PE4.34 Changes in myofibrillar proteins and fatty acid composition during production of "Uzice beef prshuta" – traditional dry-cured meat product 126.00

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***Abstract*—"Uzice Beef Prshuta" is traditional Serbian dry-cured meat product manufactured from the most valuable parts of beef carcass (round muscles, loin muscles and tenderloin). In this study electrophoretic changes in myofibrillar proteins (SDS-PAGE) and changes in fatty acid composition (gas chromatography) of *m. semitendinosus and m. longissimus lumborum et thoracis* were investigated during production of "Uzice Beef Prshuta" with application of two different salting procedures (dry salting with and without vacuum tumbling). Sampling was carried out at the following processing steps: green state; the end of salting; and the end of the production. The results obtained showed that myofibrillar proteins suffer significant proteolytic changes, irrespective of relatively short period of production (one month in this study), and from the other side there were no considerable changes of fatty acid composition during production of "Uzice Beef Prshuta".**

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***Index Terms*—fatty acids, gas chromatography, myofibrillar proteins, SDS-PAGE, "Uzice Beef Prshuta".**

I. INTRODUCTION

"Uzice Beef Prshuta" is traditional Serbian dry-cured meat product originating from the region of mountain Zlatibor (south-western part of Serbia) where it has been made for centuries. Traditional dry-cured meat products, according to their specific sensory properties, are often considered as specialties and appreciated by consumers. Among the numerous factors influencing the typical quality traits of those products, controlled degradative changes of tissue constituents, primarily of proteins and lipids, play a key role [1, 2, 3]. During the entire production, meat proteins are subjected to an intense degradation mostly by endogenous proteases, although the role of the present microorganisms must not be disregarded [2]. Intramuscular lipids are also subjected to an intense degradation, whereby lipolysis and oxidation distinguish themselves as the most important factors [2, 3, 4, 5]. Proteins and lipids are precursors of numerous volatile and non-volatile aroma compounds and thus they are essential in formation of sensory profile of matured product [1, 2, 3, 6].

The aim of this research was to study the changes in myofibrillar proteins and fatty acid composition of intramuscular lipid fraction under the impact of two salting procedures (dry salting with and without vacuum tumbling) during production of "Uzice Beef Prshuta".

II. MATERIALS AND METHODS

A. "Uzice Beef Prshuta" samples

Well fed, more than 36 months old domestic-Simmental type breed of cattle were used. Four samples of two different muscles were analyzed: two *m. semitendinosus* (**MS**) and two *m. longissimus lumborum et thoracis* (from cranial surface of 8.

thoracic vertebra to caudal surface of 6. *lumbal vertebra*; **ML**). After boning, cleaning of superficial fat and connective tissue, and shaping of muscles, pieces were rubbed with a 3.5 % (w/w) NaCl. One MS and one ML were immediately left in the salting room at 0–4 °C (traditional); other two samples were at first submitted to vacuum tumbling process for 20 hours (2 rpm with 70 % of vacuum at 0–4 °C; 10 minutes tumbling and 50 minutes resting) and then left in the same salting room. After salting process was completed, 12 days for dry salted and vacuum tumbled samples and 16 days for only dry salted samples, muscles were washed and submerged into water for 12 hours, in order to remove excess salt. The end of salting process is traditionally determined by cross section color observation. Salted muscles were subjected to smoking, drying and ripening processes (within 10 days).

Analysis of myofibrillar proteins, by determining relative molecular masses, was performed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), as described by [7], using vertical slab gels. Determination of the fatty acid compositions was conducted by means of gas chromatography (GC). Meat was sampled: at green state (raw meat); after salting; and at the end of the production (matured product).

