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Effect of commercial starter culture on physicochemical properties and biogenic amine formation in traditional dry-fermented beef sausage

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ABSTRACT

The present work aimed to investigate the effect of selected commercial starter culture on the physicochemical characteristics and formation and accumulation of biogenic amines (BAs) in dry-fermented beef sausage (of *Sjenički sudžuk* type) processed in controlled conditions during the summer production season. The results indicated a more intensive decrease in pH value in starter inoculated sausages (SC) compared to control (CO) ones, amounting 4.89 and 5.05 after 40 days of ripening, respectively. On the contrary, water activity (a_w) was found to be higher ($P < 0.05$) in SC sausages at the end of ripening. Two out of six analysed BAs (tryptamine, phenylethylamine) were not detected at any stage of the processing, while tyramine was the predominant amine in final products (CO – 101 mg/kg; SC – 123 mg/kg), regardless of the starter culture inoculation. Putrescine was the second most common amine in SC sausages (111 mg/kg), while cadaverine (58.1 mg/kg) and histamine (78.6 mg/kg) were detected only in CO sausages. This finding largely contributed to the fact that the total BA concentration was significantly higher ($P < 0.05$) in the control sausages (CO) than in inoculated sausages at the end of the production process (314 vs. 235 mg/kg). Based on the obtained results, it can be concluded that using selected starter culture in the production of dry-fermented beef sausage positively reduced the formation and accumulation of total BAs, being especially important when it comes to histamine.

1. Introduction

Traditional dry-fermented sausage, *Sjenički sudžuk*, is a meat product typical for an area around the small town of Sjenica (southwestern Serbia), and the peculiar characteristics of which arise from the combination of environmental/climatic conditions, usage of local ingredients, and manufacturing techniques specific for the Pešter plateau (approximately 1,000 m above sea level). Usually, this sausage is produced during the winter period (air temperature of approximately –1.30°C and relative humidity approximately 81%) following the original procedure and using just beef, sea salt, and spices (*Ikonić et al.*, 2019; 2023; *RHMZ*, 2023). Nevertheless, as a result of the high consumer demand, sudžuk is often produced out of the standard production season, when climate conditions are less proper for this type of manufacturing, i.e. during the summer period. Thus, the quality and safety of the "summer"

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sausages produced are questionable, primarily due to high levels of histamine and total biogenic amines (BAs) (*Ikonić et al.*, 2023; *Ruiz-Capillas & Herrero*, 2019). These anti-nutritional organic bases are very often found in fermented sausages due to the abundance of free amino acids (precursors of BAs) and decarboxylase-positive bacteria and are considered to have multiple adverse effects on human health (*Dominguez et al*., 2016; *Kononiuk & Karwowska*, 2020). Therefore, the summertime production of *Sjenički sudžuk* in small/micro processing facilities should be carried out using decarboxylase-negative starter cultures, as well as appropriate thermo-hygrometric conditions (*Ikonić et al*., 2023).

Accordingly, the aim of this research was to investigate the effect of selected commercial starter culture on the formation and accumulation of BAs in dry-fermented beef sausage (of the *Sjenički sudžuk* type) processed in controlled conditions during the summer period. Physicochemical characteristics were also followed up in order to help with the interpretation of the obtained results.

2. Materials and methods

2.1. Preparation and sampling of sausages

Sausage samples were made in the meat processing pilot plant at the Institute of Food Technology in Novi Sad according to the traditional procedure, with minor modifications, in June 2021. Fresh boneless beef (approximately 85% l ean) was salted using 32 g/kg of a mixture containing sea salt and nitrite salt (1:1) and maintained at 4°C for 7 days (pre-ripening phase). Afterward, salted beef was minced to an end particle size of approx. 4 mm and mixed with the seasonings (raw garlic paste — 4 g/kg, black pepper — 3 g/kg, red sweet paprika powder — 2 g/kg), until a homogeneous composition was achieved. Half of the obtained mixture was inoculated with 20 mg/100 g of starter culture (≥ 1.2×10^{10} cfu/g; M-CULTURE® FA 103 SSP-100, M FOOD GROUP GmbH, DE) containing *Lactobacillus curvatus, Staphylococcus carnosus,* and *Lactobacillus sakei*. Furthermore, prepared mixtures, control (CO), and starter culture inoculated (SC) were stuffed into natural casings (diameter of ~40 mm and a length of ~50 cm), forming a horseshoe shape. Raw sausages were subjected to controlled thermo-hygrometric conditions (Fig. 1) in a smoking, drying, and ripening chamber for 40 days until the required moisture content (<35%) was achieved (*Serbian Regulation*, 2023).

