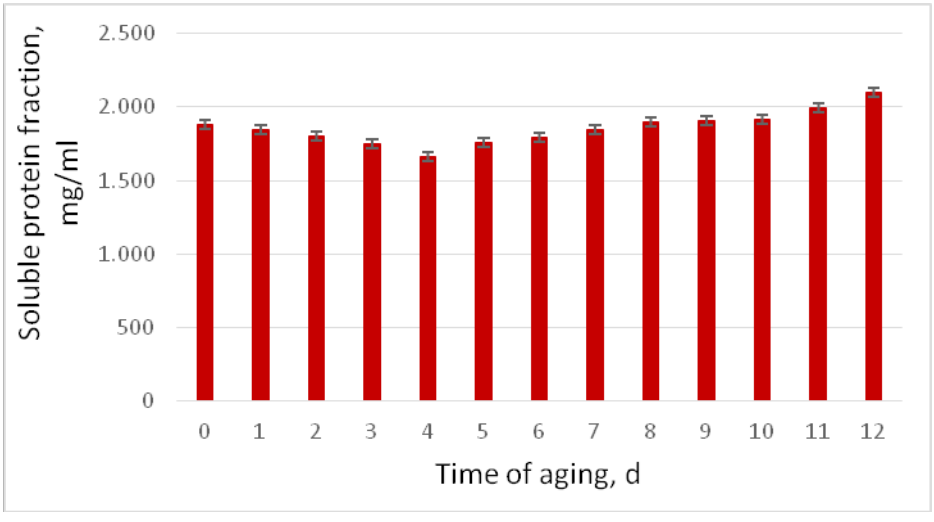

Figure 5. Changes of the soluble protein fraction extracted from horse *m. Longissimus dorsi* for a period of 12 days of aging at 0 – 4°C.

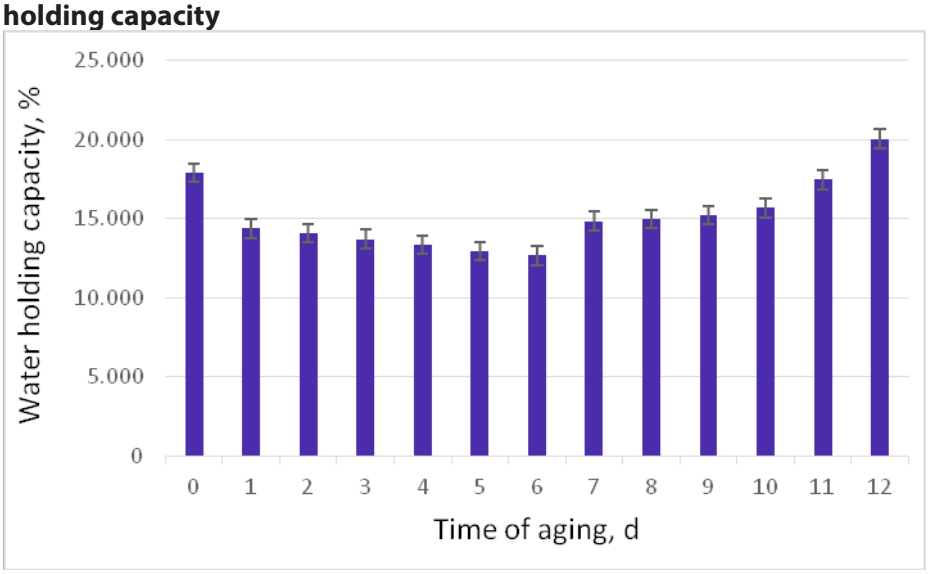

Figure 6. Changes of water holding capacity of horse *m. Longissimus dorsi* during 12 days of aging at 0 – 4°C.

