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Full Length Research Paper

Changes of biochemical and sensory characteristics in the musculus longissimus dorsi of the fallow deer in the early phase post-mortem and during maturation

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Our researches encompassed determination of biochemical, technological and sensory characteristics in the muscle of the fallow deer (*Cervus [Dama] dama* L.) in the early phase post-mortem and during maturation. In the early phase post-mortem, pH, glycogen, total amount of pigments (TP), plasticity and water-binding capability (WBC) were determined. During maturation, the following changes were followed: pH values, glycogen, WBC, plasticity, colors (sensory and instrumental measurement), the contents of alpha-amino nitrogen (NH_2-N) and non-protein nitrogen (NPN), together with the definition of sensory characteristics of texture and odor. By analyzing results, it was concluded that at 3 h post-mortem, low pH (5.49) was determined and low WBC (12.17 cm^2), which pointed to the fact that due to extreme tiring and fast decomposition of glycogen, the ultimate value of pH was reached, while glycolytic enzymes were almost deactivated. A slight increase of pH and WBC during the period of maturation could be explained as a consequence of proteolytic activity and changes in proteins, which at the same time provoked increase of alpha-amino nitrogen (NH_2-N) and non-protein nitrogen (NPC). Sensory analysis showed that the muscle of the fallow deer was most appropriate on the fifteenth day of maturation for both processing and culinary processing.

Key words: Biochemical changes, technological properties, sensory analysis of meat quality of the fallow deer.

INTRODUCTION

With meat or meat-products, consumers show a growing interest in some characteristics of the meat itself and of the system by which it has been produced. First, meat is requested to be safe, meaning that its composition will maintain man's health and that no artificial additive has been added to the animal diet or to the product. Furthermore, consumers are increasingly concerned about the animal welfare and environmental aspects of animal production systems (Volpelli et al., 2002, 2003).

The fallow deer (*Cervus [Dama] dama* L.) represents the most important game meat for consumption. Fallow deer are widely present in the world due to their good adaptability and ecological elasticity. Based on these characteristics, fallow deer have been bred in several European countries (Tešanović and Kovačević, 2003; Hoffman and Wiklund, 2006). As a wild species, fallow deer choose food freely in nature, move intensively, exposed to ecological influences and subjected to natural selection where only the fittest survive, thus their muscles are different than the muscles of domestic animals (Škrinjar and Tešanović, 2007; Tešanović, 2010). Quality traits are dependent on a number of factors: genotype, sex and age stand out among biological factors, as well as numerous non-genetic factors (Bogosavljevic-Boskovic et al., 2010).

Considering the hunting procedure, specific type of processing of carcasses and muscle characteristics of

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the species, it is expected that the post-mortem biochemical processes during the maturation in the muscle will be different than in the muscle of cattle for slaughter, and at the same time changes of muscle characteristics during muscle transformation -organ into meat – food (Tešanović and Škrinjar, 2004; Tešanović, 2010). After an animal's death, the supply of the muscle with oxygen stops. Anaerobic conditions take place, in which post-mortem biochemical processes begin, changing the properties of the muscle (Forrest et al., 1975; Rahelić, 1978; Lawrie, 1979; Pollard et al., 2002). Pavlovski (1965) points out that the course of decomposition of glycogen is different and that it depends on the species and breed, but that there are also differences within the same breeds.

Moreover, certain authors that have researched measure of glycogen and pH with game meat, pointed out that the fall of glycogen does not follow appropriate fall of pH (Šijački et al., 1990; Mojto et al., 1993; Mojto and Kartusek, 1995; Wiklund et al., 2010). Same authors have determined a low initial pH in muscles of the fallow deer, from 5.78 (5.52), which in the course of five day storage, changes a little. Hamm (1972) believes that a decrease of WBC of muscles post-mortem is conditioned 1/3 by the fall of pH, and 2/3 by the decomposition of ATP. Also, color represents one of the basic characteristics in the definition of the meat quality. Slight changes of the light can provoke greater changes of the color (MacDougall, 1982; Popov-Raljić, 1999). Petrovic et al. (1996) have determined that the color of the muscle with young beef changes during maturation within 42 days.