B. Myofibrillar proteins extraction and SDS-PAGE

Sarcoplasmic fraction was removed by method of [8]: minced samples were homogenized in quintuple quantity of 0.05 M NaCl for 1 minute and centrifuged at 10000 G for 15 minutes. After decanting the supernatant, the precipitate was two times treated as described above. Myofibrillar proteins were isolated according to [9]: the precipitate was resuspended in 3.3 times greater volume of Guba Straus solution (0.15 M KH_2PO_4 : 0.3 M KCl = 1 : 1; pH 6.5 adjusted with KOH), mixed for 20 minutes and centrifuged at 5000 G; after decanting the supernatant, extraction was repeated two times more in the same way. The collected supernatant represents the extract of myofibrillar protein fraction. After determination of protein content [10], this extract was diluted with SDS buffer (35 ml distilled water; 2.5 ml Tris HCl buffer pH 6.8 (150 ml distilled water; 37.8 g Tris; pH 6.8 adjusted with 5 N HCl; and made to 200 ml with distilled water); 1 g SDS; 2.5 ml 2-mercaptoethanol; 5.8 ml 87 % glycerol; 5 g bromophenol blue; and made to 50 ml with distilled water) to the concentration of 1 mg (crude protein)/ml and then stored at –18 °C until PAGE.

The separation of myofibrillar proteins in an electric field, according to their molecular weight, was performed on vertical slab polyacrylamide gels (14.5 cm x 16.5 cm x 1.5 mm) in a vertical slab gel apparatus LKB 2001-001 (LKB – Pharmacia, Sweeden). After defrosting and 5 minutes boiling of samples, gels were loaded with 50 µl of a sample per slot. The molecular weight standard contained phosphorylase b (94 kDa), bovine serum albumin (67 kDa), ovalbumin (43 kDa), carbonic anhydrase (30 kDa), Kunitz trypsin inhibitor (20.1 kDa) and α-lactalbumin (14.4 kDa). SDS-PAGE conditions: in the first 30 minutes: I_{const} = 60 mA, U_{max} = 500 V, T = 12 °C; in the next 6 hours: I_{const} = 90 mA, U_{max} = 500 V, T = 12 °C. Protein bands were stained by immersing gels for 45 minutes in Coomassie blue R-250 solution (0,25 g Coomassie blue R-250; 250 ml methanol; 50 ml acetic acid; 200 ml distilled water). The gels were distained for several hours with a solution containing 10 % methanol and 7 % acetic acid in distilled water, then scanned and analyzed using SigmaGel^{tm} software (Version 1.1, Jandel Corporation, Germany).

C. Gas Chromatography

The lipid fractions of "Uzice Beef Prshuta" samples were extracted by the method of Soxhlet [11] and converted to methyl esters with methanol/KOH solution [12].

The qualitative GC analysis of fatty acid methyl esters was performed as described in the standard [13] using Varian 1400 equipment with metal GC column (300 cm x 0.32 cm) and flame ionization detector. The experimental conditions were as follows: Stationary phase: LAC-3R-728 (20%); Support: Chromosorb W/AW, 80-100 mesh; Mobile phase: nitrogen, 24 ml/min; Column temperature: 180 °C; Detector temperature: 200 °C; Injecting block temperature: 200 °C.

Fatty acid methil esters were identified by comparing their retention time with the standard samples. Quantification was done by normalization and peak integration using Varian Aerograph Model 20 integrator. Results were presented as a percentage of each fatty acid relative to total fatty acid.

III. RESULTS AND DISCUSSION

A. Electrophoretic changes in myofibrillar proteins

The results obtained are interpreted regarding to molecular weight intervals and shown in Table 1. The highest shear of polypeptide bands in raw samples was detected within a range of 50-100 kDa, and the following bands were distinguished as the dominant fractions: 39550 Da; 152400 Da, and 96470 Da. Round and loin muscles used in this experiment had been boned from already chilled carcasses, therefore it is

justifiable to expect that certain changes in structural proteins had been already executed [14]. Disappearance of troponin-T and simultaneous appearance of 28-32 kDa polypeptides, and appearance of a 95 kDa polypeptide, were reported as the most noticeable changes during post mortem storage of meat[14]. Our results are in accordance with the reports, since significant quantity of polypeptides within the range of 21-35 kDa were detected in raw samples, and among the dominant polypeptide bands the fraction of 96470 Da has distinguished itself.

