FORMATION OF HISTAMINE, TRYPTAMINE, PHENYLETHYLAMINE AND TYRAMINE IN *PETROVSKÁ KLOBÁSA*, PRODUCED FROM HOT DEBONED AND COLD MEAT, DURING DRYING PERIOD

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Abstract – *Petrovská klobása* is a traditional dry fermented sausage from Province of Vojvodina (Northern Serbia). Formation of dansyl chloride derivatizated histamine, tryptamine, phenylethylamine and tyramine in dry fermented sausage *Petrovská klobása* produced from hot deboned and cold meat, were determined using HPLC–DAD on Eclipse XDB–C18 column. At the end of drying (A1 14.7 and B1 38.1) and ripening period (A1 133 and B1 120) tryptamine was the prevaling amine in both groups, except in group produced from hot deboned meat, where phenilyethylamine was the prevailing vasoactive amine (51.6 mg/kg). Histamine was not detected in any of the analyzed samples. Sum of vasoactive amines during whole production period did not exceed sum proposed (200 mg/kg) as possible indicator of hygienic conditions and GMP in the sausage production.

Key Words – Traditional dry fermented sausage, Vasoactive amines

- INTRODUCTION

Petrovská klobása is a traditional dry fermented sausage which is produced in the area nearby town of Bački Petrovac in the Autonomous Province of Vojvodina, Republic of Serbia. Due to its specific and recognizable characteristics, this product has been protected with designation of origin (PDO) according to the Serbian legislation. Traditionally, homemade *Petrovská klobása* is produced exclusively from pork meat and fat, red hot paprika powder, salt, crushed garlic, caraway and sugar. Ripened sausage is characterized by specific hot taste, aromatic and spicy flavor, dark red color and hard consistency [1, 2, 3].

During ripening process, activity of present microflora causes decarboxylation of amino acids and formation of biogenic and vasoactive amines. Thus, dry fermented sausages are rich in these compounds due to high amount of proteins [4, 5, 6, 7, 8].
The vasoactive amines (histamin, triptamin, feniletilamin and tiramin) are biologicaly active amines which possess an important physiological role in human due to its vasoactive and psychoactive properties. Also, these compounds represent a food poisoning hazard since intake of foods with their high concentration may cause a chemical intoxication [4, 8, 9, 10, 11].

Eerola et al., [6] proposed that sum of vasoactive biogenic amines (tyramine, histamine, tryptamine, phenylethylamine) as a possible indicator of hygienic conditions and good manufacturing practice (GMP) in the sausage production, which should not exceed 200 mg/kg.

The aim of this paper was to determine the formation of histamine, tryptamine, phenylethylamine and tyramine in *Petrovská klobása* produced from hot deboned or cold meat during drying period and to estimate hygienic conditions and GMP according to proposed

criteria.

- MATERIALS AND METHODS

Sausage samples
Two groups of sausages, produced from hot deboned (A1) and cold meat (B1), were examined in this study. Traditionally, *Petrovská klobása* is produced from hot deboned meat, but as in recent years food safety is an increasingly important public health issue, as well as because of some technological disadvantages connected to use of hot deboned meat, producers has to make slight changes in traditional practice in order to meet the requirements. Both group of sausages were produced in traditional manner. Minced lean pork meat 80% and fat 20 % were mixed with red hot paprika powder (2.50 %), salt (1.80 %), crushed garlic (0.20 %), caraway (0.20 %) and sugar (0.15 %). The mixture was stuffed into natural casings, pig intestine (rectum), and raw sausages were smoked using cool procedure for 10 days with pauses [1]. At the end of drying period (90th day) sausages were vacuum packed and ripened at 10^{o}C untill 120th day [2, 3].

All analyzes were performed on three sample sausages from each batch, in duplicate.

Biogenic amines determination
Tryptamine, phenylethylamine, histamine, and tyramin were determined following the high-performance liquid chromatography. Sample extraction and derivatization were done according to Eerola et al. [12]. HPLC analysis was performed by using a liquid chromatography (Agilent 1200 series), equipped with a diode array detector (DAD), Chemstation Software (Agilent Technologies), a binary pump, an online vacuum degasser, an auto sampler 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) and solvent B (water). Flow rate was 1.5 mL/min., column temperature was 40 °C and 5 μL of sample was injected (13).

Statistical analysis
Statistical analysis in biogenic amines content between groups of sausages (A1 and B1) were done at the end of drying and at the end of ripening period. One way (ANOVA), Post-hoc (Duncan test) was performed. Differences were considered significant at $P < 0.05$.

- RESULTS AND DISCUSSION

Formation of tryptamine, phenylethylamine and tyramine during drying period is shown in Fig . 1. Tryptamine content in samples varied from not detected (B1) to 133 mg/kg, with concentration of 14.7 (A1) and 38.1 (B1) mg/kg at the end of drying and of 120 (B1) and 133 (A1) mg/kg at the end of ripening, when tryptamine was the prevailing biogenic amine in both groups of sausages. The differences between both groups at the end of drying, as well as at the end of ripening were significant (P<0,05).