The identified changes in water holding capacity (WHC) of the horsemeat samples (Fig. 6) correspond to the data obtained for the pH (Tab. 1). From 1 day to 6 day of the experiment, the WHC decreased by 45.8% (Fig. 6, $p \leq 0.05$). From 6 day to 12 day of the storage of samples of horse meat (0 - 4°C) the WHC increased by 7.5% ($p < 0.05$) (Fig. 6).

pH value

Immediately after slaughter (4h post mortem) the horse meat was characterized by a normal pH of the order of 6.75 (Tab. 1). A statistically significant decrease of pH of 14.07% ($p \leq 0.05$) was found on the 4 day of the horse meat storage at 0 - 4°C. These results are indirect evidence of a rigor mortis stage. Similar results for the pH at 45 min, 24 and 48 h post mortem in the lumbar segment of Longissimus dorsi muscle (Longissimus dorsi) and m. Semitendinosus are found by Litwinczuk et al. (2008). Unlike our study Lourdes Perez et al. (1998) and Litwinczuk et al. (2008) found a 8.39% lower pH at 24 h post mortem. This is probably due to the fact that the samples in the studies were taken from horses considerably older and probably in purer health.

From 4 days to 8 days of the refrigeration storage the rigor mortis progressively passed and the pH of the horse meat increased by 8.62% ($p \leq 0.05$) (Tab. 1).

From 8 days to 12 days at the end of the cold storage period of the horse meat at 0 - 4°C the pH again decreases and reaches acidic values commensurate with those in the post-mortem state (Tab. 1). The resulting decrease in pH at the end of meat storage (0 - 4°C) is probably due to the development of lactic acid microflora. Contrary to our data Seong et al. (2014) found a constant increase in the pH of vacuum packed and 30 days aged at 4°C m. Longissimus dorsi. Similar as our results were found in thawed horse meat after 30 days storage at -20°C.

Colour determination

Table 1. Changes of pH and colour characteristics of horse m. *Longissimus dorsi* for a period of 12 days of aging at 0 - 4°C.

Time for post mortem aging d	pH	The color brightness L*	The color redness a*	The color yellowness b*
0	$6.75^{e} \pm 0.05$	$30.01^{a} \pm 0.26$	$16.91^{e} \pm 0.10$	$3.45^{b} \pm 0.38$
1	$6.20^{b,c} \pm 0.05$	$30.35^{a} \pm 0.14$	$17.01^{e} \pm 0.37$	$3.58^{b} \pm 0.17$
2	$6.15^{b,c} \pm 0.05$	$30.82^{a} \pm 0.79$	$17.86^{f} \pm 0.21$	$3.61^{b} \pm 0.35$
3	$6.10^{b} \pm 0.05$	$31.71^{b} \pm 0.13$	$19.93^{g} \pm 0.43$	$4.89^{d} \pm 0.28$
4	$5.80^{a} \pm 0.08$	$31.34^{b} \pm 0.52$	$19.67^{g} \pm 0.32$	$4.62^{d} \pm 0.17$
5	$6.16^{b,c} \pm 0.05$	$30.42^{a} \pm 0.34$	$15.55^{d} \pm 0.36$	$4.21^{c} \pm 0.23$
6	$6.20^{b,c} \pm 0.05$	$30.29^{a} \pm 0.72$	$14.62^{c} \pm 0.73$	$3.78^{b} \pm 0.27$
7	$6.25^{c} \pm 0.05$	$30.30^{a} \pm 0.56$	$14.42^{c} \pm 0.41$	$3.75^{b} \pm 0.21$
8	$6.30^{c,d} \pm 0.02$	$30.36^{a} \pm 0.44$	$12.62^{b} \pm 0.20$	$3.21^{a,b} \pm 0.07$
9	$6.12^{c} \pm 0.07$	$30.52^{a} \pm 0.27$	$12.17^{a} \pm 0.23$	$3.08^{a,b} \pm 0.23$
10	$6.00^{b} \pm 0.06$	$30.42^{a} \pm 0.68$	$12.14^{a} \pm 0.28$	$2.99^{a,b} \pm 0.23$
11	$5.85^{a} \pm 0.08$	$30.50^{a} \pm 0.45$	$11.98^{a} \pm 0.58$	$2.71^{a} \pm 0.20$
12	$5.80^{a} \pm 0.10$	$30.78^{a} \pm 0.56$	$11.84^{a} \pm 0.15$	$2.70^{a} \pm 0.23$

Means within each column having different letters were significantly different according to Duncan's test at $p < 0,05$.

No statistically significant differences ($p > 0.05$, Tab. 1) were observed when the colour brightness (L*) measured at the beginning (4 h, 0 - 4°C) and at the end of the experiment (12 days, 0 - 4°C) of horse m. Longissimus dorsi were compared. Statistically significant increase of the colour brightness (L*) by about 5.66% ($p \leq 0.05$) was found on the 3 and 4 day of the storage of the horse meat at 0 - 4°C (Tab. 1). From the 4 d (Tab. 1) until the end of the study period the colour brightness decreases with no statistically significant difference ($p > 0.05$) of the originally determined value on the 12days. These conclusions are consistent with the results reported by Seong et al. (2014) for vacuum-packed and 30 days aged at 4°C d horse m. Longissimus dorsi but differ significantly from the results reported by Ruiz et al. (2018) for 9 d aged at 4°C foals m. Longissimus dorsi.

After the 4 days of horse meat storage (Tab. 1) the color redness (a*) increases by approximately 17.86% ($p \leq 0.05$). After the 4 days of the meat refrigeration a reverse trend was established and by the end of the study the colour redness was reduced by 7.83 ($p < 0.05$).

A statistically significant increase in the colour, with an approximately 41.74% ($p \leq 0.05$) increase in the yellowness (b*), was found on the 3 day of the horse meat storage at 0 - 4°C (Tab. 1). From the 4 day to the 12 day of the storage at 0 - 4°C a reverse trend was established as well.

On the 12 day of the experiment the colour yellowness (b*) decreased with 1.92 units ($p \leq 0.05$) from the baseline. These results are not in agreement with those reported by Seong et al. (2014) who found that the yellowness of the vacuum packaged and 30 days aged at 4°C d horse m. Longissimus dorsi significantly increases and those of Ruiz et al. (2018) for 9 days aged at 4°C d foals m. Longissimus dorsi.

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ОСОБЕНОСТИ НА СТАРЕЕЊЕТО НА КОЊСКОТО МЕСО

Стефан Г. Драгоев[1]*, Десислава Б. Влахова-Вангелова[1], Десислав К. Балев[1], Кољо Т. Динков[2], Ацо Кузелов[3]

[1] Универзитет за прехранбени технологии, Технолошки факултет, Катедра за месо и риба технологија, бул. „Марица“ 26, Пловдив, Бугарија

[2] Универзитет за прехранбени технологии, Технички факултет, Одделение за процеси и апарати, бул. „Марица“ 26, Пловдив, Бугарија

[3] Универзитет „Гоце Делчев“, Земјоделски факултет, Одделение за прехранбена технологија и преработка на производи за животни, „Крсте Мисирков“ 10-А, Штип, Македонија

**Контакт автор: logos2000lt@gmail.com*

Резиме

Во текот на последната деценија коњското месо отиде подлабоко во видното поле на потрошувачите и научниците. Целта на оваа студија е да се идентификуваат специфичните карактеристики за време на стареењето на коњското месо. Промените во микроструктурата, морфологијата, протеинската автолиза, растворливите протеини, pH, капацитет за задржување на вода (КЗВ), загубата на вода и бојата беа испитувани кај m. Longissimusdorsi од коњ за време на 12 дена на на температура од 0 до 4°C. На 3 ден postmortem А и I-зони потешко се разликуваа. Беше забележано скратување на саркомерата. Периодот на постморталната вкочанетост (rigormortis) во коњското месо се јавува помеѓу 3 и 5 ден. Во овој период мускулните влакна се контрахирани, компонентата на црвената боја е намалена за 2-3 единици, pH и КЗВ се минимални - 5,80 и 13-14%, соодветно, и загубите на вода беа најмногу околу 20%. По 5 ден postmortem беа забележани поединечни пукнатини - показател за тековните автолитички процеси. Растворливоста на протеините се стабилизира на околу 1750 mg/ml. Зголемен дел од фракциите на протеините со молекуларна тежина од 28 до 23 KDa, сметано како индикатор за зголемена кршливост на месото беше пронајдена по 5 дена. Од 5 до 12 ден се откриени повисоки нивоа на α-актинин, дезмин и лесен меромиозин. По 7 ден postmortem деструктивните промени се продлабочуваат. Z-линии беа многу искинати. А- и I-дисковите тешко се разликуваа. Миофибрилите, исто така, беа многу фрагментирани и I-зоните не можеа лесно да се разликуваат.

Клучни зборови: *m. Longissimus dorsi, микроструктура, морфологија, протеинска автолиза, растворливи протеини, загуба на вода, боја.*