It has been observed that after the first phase of post-mortem processes in meat – rigor mortis phase, the second phase develops (meat maturation), in the course of which a decomposition of the structural elements of the muscular tissue evolves due to the influence of endogen enzymes, thereby giving meat desirable sensory characteristics. It is considered that connectin has a great role in tenderization of meat in the course of maturation (Peterson and Parrish, 1987; Bandman and Zdanis, 1988; Dransfield, 1994; Koohmaraie et al., 1987; Sentandreu et al., 2002; Volpelli et al., 2005). Nishimura et al. (1996) claim that proteoglycans bound to collagen perimysium and endomysium decompose during maturation and that that is the cause of loosening of the web of collagen fibers, leading to increase of the tenderness of meat during maturation.

The structure of the muscle of the fallow deer and post-mortem biochemical changes are linked to the condition and activity during lifetime, hence they are different from the muscles of domestic animals (Wiklund et al., 2002; Hoffman and Wiklund, 2006). For these reasons, these researches have a task to contribute to the research of certain post-mortem changes in the muscle of the fallow deer (*Cervus [Dama] dama* L.) and to determine at which period during maturation this type of meat has most desirable characteristics for processing and culinary processing.

MATERIALS AND METHODS

Muscles of musculus longissimus dorsi (LD) of the fallow deer (*C. [Dama] dama*), about three years old was used for the research, and it was determined in an organized selective hunt in the hunting area "Karadjordjevo" (The Western Balkans). From the twenty seven species found, nineteen corresponded to the targeted age group.

pH determination

Measuring of pH value was done by the mobile pH meter of the company Gronert Ultra X type TM-5, with reinforced combined electrode for direct measuring, Ingold pH Elektroden Industrie Geber, after 3, 8, 24 and 48 h post-mortem, as well as after 7, 15 and 30 days post-mortem.

Glycogen determination

The content of glycogen was determined by spectrophotometric measuring of intensity of formed green coloring with the antron at 620 nm.

Determination of water-binding and plasticity

The capability of water-binding (WBC) and plasticity were determined by the compression method according to Gran and Hamm (1953).

Determination of pigments

The total content of the pigments (TP) was determined according to Mohler's modification of Hornsaj's method (Rede and Rahelić, 1971).

Determination of non-protein nitrogen

The content of non-protein nitrogen (NPN) was determined from the homogenized granulated sample previously poured over by 10% trichloroacetic acid. The sample was filtered after that and in a clear filtrate the content of nitrogen was determined according to Kjeldahl, as well as the content of non-protein nitrogen which was calculated according to formula:

NPN (%) = (VHCL F - VNaOH) 0.14

Determination of alpha-amino nitrogen

The content of alpha-amino nitrogen (NH_2-N) was determined by formal titration, method according to Sorensen, modified by Petrov. The content of NH_2-N was calculated according to the formula:

NH_2-N (mg/100 g) = ml NaOH FNaOH 1.4100

Where 1 ml 0.1 mol/1 NaOH corresponds to 1.4 mg NH_2-N.

Determination of the color

The color of the sample was determined with the help of photo-

Table 1. Sensory evaluation of the color, texture and odor of the fallow deer (*Cervus [Dama] dama L.*)

Mark	Colour	Texture	Odour
1.0	very bright red	very tough	very weak
2.0	red	tough	weak
3.0	slightly dark red	moderate tough	moderate weak
4.0	moderate dark red	insufficiently fine	insufficient
5.0	dark red	moderate fine	moderate
6.0	very dark red	fine	prominent
7.0	black red	very fine	very prominent

electric tristimulus colorimeter MOM – color 100, Budapest. Results were expressed on the basis of CIE system (Pribiš and Rede, 1982) and CIE lab system (Robertson, 1977; Popov-Raljić, 1999).

