Differences in biogenic amines content of dry-fermented sausage *Petrovská klobása* produced in traditional manner from hot deboned and cold meat

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Abstract— **Contents (mg/kg dm) of nine biogenic amines were determined in the raw sausage mixture and at the end of drying period of *Petrovská klobása*, produced from hot deboned and cold meat.**

Analyses of biogenic amines were performed by HPLC-DAD. Chromatographic separation derivatizated amines were completed in 8 minutes.

Content of tryptamine, phenilethylamine and spermin was significantly (*P*<0.05) lower in sausage mixture made of hot deboned meat (24.3; 33.8; 55.3, respectively) than in the sausage mixture made of cold meat (53.7; 53.9; 62.9, respectively). Also, content of tryptamine and spermine was significantly (*P*<0.05) lower in sausages made of hot deboned meat (21.1; 59.3, respectively) in comparison to sausages made of cold meat (52.4; 139, respectively). On contrary, content of phenilethylamine was significantly (*P*<0.05) higher in sausages made of hot deboned meat (73.8) than in sausages made of cold meat (39.4).

During the ripening process, content of phenilethylamine significantly (*P*<0.05) increased in sausages made of hot deboned meat, while in sausages made of cold meat this content significantly (*P*<0.05) decreased and content of spermin significantly (*P*<0.05) increased.

Putrescin (7.93) was detected only in sausages produced of hot deboned meat, while tyramin (9.50) was detected only in sausages produced of cold meat. Cadaverine, histamine, serotonin and spermidin were not detected in any mixture or sausage sample.

Total content of biogenic amines was significantly (*P*<0.05) lower in sausage mixture made of hot deboned meat in comparison with sausage mixture made of cold meat. During ripening, content of total biogenic amines significantly (*P*<0.05) increase in both groups of sausages.

Keywords— **traditional fermented sausage (*Petrovská klobása)*, biogenic amines**

I. INTRODUCTION

Traditional dry fermented sausage *Petrovská klobása* has been produced in the area nearby town of Bački Petrovac in the Autonomous Province of Vojvodina, Republic of Serbia. Production is carrying out in small household enterprises during December, when temperatures are around 0 ^{o}C and lower, using traditional methods. Ripened sausage is characterized by specific hot taste, aromatic and spicy flavor, dark red color and hard consistency [1, 2, 3]. Due to its specific and recognizable characteristics, this product has been protected with designation of origin (PDO) according to the Serbian legislation.

In contrary to industrial production of fermented sausages, traditional production of *Petrovská klobása* does not include the use of starter cultures and additives (nitrate, nitrite, glucono delta-lactone, etc.) Fermentation and ripening processes, as well as final quality of the product, are influenced by indigenous microflora of raw materials and environment. During ripening process, activity of present microflora causes decarboxylation of amino acids and the formation of biogenic amines [4, 5, 6].

Biogenic amines are organic bases with aliphatic (putrescine, cadaverine, spermine, spermidine), aromatic (tyramine, phenylethylamine) or heterocyclic (histamine, tryptamine) structures [5]. High levels of these compound present in foods may cause migraine, headache, gastric and intestinal problems and pseudo allergic responses [4, 7]. Also, they can be a useful index of spoilage or ripening stage [8, 9]. So, it is important to monitor them.

Traditional fermented sausages produced in European countries are characterized by large variations in biogenic amine content and composition [10, 11], while according to available literature, there is no data concerning biogenic amines content in traditional dry fermented sausages from Serbia. Large

variations are explained by complex interaction of factors that determine aminogenic activity and formation of biogenic amines [10, 12].

Traditionally *Petrovská klobása* is made from hot deboned meat. As in recent years food safety is an increasingly important public health issue and some technological disadvantages of hot deboned meat processing are evident, producers has to make slight changes in tradition to meet the requirements.

The aim of this paper was to determine the content of nine biogenic amines in *Petrovská klobása* mixture and at the end of drying period, with regard to raw material (hot deboned meat or cold meat).

II. MATERIAL AND METHOD

A. Material

Sausages used in this study were produsedfrom hot deboned (HD) and cold (C) pork meat and fat, red hot paprika powder, salt, crushed garlic, caraway and sugar according to traditional procedure.

Both mixtures were stuffed into pig natural casings and processed to smoking, drying and ripening in traditional room.

Analyses were performed on the mixtures (HD_0 and C_0) before stuffing and at the end of drying process at 90. day (HD_{90} and C_{90}).

Homogenized samples were stored at – 20 °C until analysed.

B. Method

Sample preparation and extraction were done according to Eerola et al. [13]. Briefly, 2 g of each sample were weight and put into test tube. Appropriate amount of internal standard was added and sample was homogenized in 10 ml 0.4 M perchloric acid using Ultraturrax blender. The homogenate was centrifuged for 10 min at 3000 rpm, and supernatant was rinsed into 25 mL bottle through filter paper. Extraction was repeated with 10 mL 0.4M perchloric acid solution, mixed with Vortex and centrifuged as before. Supernatants were combined and adjust to 25 mL with 0.4M perchloric acid.

1 mL of sample extract was made alkaline by adding 200 µL 2M NaOH and buffered by adding 300 µL saturated $NaHCO_3$. 2 mL of dansyl chloride solution was added and reaction mixture was transferred to incubation on 40 ^oC for 45 minutes. Residual dansyl chloride was removed by adding 100 µL ammonia. After 30 minutes mixture was adjusted to 5 mL with acetonitrile and filtered through 0.45 µm filter.

HPLC analysis was performed by using a liquid chromatograph (Agilent 1200 series), equipped with a diode array detector (DAD), Chemstation Software (Agilent Technologies, USA), a binary pump, an online vacuum degasser, an autosampler and a thermostated column compartment, on an Agilent, Eclipse XDB-C18, 1.8 µm, 4.6 x 50 mm column.