Samples were taken before stuffing (0) and during processing (after 3, 7, 15, 23, and 40 days). Three randomly selected sausages from each batch were taken for physicochemical analyses on each sampling day. The remaining sausages were homogenized, vacuum packed and stored at –20°C for further analyses. Analyses for all samples were carried out in duplicate.

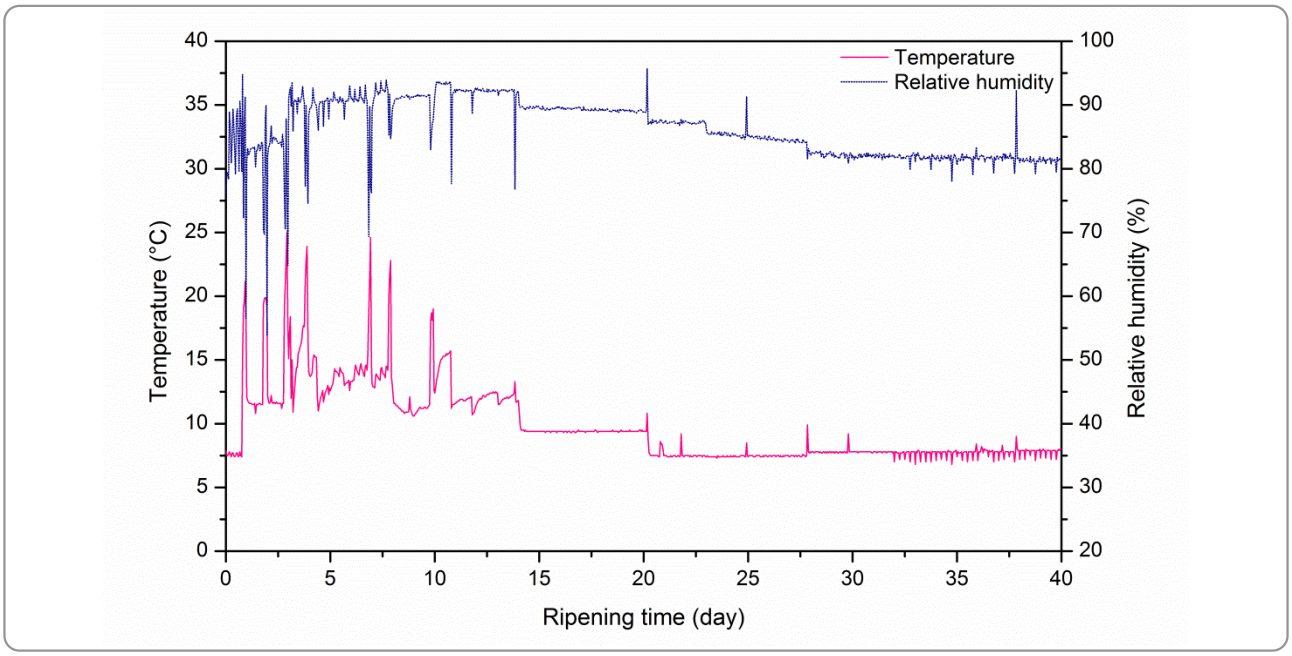

Figure 1. Thermo-hygrometric conditions recorded throughout the manufacturing of dry-fermented beef sausage

2.2. Physicochemical analyses

Sausage pH was measured using a digital pH meter Testo 205 (Testo SE & Co. KGaA, Titisee-Neustadt, DE), equipped with a combined penetration tip with the temperature probe. Water activity (a_w) determination was carried out using a LabSwift-aw measuring instrument (Novasina AG, Lachen, CH).