Sensory analyses

Sensory validation of color, texture (Tešanović et al., 2010) and odor of raw samples of M. Longissimus dorsi of the fallow deer, was done by a group of five experienced degustators (ISO 8568 – 1, 1993; ISO 8586 – 2, 1994), (Table 1). The design of the sensory facility at University of Novi Sad is consistent with ISO guidelines and equipped with five individual booths and uniform fluorescent lighting.

Statistical analysis

Obtained databases were analyzed using computer program Statsoft, Inc. (2003) STATISTICA (data analysis software system), version 6. Obtained results were statistically processed and expressed as: average value (x); standard deviation (Sd); coefficient of variation (Cv).

RESULTS AND DISCUSSION

Content of pH, glycogen, WBC and plasticity changes

Changes of the pH, glycogen content, WBC and plasticity early post-mortem and during maturation of musculus longissimus dorsi of the fallow deer (Cervus Dama dama L.) are shown in Table 2. On the basis of obtained results, it could be noticed that at about 3 h post-mortem, pH of the muscle longissimus dorsi was very low (pH = 5.49), with the relatively high coefficient of variation of 2.60%, a great variation of individually measured values. After 48 h post-mortem, pH was stabilizing around slightly higher values (pH = 5.63), after which during maturation within 15 days it slightly increased (pH = 5.75) and then decreased slightly within 30 days of maturation, until it reached the initial value (pH = 5.48), with much less variations of individually measured values (Cv from 0.35 to 1.84%).

Results of determination of the changes of glycogen content in early post-mortem as well as in the course of maturation of M. longissimus dorsi of the fallow deer are shown in the Table 2. From the results, it can be seen that about 3 h after being hunted, the content of glycogen in the muscles LD was 359.0 mg/100 g on average and that later decreased, until 48 h post-mortem when it was 195.5 mg/100 g. After 7, 15 and 30 days, glycogen was found in the muscles, completely decomposed. In the same table, results of the determination of WBC early post-mortem and during maturation of musculus longissimus dorsi of the fallow deer, as well as the values for plasticity (cm^2) are shown.

It can be concluded that WBC in the course of maturation of muscle longissimus dorsi of the fallow deer extremely increased within the 7^{th} day of maturation (from 12.17 to 10.17 cm^2), and after the 15^{th} day it slightly decreased, so that after the 30^{th} day of maturation it decreased to the value (12.77 cm^2). At the same time the lowest plasticity was determined in the muscle at 8 h post-mortem (4.36 cm^2), increased within the 15^{th} day of maturation (5.23 cm^2) and then after the 30^{th} day of maturation it slightly dropped (5.12 cm^2). The results therefore show that (Table 2) the pH had increased within the 15th day of maturation, after which it slightly decreased, and the same pH value as the one measured at 3 h post-mortem was reached on the 30^{th} day of maturation due to the fact that in this period the content of glycogen had slightly decreased and pH had slightly increased. Since similar increase of pH during maturation was registered by other authors in the muscles of domestic animals, it can be concluded that that change of pH during maturation is the result of proteolytic activity and changes in proteins (Wiklund et al., 1995; Petrović et al., 1996) and that within 3 h post-mortem in the muscles of the fallow deer, pH value is already reached and the activity of glycolytic enzymes is mainly stopped.

Hofmann (1986) concludes that pH value influences the color, tenderness, odor, water-binding capability and maintenance of meat and therefore it is an important indicator, not only of the state (degree of development of biochemical processes), but of meat quality as well (Hofmann, 1986). pH changes were followed by the changes of WBC, which was confirmed by the correlative and regressive analysis of the mutual dependability of the changes of these two properties. Petrovic et al. (1996) concluded by examining WBC of the bovine muscles that it is 1 and 8 h post-mortem better than WBC of the muscles of the fallow deer, which is established by examinations at 3 and 8 h post-mortem, and that later in

Table 2. Content of pH, glycogen, WBC and plasticity changes in early post-mortem and during maturation of musculus longissimus dorsi of the fallow deer (*Cervus [Dama] dama L.*) (n=19).

Examined property		Time of post-mortem						
		Hour				Day		
		3	8	24	48	7	15	30
pH	x	5.49	5.58	5.58	5.63	5.68	5.78	5.48
	Sd	0.143	0.069	0.052	0.054	0.023	0.020	0.101
	Cv	2.60	1.26	0.93	0.96	0.40	0.35	1.84
Glycogen (mg/100)	x	359.0	322.4	281.5	195.5	-	-	-
	Sd	36.469	29.518	43.142	25.351	-	-	-
	Cv	10.16	9.16	15.33	12.97	-	-	-
WBC (cm)	x	12.17	12.61	11.17	11.37	10.17	10.22	12.77
	Sd	2.411	1.530	1.811	1.651	0.673	1.638	2.218
	Cv	19.81	12.13	16.21	14.52	6.62	16.03	17.37
Plasticity (cm^2)	x	5.01	4.36	5.03	5.02	5.04	5.23	5.12
	Sd	0.946	0.250	0.648	0.332	0.256	0.334	0.497
	Cv	18.88	5.73	12.88	6.61	5.08	6.39	9.71

x, Average; Sd, standard deviation, Cv, index of variation.

the course of maturation very similar value of WBC is determined. This actually indicates that in the muscle of the fallow deer, as a consequence of the quick decrease of pH due to extreme tiring of the muscle and fast decomposition of glycogen immediately before hunting, fast decrease of WBC occurs, that is; the condition similar to BMV develops (Šijački et al., 1990; Petrović et al., 1996).

The content of pigments in total in the muscle of the fallow deer (n=5), (*C. Dama dama L.*) of 327.76 mg/100 g was determined. The standard deviation was (s = 34.47), and coefficient of the variation (Cv = 10.52). If the obtained results are compared to the findings of myoglobin (Mb) in the meat of other species of animals as mentioned by Lawrie (1996), this type of meat is categorized according to the content of pigments and at the same time according to color, after meat of beef and mutton (0.25% Mb), and it comes before horse meat (0.50 Mb). Therefore, it is categorized as very dark meat, which also confirmed the results of sensory analysis of the color of raw muscles longissimus dorsi, as well as instrumental determination of a variety of indicators of the color.

The changes of the content of non-protein nitrogen and alpha- amino nitrogen

Research results of the changes of the content of non-protein nitrogen (NPN) and alpha- amino nitrogen (NH_2N) in the muscle longissimus dorsi of the fallow deer during maturation, as well as basic parameters of the statistical processing of these results are shown in Table 4. As shown in the table, at the 1st day of maturation, the content of NPN in the muscle longissimus dorsi of the fallow deer was the lowest and it equaled 0.36%, then it increased permanently until the 30th day of maturation when it was 0.42% and Cv varied from 1.9% (30th day of maturation) to 4.75% (15th day of maturation). In the same table, results of the examination of changes of the contents of alpha-amino nitrogen NH_2-N in the muscle LD of the fallow deer during maturation are shown. From these data, it can be observed that at the 1st day of maturation it was found that muscles LD contained 128.82 mg/100 g NH_2-N, and that on the 15th day of maturation it increased (147.04 mg/100 g).On the 30th day of maturation in comparison to the 15th day, the content of NH_2-N in the muscle increased dramatically (180.60 mg/100 g).

The color of the sample

Since color is one of the basic characteristic of the meat quality, in Table 3 results of the color characteristics of the muscle longissimus dorsi of the fallow deer (*C. [Dama] dama L.*) are shown. They were determined by the instrumental method on the photoelectric tristimulus colorimeter MOM-color 100. Results were expressed in the CIE system through: the brightness or an average reflectance (%), dominant wave length (nm) and the color stability S (%), and in the CIE lab system on the basis of: psychometric light L*, psychometric tone a* and psychometric chrome b*.

Table 3. Some indicators of color changes of the raw samples of the muscle longissimus dorsi of the fallow deer during maturation (n=19).