Solvent gradient was performed by varying the proportion of solvent A (acetonitrile) to solvent B (water) as follows: initial 50% B; linear gradient to 10% B in 7.6 min, 10% B to 10 minutes; linear gradient to 50% B in 2 min. System was equilibrated 3 minutes before next analysis. Flow rate was 1.5 mL/min and column temperature was 40 ^oC. 5 µL of sample was injected. The spectra were acquired in the range 190-400 nm (Tasić et al, in press).

Detection limits of the amines were determined to be 0.1 µg/g for putrescine and spermidine, 0.17 µg/g for cadaverine and tyramin, 0.25 µg/g for tryptamine, phenylethylamine and histamine and 0.5 µg/g for serotonin and spermine.

One way (ANOVA), Post-hoc (Duncan test) was performed using the software package Statistica 9.1 for Windows, Stat Soft, Tulsa, Oklahoma, USA. Differences were considered significant at $p < 0.05$.

III. RESULTS AND DISCUSION

Biogenic amines in traditional fermented sausage *Petrovská klobása* were determined using HPLC method for analysis. Chromatographic separation of derivatizated amines was completed in 8 minutes, and all the amines were well separated.

Content of nine biogenic amines and total content of biogenic amines (mg/kg dm) in the mixtures and in the traditional dry fermented sausages, produced from hot deboned (HD) and cold meat (C) at the end of drying period, are shown in Table 1 and Fig. 1, respsctively.

Content of tryptamine, phenilethylamine and spermin was significantly (P<0.05) lower in sausage

mixture made from hot deboned meat (24.3; 33.8; 55.3, respectively) than in the sausage mixture made from cold meat (53.7; 53.9; 62.9, respectively). Also, content of tryptamine and spermine was significantly (P<0.05) lower in sausages made from hot deboned meat (21.1; 59.3, respectively) in comparison to sausages made from cold meat (52.4; 139, respectively).

On contrary, content of phenilethylamine was significantly (P<0.05) higher in sausages made from hot deboned meat (73.8) than in sausages made from cold meat (39.4). During the ripening process, content of phenilethylamine significantly (P<0.05) increases in sausages made from hot deboned meat, while in sausages made from cold meat this content significantly (P<0.05) decreases.

Putrescin (7.93) was detected only in sausages produced from hot deboned meat (HD_{90}), while tyramin (9.50) was detected only in sausages produced from cold meat (C_{90}).

Table 1 Content of biogenic amines in the mixtures and in the traditional dry fermented sausages, produced from hot deboned (HD) and cold meat (C) at the end of drying period

Biogenic amines (mg/kg dm)	HD_0	C_0	HD_{90}	C_{90}
	0^{th} day		90^{th} day	
tryptamine	24.3[a]	53.7[b]	21.1[a]	52.4[b]
phenetamin	33.8[a]	53.9[c]	73.8[d]	39.4[b]
putrescine	ND[a]	ND[a]	7.93[b]	ND[a]
cadaverine	ND	ND	ND	ND
histamine	ND	ND	ND	ND
serotonin	ND	ND	ND	ND
tyramin	ND[a]	ND[a]	ND[a]	9.50[b]
spermidin	ND	ND	ND	ND
spermin	55.3[a]	62.9[b]	59.3[a,b]	139[c]

Cadaverine, histamine, serotonin and spermidin were not detected in any mixture or sausage sample. The fact that histamine was not detected should be pointed out, since histamine is the most important biogenic amine from toxicology point of view [4, 6, 7, 11]. and the only biogenic amine with content subjected to a legal regulation in Europe [15] with maximum of 100 mg/kg in some fish species and 200 mg/kg in fishery products [14].

Total content of biogenic amines was significantly (P<0.05) lower in sausage mixture made of hot deboned meat (113) in comparison with sausage mixture made of cold meat (170). During ripening, content of total biogenic amines significantly (P<0.05) increase in both groups of sausages.

Higher content of biogenic amines in C mixture could be explained by different time (24 or 2 h pm) of endogenous peptidases activity that is mainly active during this stage [16, 17].

Obtained differences in biogenic amines content could be explained by development of different microflora in sausages produced from hot deboned meat or cold meat, as well as by complex interaction of factors that determine aminogenic activity and formation of biogenic amines.

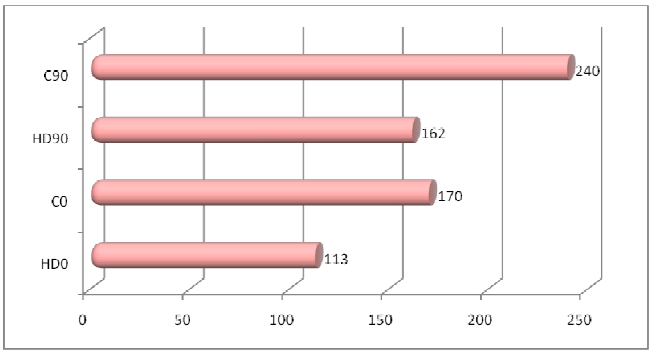

Fig. 1 Total biogenic amines content (mg/kg dm) in the mixtures and in the traditional dry fermented sausages, produced from hot deboned (HD) and cold meat (C) at the end of drying period

IV. CONCLUSIONS

Tryptamine, phenilethylamine and spermin were determined in all analised samples, while cadaverine, histamine, serotonin and spermidin were not detected in any mixture or sausage sample.

Putrescin was detected only in sausages produced from hot deboned meat and tyramin only in sausages produced from cold meat.

Total content of biogenic amines significantly increased in both groups of sausages during ripening and it was significantly lower in sausages made of hot deboned meat.

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