Results obtained at the end of the salting stage compared with the green state, do not indicate clear pattern in quantity changes of detected molecular mass intervals regarding to all experimental groups. A noticeable reduction of 50-100 kDa fraction was more profound with MS than with ML samples. Also, an increase in 14-19 kDa and 38-47 kDa fractions was observed for all experimental groups. The share of 152400 Da band remained approx. the same as it was in raw samples. On the other side, concentrations of 96470 Da and 39550 Da polypeptides decreased in time. At the end of salting the following bands were distinguished as the dominant fractions: 55200 Da – amount increased comparing with raw samples; 16200 Da –not even detected in raw samples; and 14400 Da.

The stage of smoking/drying/ripening was carried out at relatively higher temperatures (up to 18 °C with max. allowed of 20 °C) which certainly stimulate the activity of endogenous proteolytic enzymes, especially cathepsins [2]. García et al. [15] reported a progressive increase in polypeptides of about 75, 70 and 65 kDa during production of "Cecina", traditional Spanish dry-cured beef product, especially after postsalting stage, i.e. when processing temperature is risen. Our results referring to the end of production show decreasing of 100-200 kDa and 14-19 kDa, as well as increasing of 38-47 kDa and 21-35 kDa fractions, while 50-100 kDa polypeptides were present in significant quantities. The following bands are distinguished as the dominant fractions at the end of this stage: 55200 Da; 23500 Da – amount increased comparing with results at the end of salting stage; and 17200 Da – not detected neither in raw samples nor at the end of salting.

B. Changes in fatty acid composition

Preparation of muscles for further processing of "Uzice Beef Prshuta" includes cleaning of superficial fat and connective tissue in detail. Consequently, total extracted lipid fraction, used for fatty acid composition determination, consisted of intramuscular lipids, which refer to lipids contained in both intramuscular adipose tissue and muscle fibers [3]. According to the data reported by [16], total lipid fraction extracted from raw ML shaped for further processing of "Uzice Beef Prshuta" is composed of 87.16 % of neutral lipids, 11.35 % of phospholipids, and 1.46 % of glucolipids, with no significant changes observed during production.

Method used in this study for lipid extraction was the Soxhlet method by which neutral lipids are being extracted to the greatest extent, while polar lipids, to which phospholipids belong, are being partly extracted [17]. That is why the results obtained (Table 2) in some cases are lower than it is expected and those which can be found in literature [18, 19].

In the fat of meat animals, only 3 or 4 acids are present in substantial amount: palmitic (C16:0), stearic (C18:0) and oleic (C18:1) [20, 21]. Our results for raw meat (Table 2) show that average sum values of those three fatty acids contents were: 82.2 % in MS, and 88.0 % in ML. Concerning all four experimental groups, there were no considerable changes in those values during processing, whereby all samples have shown decrease in palmitic and increase in oleic acid content, while changes in stearic acid were detected to a lower extent.

Among the others, meat quality is influenced by a presence of polyunsaturated fatty acids (PUFA), i.e. by the ratio of PUFA and monounsaturated fatty acids (MUFA). Vast majority of total fatty acids of beef intramuscular lipids are saturated (SFA) and MUFA [20, 21, 22, 23], and our results (Table 2) are in accordance with those reports: the ratio of SFA:MUFA:PUFA in raw MS is 1:0.94:0.05, while in raw ML is 1:0.73:0.03. During processing (Table 2) there were no considerable changes in fatty acid content within all four experimental groups, especially in PUFA.

IV. CONCLUSION

During production of "Uzice Beef Prshuta", myofibrillar proteins suffer significant proteolytic changes, irrespective of relatively short period of production for dry-cured meat products (one month in this study). From the other side, no considerable changes in fatty acid composition in intramuscular lipid fraction were observed under the tested conditions, regardless of the salting methods applied, which confirms that the production of this autochthonous meat product occurs in the conditions that largely prevent oxidative changes of present unsaturated fatty acids.

ACKNOWLEDGEMENT

The authors would like to thank to MP "Zlatibor", Cajetina, and to Mr. Borivoje Bestic, the former owner of the plant.