Reading and calculated value			Time post-mortem (day)			
			1	7	15	30
	Brightness (%)	x	7.17	7.48	7.86	8.65
		Sd	1.791	1.427	1.406	1.780
		Cv	24.98	18.08	17.89	20.58
CIE	Dominant wave length (nm)	x	615.6	615.2	615.4	612.0
		Sd	0.548	0.447	0.548	7.842
		Cv	0.09	0.07	0.01	1.28
	Color purity P (%)	x	16.85	17.77	17.30	18.02
		Sd	1.281	1.064	1.304	2.312
		Cv	7.6	5.99	7.54	12.83
	Psychometric light. L	x	31.93	32.73	33.54	35.09
		Sd	3.835	2.993	3.025	3.642
		Cv	12.01	9.14	9.02	10.28
CIE Lab	Psychometric tone a	x	13.79	14.43	13.89	12.97
		Sd	0.848	0.791	0.372	2.026
		Cv	6.15	5.48	2.68	15.62
	Psychometric chrome b	x	5.90	6.74	6.89	7.63
		Sd	1.390	1.068	0.858	1.228
		Cv	23.56	15.85	12.45	16.09

x=average, Sd - standard deviation, Cv -index of variation.

Table 4. Changes of the content of NPN and NH_2-N in the muscle longissimus dorsi of the fallow deer during maturation (n = 19).

Indicator		Time of post-mortem (day)			
		1	7	15	30
NPN (%)	x	0.36	0.38	0.40	0.42
	Sd	0.013	0.011	0.019	0.008
	Cv	3.61	2.89	4.75	1.9
NH_2 N (mg/100)	x	128.82	142.57	147.04	180.60
	Sd	3.616	4.117	7.040	7.258
	Cv	2.81	2.89	4.79	4.02

x, Average, Sd, standard deviation, Cv, index of variation.

From the results obtained as shown in Table 3, muscles longissimus dorsi of the fallow deer were the darkest on the 1st day of maturation (y = 7.17%), and then in the course of maturation muscles became more brighter, especially in the period of time from 15 to 30 days of maturation (values of the average reflectance increased from 7.86 to 8.65), with the great variations of individually determined values (Cv from 24.98 to 17.89%). However, values of the dominant wave length (λ) of the muscle longissimus dorsi during maturation did not change until the 15th day (615.6, 615.2 and 615.4 nm, from 1st until 15th day, respectively), they were then reduced in the period from the 15th till the 30th day of maturation (to 612.0 nm), with a very slight variation of individually measured values that were in the range of calculated average values (Cv from 0.07 to 1.28%). More

Table 5. Results of the sensory analysis of color, odor and texture of the raw muscle longissimus dorsi of the fallow deer during maturation (n=19).

Characteristic		Time of post-mortem (day)			
		1	7	15	30
Color	x	5.60	5.40	4.6	4.6
	Sd	1.084	0.894	1.140	-
	Cv	19.36	16.55	24.78	-
Odor	x	1.4	2.9	4.77	6.65
	Sd	-	-	-	-
	Cv	-	-	-	-
Texture	x	6.30	6.10	6.40	6.50
	Sd	0.447	0.224	-	-
	Cv	7.10	3.67	-	-

x, Average; Sd, standard deviation; Cv, index of variation.

also, the purity of the muscle LD color during maturation had increased incessantly from the 1st day of maturation, when it was 16.85%, to the 30th day of maturation when it was 18.02% together with (Cv from 5.99 to 12.83%). Similar, but numerically different values were obtained also by determination of color characteristics in CIE lab system.