2.3. Biogenic amines (BAs) analysis

Determination of six dietary BAs, i.e. tryptamine, phenylethylamine, putrescine, cadaverine, histamine, and tyramine, was done according to the procedure described by *Tasić et al.* (2012). BA concentrations are expressed as mg/kg of sample.

2.4. Statistical analyses

Factorial (two-way) analysis of variance (Statistica 14.0, TIBCO Software Inc., Palo Alto, CA, USA) was used to examine the effects of starter culture inoculation and ripening time on the detected variables. Duncan's post-hoc test was run to compare mean values. Differences were considered significant at $P < 0.05$.

3. Results and discussion

3.1. Effect of the starter culture on pH and water activity (a_w) values

The effect of starter culture on pH and a_w is presented in Fig. 2. After 7 days of ripening, the SC batch showed a pH value significantly ($P < 0.05$) lower than the one registered in CO, indicating a faster fermentation process and more intensive pH decline in starter inoculated sausage. However, the minimal pH values reached after 15 (SC – 4.81) and 23 days (CO – 4.84) were almost the same ($P > 0.05$). Similar results were reported by *Van Ba et al.* (2016) and *Ren et al.* (2022), who detected faster pH drops and lower pH values in all inoculated sausages. Consequent to proteolytic reactions, i.e., generation of several low molecular weight compounds (peptides, amino acids, ammonia, amines, etc.), final pH values in both batches were significantly higher ($P < 0.05$) than previously detected minimal values (*Ikonić et al.*, 2023; Rocchetti *et al.*, 2021).

Due to the drying process, the a_w value decreased continuously up to the end of the ripening (40 days) in both CO and SC batches, finally amounting 0.86 and 0.89, respectively. Hence, the

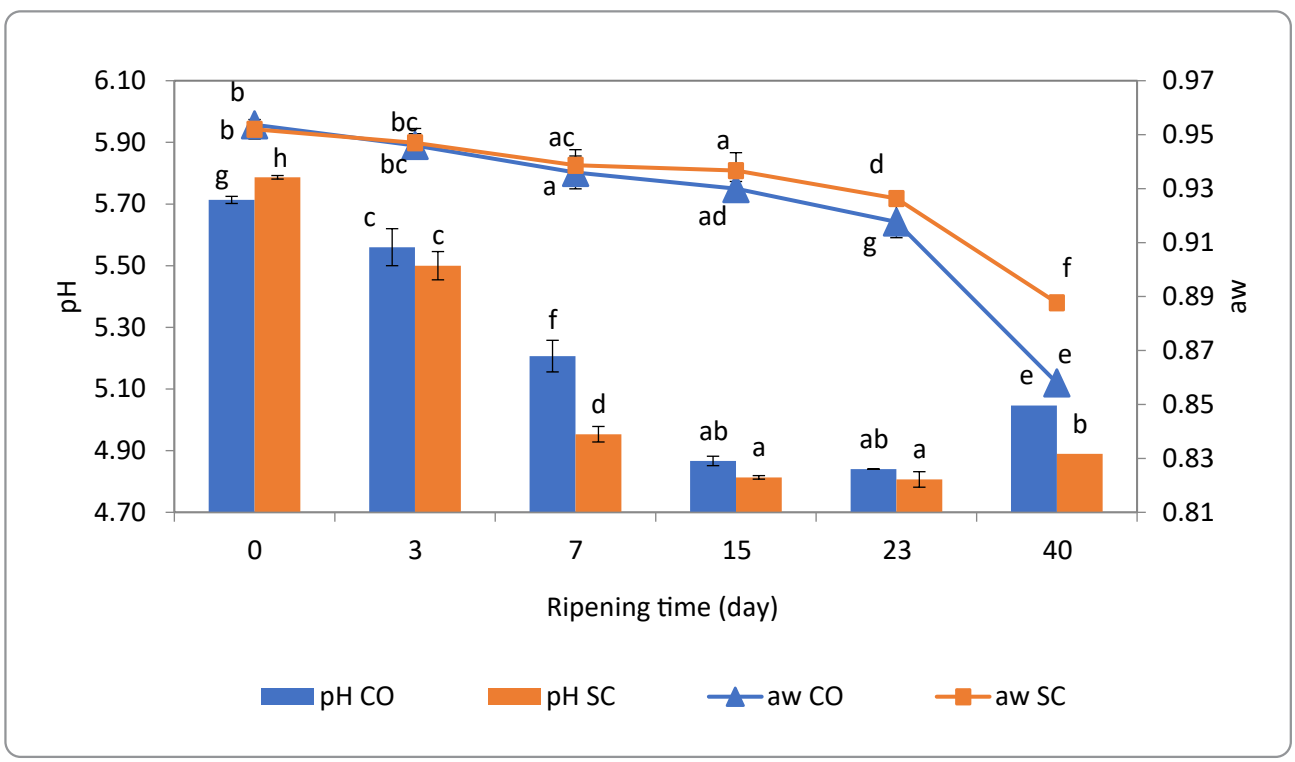

Figure 2. Evolution of pH and water activity (a_w) in control (CO) and starter inoculated (SC) dry-fermented beef sausage during ripening (mean ± standard deviation). Mean values for both physicochemical indicators marked by diverse letters differ significantly ($P < 0.05$).

final a_w in the CO sample was significantly lower than that of the SC sample, which agrees well with *Van Ba et al.* (2016). This finding could be the consequence of the pH in CO sausage being closer to the isoelectric point of actomyosin (≈5.0), thus resulting in lower water holding capacity than in the higher pH SC sausages (*Huff-Lonergan*, 2010).

3.2. Effect of the starter culture on biogenic amine formation

In the present study, four dietary BAs, i.e. putrescine, cadaverine, histamine, and tyramine, were detected in the sausage samples, and their concentrations are presented in Table 1. Tryptamine and phenylethylamine were not detected in any sample. On the contrary, tyramine was the prevailing amine found in both batches starting from day 15 until the end of the ripening period. The ripening time significantly influenced ($P < 0.05$) the concentration of this BA in both batches, as did starter culture inoculation, which resulted in the more intensive accumulation of tyramine in SC samples in the second half of the ripening period. This finding is in accordance with reports of several authors who previously found a highly positive correlation between the concentration of tyramine and the lactic acid bacteria counts in fermented sausages (*Dominguez et al.*, 2016; *Ikonić et al.*, 2023; Šojić et al., 2023).

Putrescine was the second amine found in both batches following the third day of ripening. Its concentration was affected ($P < 0.05$) by both ripening time and starter culture inoculation, resulting in a significant increase until the end of ripening time (when putrescine was 75.6 mg/kg – CO; 111 mg/kg – SC). This result is in concordance with the report of *Dominguez et al*. (2016), who found higher putrescine concentrations in two starter-inoculated batches compared to the non-inoculated control batch. On the other hand, several other authors suggested that starter culture inoculation is beneficial for putrescine reduction (*Ren et al*., 2022; *Van Ba et al.*, 2016). Conversely, in our study, cadaverine was found only in CO sausages at a later ripening stage, i.e. after 23 days. According to others (*Ikonić et al.*, 2023; *Ren et al.*, 2022; *Sun et al.*, 2018), the accumulation of putrescine and cadaverine in meat products could indicate lower processing hygiene and usage of low-quality raw materials, since formation and accumulation of these Bas is related to the growth of contaminant bacteria, such as *Enterobacteriaceae*.

Table 1. Evolution of biogenic amine (BA) concentrations (mg/kg) in control (CO) and starter inoculated (SC) dry-fermented beef sausage during ripening (mean ± standard deviation).

Ripening time (day)	Batch	Putrescine	Cadaverine	Histamine	Tyramine	Total BAs
0	CO	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}
	SC	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}
3	CO	ND^{a}	ND^{a}	ND^{a}	ND^{a}	ND^{a}
	SC	ND^{a}	ND^{a}	ND^{a}	37.2 ± 7.2^{b}	37.2 ± 7.2^{e}
7	CO	48.8 ± 6.4^{bc}	ND^{a}	68.8 ± 8.3^{b}	54.6 ± 6.7^{f}	172 ± 17^{b}
	SC	43.6 ± 6.7^{c}	ND^{a}	ND^{a}	38.1 ± 4.1^{b}	81.7 ± 8.4^{f}
15	CO	60.6 ± 9.3^{b}	ND^{a}	66.7 ± 4.5^{b}	69.8 ± 12^{c}	197 ± 19^{c}
	SC	80.1 ± 9.1^{d}	ND^{a}	ND^{a}	83.8 ± 14^{d}	164 ± 6.3^{b}
23	CO	59.1 ± 11^{b}	32.2 ± 8.2^{b}	69.3 ± 5.8^{b}	74.2 ± 5.3^{cd}	235 ± 15^{d}
	SC	94.4 ± 7.9^{e}	ND^{a}	ND^{a}	114 ± 7.8^{e}	209 ± 9.1^{c}
40	CO	75.6 ± 5.5^{d}	58.1 ± 6.4^{c}	78.6 ± 1.2^{c}	101 ± 7.9^{g}	314 ± 7.5^{g}
	SC	111 ± 8.5^{f}	ND^{a}	ND^{a}	123 ± 6.3^{e}	235 ± 4.9^{d}
Ripening time		*	*	*	*	*
Batch		*	*	*	*	*
Ripening time x Batch		*	*	*	*	*

$^{a-g}$ Mean values within the same column marked by diverse letters differ significantly ($P < 0.05$); ND – not detected; * $P < 0.05$

Formation and accumulation of histamine, the most dangerous amine for human health, was detected only in control (non-inoculated) sausages after 7 days of ripening, amounting to 68.8 mg/kg. After the following two sampling periods (15 and 23 days), its concentration practically remained constant ($P > 0.05$), but by the end of the ripening period (40 days), it increased significantly to 78.6 mg/kg. Thus, the detected histamine concentration, even in sausages of the CO batch, was lower than its allowable limit (100 mg/kg) (*Dominguez et al.*, 2016; *Ikonić et al.*, 2023).

Regarding the total BA concentration, it ranged from 0 to 314 mg/kg in the CO batch and from 0 to 235 mg/kg in batch SC. It increased significantly ($P < 0.05$) as ripening time elapsed. Also, the influence of added starter culture on the total BA concentration was significant ($P < 0.05$). Nevertheless, both values registered after 40 days of ripening were much lower than the maximum threshold of 1000 mg/kg of total biogenic amines, which is considered dangerous for human health. According to the obtained results, this study confirmed previously published findings regarding the positive effect of starter culture on the reduction of BA formation during the production of fermented sausages (*Dominguez et al.*, 2016; *Ren et al.*, 2022; *Van Ba et al.*, 2016).

4. Conclusion

Putrescine and tyramine were detected in both analysed batches, having significantly higher concentrations in starter-inoculated sausages (SC). Cadaverine and histamine were detected exclusively in CO sausages. However, histamine concentration always remained within the allowable limit (100 mg/kg), above which it is considered dangerous for human health. Also, the total BA concentrations in both batches of sausages (314 mg/kg – CO; 235 mg/kg – SC) were much lower than the maximum threshold of 1000 mg/kg. Thus, the summertime production of dry-fermented beef sausage (of *Sjenički sudžuk* type) in small/micro processing plants is possible if appropriate control and regulation of thermo-hygrometric conditions are applied. Additionally, the results showed that starter culture inoculation is conducive to decreasing the accumulation of BAs, especially histamine.

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