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Table 1
Polypeptide compositions of myofibrillar protein fractions extracted from "Uzice Beef Prshuta" samples at different stages of processing

Processing stages	Molecular mass intervals (kDa)	Share of a fraction in regard to total detected polypeptides (%)			
		"MS"-*m. semitendinosus*		"ML"-*m. longissimus lumborum et thoracis*	
		Dry salting and vacuum-tumbling	Dry salting	Dry salting and vacuum-tumbling	Dry salting
Raw meat	100-200	9.78	18.27	20.64	16.05
	50-100	41.14	40.78	37.56	31.62
	38-47	18.19	22.05	18.95	18.19
	21-35	14.91	10.27	10.94	20.94
	14-19	15.99	8.63	11.90	13.19
The end of salting stage	100-200	15.37	13.69	19.12	18.72
	50-100	24.74	26.26	30.71	27.34
	38-47	21.32	23.29	23.23	19.21
	21-35	17.07	14.36	6.94	6.53
	14-19	21.51	22.41	19.99	28.20
Matured product	100-200	3.64	7.99	13.02	14.46
	50-100	9.28	34.99	20.81	28.99
	38-47	50.34	33.09	30.89	28.63
	21-35	27.99	17.20	18.67	14.37
	14-19	8.74	6.73	16.61	13.55

Table 2
Fatty acid compositions of intramuscular lipid fractions extracted from "Uzice Beef Prshuta" samples at different stages of processing

Meat samples	Fatty acid	Fatty acid content. Share of total detected fatty acids (%)					
		Dry salting and vacuum-tumbling			Dry salting		
		raw meat	the end of salting	matured product	raw meat	the end of salting	matured product
MS	14:0	3.0	3.2	3.4	5.1	3.4	4.5
	14:1	0.9	1.2	1.4	1.8	1.2	0.9
	15:0	0.2	0.9	0.6	0.9	0.5	0.3
	15:1	traces	-	0.1	0.2	0.1	0.1
	16:0	34.8	33.6	28.1	34.9	34.0	33.2
	16:1	6.7	6.7	7.6	7.8	7.9	7.4
	17:0	0.9	0.9	1.4	0.9	0.7	0.6
	17:1	0.6	0.6	0.7	0.6	0.6	0.3
	18:0	9.8	11.0	10.4	9.6	8.9	9.2
	18:1	40.3	39.1	43.3	34.9	39.4	40.1
	18:2	1.7	1.9	2.2	2.4	2.5	2.6
	18:3 α	0.5	0.5	0.4	0.5	0.6	0.4
	20:0	0.1	-	-	-	traces	-
	20:1	0.3	0.4	0.2	0.3	0.2	0.3
	20:4	0.1	traces	0.1	-	traces	0.1
	SFA	48.8	49.6	43.9	51.4	47.5	47.8
	MUFA	48.8	48.0	53.3	45.6	49.4	49.1
	PUFA	2.3	2.4	2.7	2.9	3.1	3.1
ML	14:0	2.5	2.6	2.7	3.5	3.5	3.3
	14:1	0.6	0.7	0.7	0.6	0.6	0.3
	15:0	0.4	0.5	0.6	0.6	0.5	0.3
	15:1	0.1	-	0.1	traces	0.1	traces
	16:0	37.5	33.3	30.8	36.9	33.3	33.6
	16:1	3.9	4.8	5.1	3.9	4.7	4.5
	17:0	1.2	1.4	1.4	0.9	1.2	1.0
	17:1	0.8	1.0	0.8	0.6	0.6	0.5
	18:0	13.6	13.4	13.0	16.1	18.1	16.4
	18:1	37.3	40.4	42.7	34.6	35.6	37.9
	18:2	1.4	1.4	1.3	1.2	1.3	1.1
	18:3 α	0.3	0.3	0.3	0.6	0.5	0.6
	20:0	traces	-	0.1	traces	-	-
	20:1	0.3	0.2	0.3	0.2	0.1	0.2
	20:4	0.04	-	0.1	0.1	0.1	0.1
	SFA	55.2	51.2	48.6	58.0	56.6	54.6
	MUFA	43.0	47.1	49.7	39.9	41.7	43.4
	PUFA	1.7	1.7	1.7	1.9	1.9	1.8

MS: *m. semitendinosus*; ML: *m. longissimus lumborum et thoracis*; SFA: saturated fatty acids; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids.