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Formation of Vasoactive Amines in Dry Fermented Sausage Petrovská Klobása during Drying and Ripening in Traditional and Industrial Conditions

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***Abstract*—**Formation of histamine, tryptamine, phenylethylamine and tyramine (vasoactive amines) in dry fermented sausage *Petrovská klobása* during drying and ripening in traditional room (B1) and industrial ripening chamber (B3) were investigated. Dansyl chloride derivatized vasoactive amines were determined using HPLC-DAD on Eclipse XDB-C18 column.

Histamine, the most important amine from food safety point of view, was not detected in any analyzed sample. Unlike most of the other fermented sausages, where tyramine is reported as the most abundant amine, in Petrovská klobása tryptamine was the most abundant vasoactive amine in both groups of sausages even though concentrations of tryptamine and tyramine in B3 sausages at the end of ripening were nearly the same (39.8 versus 39.6mg/kg). Sum of vasoactive amines in samples varied from not detected ND (B3) to 176 mg/kg (B1), with concentration of 36.1 (B3) and 73.6 (B1) mg/kg at the end of drying and 96 (B3) and 176 (B1) mg/kg at the end of ripening period. Although the sum of vasoactive amines has increased from the end of drying (45. and 90. day) to the end of ripening period (120. day), during whole production period these values did not exceed 200 mg/kg proposed as possible indicator of hygienic conditions and GMP in the sausage production.

***Keywords*—**Vasoactive amines, traditional dry fermented sausage *Petrovská klobása.*

I. Introduction

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 [1]-[5].

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 should not exceed 200mg/kg.

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The most important biogenic amine from toxicology point of view is histamine [1], [2], [7], [8]. It is a vasoactive substance and it can cause urticaria, hypotension, headache, flushing, abdominal cramps and other problems for human health [9]. Poisoning with histamine is a chemical intoxication resulting from ingestion of foods that contain high levels of histamine. This is the only biogenic amine whose content is subjected to legal regulation in Europe [10] with top limit of 100mg/kg in some fish species.

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 [11]-[13].

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 [1], [2], [6], [7], [14].

The aim of this study was to determine the formation of vasoactive amines in dry fermented sausage Petrovská klobása with regard to processing condition (traditional room or industrial ripening chamber).

II. Material and Methods

A. Material

Two groups of sausages dried and ripened in traditional (B1) and industrial (B3) conditions were examined in this study. Both groups of sausages were produced in traditional manner. Minced lean pork meat 80% and pork 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%). All ingredients were mixed approximately 10min using traditional technique. The mixture was stuffed into natural casings, pig intestine (rectum), around 40cm long and near 5cm in diameter. Raw sausages from B1 group were processed in traditional smoking/drying room, while sausages from B3 group were processed in industrial ripening chamber.

According to Serbian legislation [15] moisture content for dry fermented sausages have to be less than 35%. Sausages from B1 group dried in traditional room (temperature from 2.6°C to 12.4°C; relative humidity from 43.3% to 93.0%) needed 90 days to reach required moisture content, while sausages from B3 group dried in industrial ripening chamber (temperature of 10°C; relative humidity ~75%) reached this value after 45 days. At the end of drying period 90th day (B1) and 45th day (B3), sausages from both groups were ripened at 10°C, 75% RH further on until 120th day.

B. Methods

1. 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. [16]. 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, 1.8µm, 4.6 x 50mm column.

Solvent gradient was performed by varying the proportion of solvent A (acetonitrile) and solvent B (water). Flow rate was 1.5mL/min., column temperature was 40°C and 5µL of sample was injected [17].

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

2. Statistical Analysis

Statistical analysis in biogenic amines content between groups of sausages (B1 and B3) was 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 $P < 0.05$.

III. Results and Discussion

Histamine was not detected in any of the analyzed samples (Table I). 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 I
HISTAMINE CONTENT IN SAUSAGES DRIED AND RIPENED IN TRADITIONAL AND INDUSTRIAL CONDITIONS

	Histamine (mg/kg)									
	0	2	6	9	12	15	30	60	90	120
B1	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
B3	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