The sensory analysis

The sensory quality of venison has not been studied extensively (Hutchison et al., 2010). Results of the sensory analysis of the color, odor and texture of the muscle longissimus dorsi of the fallow deer during maturation are shown in Table 5. Results indicate that sensory color of the muscle of LD of the fallow deer, the 1st day of maturation was marked 5.60, as dark to very dark red. Results of the determination of the dominant wave length (Table 3), indicated that the value was (λ =615.6 nm) which based on the chrome diagram corresponded to the red-orange part of the spectrum. If results of the sensory analysis of muscle color of the fallow deer are compare to the color of bovine muscles in the research done by Petrovic et al. (1996), it can be concluded that muscles of the fallow deer are darker color than the bovine muscles, because the 1st day of maturation muscle longissimus dorsi of bovine was marked as moderate dark red (3.67); that is dark red (4.11 and 4.39 points). Mojto et al. (1993) determined by sensory analysis that in relation to other types of game meat, meat of the fallow deer was the darkest, and meat of the wild boar was the brightest. Comparatively, the color of the muscle of the fallow deer determined instrumentally and marked sensory on the 1st day of maturation was of extremely dark color, which meant that the development of BMV phenomenon did not take place in the muscle of the fallow deer, but that only the fast development of the full rigor mortis in the early phase post-mortem took place (Rede and Rahelić, 1971).

The fact that the presumed structural change took place, due to proteolytic activity during maturation, was also confirmed by the results of determination of content changes in NPN and NH_2-N of the muscle of the fallow deer during maturation (Table 4). These results undoubtedly showed that the contents of NPN and NH_2-N significantly increased during maturation, which had as a consequence the already mentioned changes of pH and changes of sensory characteristics of the raw muscle during maturation (Table 5). Consequently, apart from color changes, the odor of the raw meat of the fallow deer improved significantly during maturation, as well as the texture. This same conclusion confirms Wagner (1986), who stated that hydrolytic processes during maturation positively intensify aroma, while oxygenizing processes in the fatty tissues have a negative influence. Petrovic et al. (1996) also stated that the processes of maturation improve tenderness, aroma and the taste of meat, and that meat in the phase of complete maturity acquires optimal, above mentioned sensory characteristics.

Conclusion

In the examined muscles of the fallow deer, biochemical changes during maturation were determined which was closely linked to the activity of the muscle and the way of life before death. It was determined that:

1) pH of the muscle longissimus dorsi of the fallow deer at 3 h post-mortem was 5.49, gradually increased until the 15th day of maturation after which it slightly decreased, and that at the 30th day of maturation, the same pH value as well as the one measured at 3 h post-

mortem was determined.
2) In the muscle longissimus dorsi of the fallow deer the content of glycogen which equaled 359.00 mg/100 g was found at 3 h post-mortem, within 48 h post-mortem glycogen decomposed to the value of 195.5 mg/100 g, and that the at 7^{th} day post-mortem, glycogen was not present in the muscle any more.
3) During maturation of the muscle longissimus dorsi of the fallow deer, WBC increased until the 7^{th} day of maturation (from 12.17 to 10.17 cm^2), then until the 15^{th} day it decreased slightly, and the 30^{th} day it reached the value of 12.77 cm^2. At the same time the lowest plasticity was determined in the muscle at 8 h post-mortem (4.36 cm^2). After that plasticity had increased to the 15^{th} day of maturation (5.23 cm^2), and then the 30^{th} day of maturation it decreased (5.12 cm^2).
4) Muscle longissimus dorsi of the fallow deer on average contained 327.76 µg/g of the total amount of pigments.
5) The content of non-protein nitrogen (NPN) and alpha-amino nitrogen (NH_2-N) in the muscle longissimus dorsi of the fallow deer increased, so that the 1^{st} day of maturation the content of NPN amounted to 0.36%, and the 30^{th} day it was 0.42%. The content of NH_2-N of 128.82 mg/100 g, (1^{st} day of maturation) to 180.60 mg/100 g (30^{th} day of maturation) was determined.
6) By instrumental determination of a variety of indicators of the color of the muscle longissimus dorsi of the fallow deer, as well as by sensory analysis, it was determined that the at 1^{st} day of maturation muscles were very to very dark red, that they were in accordance to the dominant wave length (615.6 nm), reddish to orange color and that in the course of maturation they became brighter and the share of red color; psychometric tones (a) in them decreased, while share of yellow color of the psychometric chrome (b) increased.

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