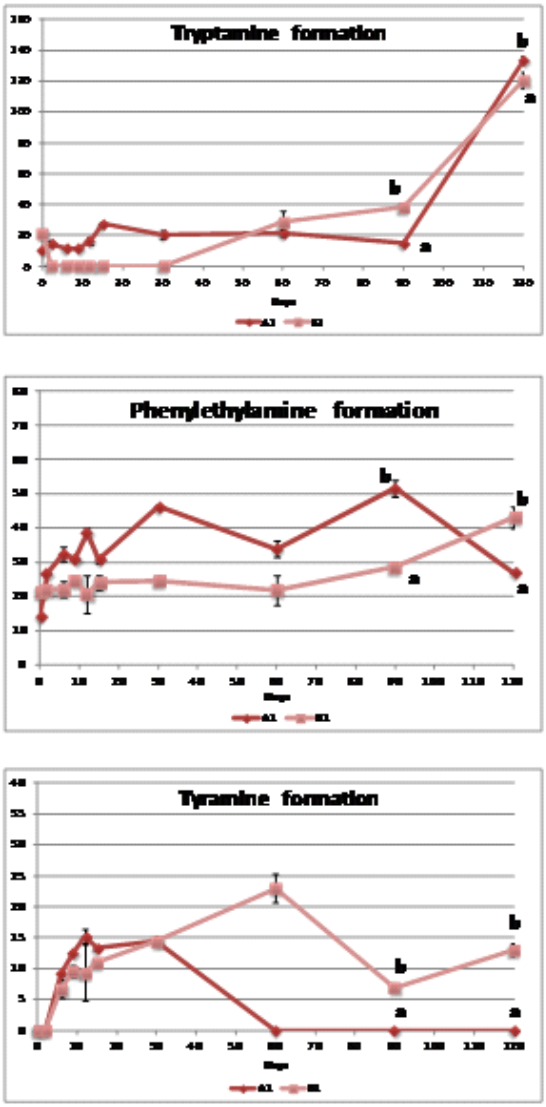

Figure 1. Formation of tryptamine, phenylethylamine, and tyramine (mg/kg) in sausages produced from hot deboned and cold meat during drying and ripening

Phenylethylamine content in samples varied from 14,1 (A1) to 51,6 (A1), with concentration of 28.6 (B1) and 51.6 (A1) mg/kg at the end of drying and of 27.0 (A1) and 43.1 (B1) mg/kg at the end of ripening. The differences between both groups at the end of drying, as well as at the end of ripening were significant (P<0,05).
Tyramine content in samples varied from not detected (A1, B1) to 23.0 (B1), with concentration of not detected (A1) and 6.90 (B1) mg/kg at the end of drying and of not detected (A1) and 13.1 (B1) mg/kg at the end of ripening. The differences between both groups at the end of drying, as well as at the end of ripening were significant (P<0,05).

Tyramine, reported as the most abundant amine in fermented sausages [7, 8, 10, 14, 15, 16, 17], at the end of drying and at the end of ripening period was determined in sausages produced from cold meat in concentrations less than 15 mg/kg, while in sausages produced from hot deboned meat this amine was not even detected.
Unlike most of the other fermented sausages, in *Petrovská klobása* tryptamine was the prevailing vasoactive amine in both groups of sausges at the end of drying and at the end of ripening period, except in sausages produced from hot deponed meat (A1) at the end of drying where phenylethylamine was the prevailing vasoactive amine. Traditionally, *Petrovská*

klobása is made of hot deboned meat, but as in recent years food safety is an increasingly important public health issue, as well as because of some technological disadvantages of hot deboned meat processing, producers have to make slight changes in tradition to meet the requirements [13].

Histamine was not detected in any of the analyzed samples (Table 1). Since histamine is well known for its toxity, the absence of this vasoactive biogenic amine is very important from toxicological and food safety point of view.

Table 1. Histamine content in sausages produced from hot deboned and cold meat during drying and ripening

	Histamine									
	0	2	6	9	12	15	30	60	90	120
A1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

Sum of vasoactive amines in samples varied from 21.8 (B1) to 176 (B1), with concentration of 66.4 (A1) and 73.6 (B1) mg/kg at the end of drying and of 160 (A1) and 177 (B1) mg/kg at the end of ripening. The differences between both groups at the end of drying, as well as at the end of ripening were significant ($P<0,05$).

Based on results obtained in this study, regarding vasoactive amines as food safety parameter, it is obvious that both, hot deboned meat and cold meat could be used for sausage production. Formation and changes in the type and content of vasoactive amines can be different even final product is produced from the same raw material, thus raw materials used in this experiment, hot deboned and cold meat, certainly took an important role and had impact on the dynamics of proteolysis and vasoactive amines formation.

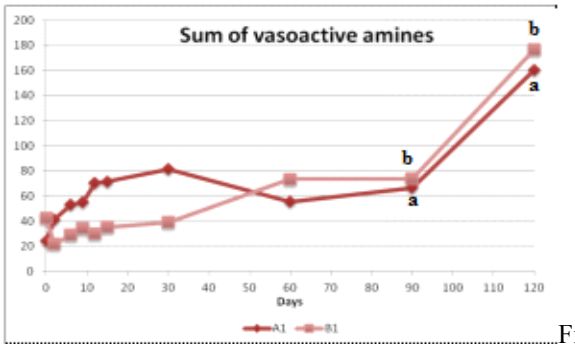

Figure 2. Formation of vasoactive amines (mg/kg) in sausages produced from hot deboned and cold meat during drying and ripening

The sum of vasoactive amines during whole drying period of 90. days were around and lower than 80 mg/kg in both examined groups, after which increased more than two times by the end of ripening period. Although the sum of vasoactive amines has increased since the end of drying (90. day) to the end of ripening period (120. day), during whole production period these values did not exceed 200 mg/kg proposed by Eerola et al. [6] as a possible indicator of hygienic conditions and GMP in the sausage production.

- CONCLUSION

Tryptamine was the prevailing vasoactive amine in both examined groups of sausages at the end of drying and at the end of ripening period, except in sausages produced from hot deboned meat.

Histamine was not detected in any of the analyzed samples which is very important from toxicological and food safety point of view.

Sum of vasoactive amines during whole production period did not exceed sum proposed (200 mg/kg) as possible indicator of hygienic conditions and GMP in the sausage production.

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