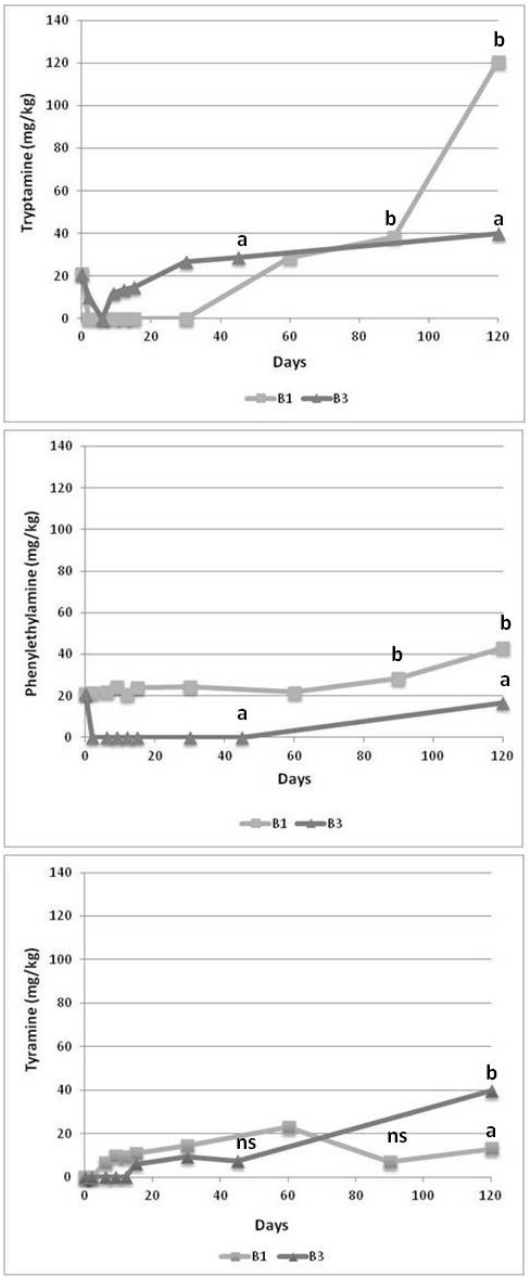

Fig. 1 Formation of tryptamine, phenylethylamine, and tyramine (mg/kg) in sausages dried and ripened in traditional and industrial conditions

Formation of tryptamine, phenylethylamine and tyramine during drying and ripening period is shown at Fig. 1. Tryptamine content in samples varied from not detected ND (B1, B3) to 121 mg/kg (B1), with concentration of 28.7 (B3) and 38.1 (B1) mg/kg at the end of drying and of 39.8 (B3) and 121 (B1) mg/kg at the end of ripening. The differences in tryptamine content between groups at the end of drying as well as at the end of ripening period were significant ($P<0.05$).

Phenylethylamine content in samples varied from not detected ND (B3) to 43.1 (B1), with concentration of ND (B3) and 28.6 (B1) mg/kg at the end of drying and of 16.6 (B3) and 43.1 (B1) mg/kg at the end of ripening. The differences in

phenylethylamine content between groups at the end of drying as well as at the end of ripening period were significant (P<0.05).

Tyramine content in samples varied from not detected ND (B1, B3) to 39.6 (B3), with concentration of 6.90 mg/kg (B1) and 7.34 mg/kg (B3) mg/kg at the end of drying and 13.1 mg/kg (B1) and 39.6 (B3) mg/kg at the end of ripening period. The differences between groups in tyramine content at the end of drying were not significant (P>0.05), while at the end of ripening period differences were significant (P<0.05).

Unlike most of the other fermented sausages, where tyramine is reported as the most abundant amine [4], [5], [7], [8], [18]-[20], in *Petrovská klobása* tryptamine was the most abundant vasoactive amine in both groups of sausges at the end of drying and at the end of ripening period, even though concentrations of tryptamine and tyramine in B3 sausages at the end of ripening were nearly the same (39.8 versus 39.6 mg/kg).

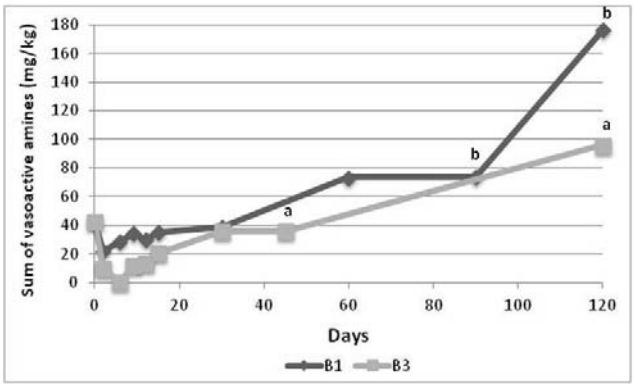

Fig. 2 Sum of Vasoactive Amines (Mg/Kg) In Sausages dried and ripened in traditional and industrial conditions

Sum of vasoactive amines in samples varied from not detected ND (B3) to 176 mg/kg (B1), with concentration of 36.1 (B3) and 73.6 (B1) mg/kg at the end of drying and of 96 (B3) and 176 (B1) mg/kg at the end of ripening period. The differences in the sum of vasoactive amines between groups at the end of drying, as well as at the end of ripening period were significant (P<0.05).

The sum of vasoactive amines during whole drying period of 45. days (B3) and 90. days (B1) was maximum 42.4 and 73.6 mg/kg, respectively. After this period sum of vasoactive amines increased more than two times by the end of ripening period. Although the sum of vasoactive amines has increased from the end of drying (45. and 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.

Based on results obtained in this study, regarding vasoactive amines as food safety parameter, it is obvious that sasuages could be dried and ripened in traditional, as well as in industrial conditions.

IV. Conclusion

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

Tryptamine was the prevailing vasoactive amine in sausages dried and ripened in traditional (B1) and industrial (B3) conditions at the end of drying and at the end of ripening period.

Sum of vasoactive amines during whole production period did not exceed proposed value of 200 mg/kg.

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