

Evaluation of starter cultures for biogenic amine formation and microbial quality in reduced-salt fermented rainbow trout sausages

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Abstract

In this study, the effects of *Lactobacillus sakei* subsp. *sakei*, *Staphylococcus xylosum*, and *Pediococcus pentosaceus* starter cultures on biogenic amine formation and overall product quality in reduced-salt fermented fish sausages were evaluated. Six experimental groups were prepared: two control groups containing 2.5% and 1.5% salt, and four treatment groups inoculated at a level of 10^7 CFU/g with single or mixed cultures (*L. sakei* subsp. *sakei*, *S. xylosum*, *P. pentosaceus*, and a mixed culture). In all inoculated groups, the salt content was reduced to 1.5%. Samples were analyzed on days 0, 3, and 6 to determine biogenic amine levels, physicochemical properties, and microbial quality. Compared with the controls, reduced-salt sausages inoculated with starter cultures exhibited lower pH, thiobarbituric acid reactive substances values and reduced biogenic amine accumulation. Sensory analysis revealed that the addition of starter cultures, particularly *P. pentosaceus* and the mixed culture, significantly improved aroma attributes and increased overall product acceptability. Notably, production of histamine, putrescine, cadaverine, spermine, and spermidine was significantly suppressed in sausages inoculated with *P. pentosaceus*. The findings demonstrate that the use of starter cultures in reduced-salt fermented fish sausages effectively inhibits Enterobacteriaceae growth and improves sensory quality, with *P. pentosaceus* showing the most pronounced beneficial effects. It is recommended that future studies investigate the use of different fish species and evaluate longer fermentation and storage periods to more comprehensively elucidate the sustainable technological and safety-related effects of starter cultures in reduced-salt fermented products.

Keywords: biogenic amines, fermentation, low salt products, lactic acid bacteria, starter culture, rainbow trout sausage

Introduction

In Turkey, fermented fish sausages are not traditional; however, there is an increasing interest in developing healthier, fish-based alternatives to meat sausages using local species such as rainbow trout (Kılınç and Çaklı, 2021; Kahraman Yılmaz and Berik, 2022). Food preservation through fermentation, an ancient technique, has been shown to enhance both flavor and nutritional

(Afifah *et al.*, 2023; Kuley *et al.*, 2018; Mu *et al.*, 2023; Sun *et al.*, 2020; Zang *et al.*, 2020). Fermented sausages are produced from meat and fat mixtures with spices, sugar, salt, and curing agents, then filled into casings and subjected to microbial fermentation. Their long shelf life is due to low water activity (0.7–0.8), low pH (4.5–5.5), and high salt content (Lücke, 2003; Janković *et al.*, 2017; Sadeghi *et al.*, 2021; Sallan *et al.*, 2023).

Biogenic amines are low-molecular-weight, basic nitrogenous compounds formed by amino-acid decarboxylation and are commonly present in protein-rich foods such as meat, fish, cheese, and fermented products acids (Lee et al., 2016; Valsamaki et al., 2000; Xu et al., 2018). High levels, particularly histamine, can cause adverse effects in susceptible individuals (Ma et al., 2021; Valsamaki et al., 2000; Zarei et al., 2011). During storage, certain bacteria decarboxylate free amino acids in fish muscle, especially in scombroid and some nonscombroid species, leading to amine accumulation (Humaid and Jamal, 2014). Compounds such as cadaverine and putrescine, which increase with bacterial spoilage, can potentiate histamine (Ghayoomi et al., 2023; Lehane and Olley, 2000; Lee et al., 2016; Li et al., 2023; Özoğul et al., 2004). Storage temperature, pH, water activity, salt content, and additive use are key factors influencing biogenic amine formation (Čuboň et al., 2019; Ma et al., 2022; Tagawa et al., 2023). The Food and Drug Administration (FDA) has set a maximum permissible histamine level of 50 mg/kg in scombroid and scombroid-like fish (FDA, 1996).

Fermented fish products may contain high amounts of histamine (Mah et al., 2002; Lee et al., 2016; Lu et al., 2015). Although high salt content prevents biogenic amine (Ekici and Khalid Omer, 2020; Prester, 2011), excessive salt is associated with hypertension and cardiovascular disease (Akgün et al., 2018; Kremer et al., 2009; Singracha et al., 2017). In this context, reducing salt in fermented products is important. The use of lactic acid bacteria as starter cultures in reduced-salt products helps inhibit excessive biogenic amine accumulation by rapidly acidifying the environment and producing antimicrobial metabolites such as organic acids, fatty acids, hydrogen peroxide, diacetyl, and bacteriocins (Kuley et al., 2018; Lu et al., 2015).

The main aim of this study was to determine the effects of three starter culture groups individually (*Lactobacillus sakei* subsp. *sakei*, *Staphylococcus xylosum*, *Pediococcus pentosaceus*) and as a mixed culture on biogenic amine formation and overall product quality in reduced-salt fermented fish sausages.

Materials and Methods

Preparation of starter cultures

Three strains of lactic acid bacteria (*L. sakei* subsp. *sakei* ATCC 15521, *P. pentosaceus* ATCC 33316, and *S. xylosum* ATCC 29971) were used as starter cultures. The selected starter cultures were chosen based on their recognized roles in fish fermentation. *L. sakei* and *P. pentosaceus* are lactic acid bacteria known for rapid acidification and antimicrobial activity, while *S. xylosum* contributes to

flavor development and nitrate reduction. These strains have been widely applied in similar fermented meat and fish products to control spoilage and biogenic amine formation. Freeze-dried strains of the starter cultures of *L. sakei* subsp. *sakei* and *P. pentosaceus* were inoculated in Man Rogosa Sharpe (MRS, Merck) Broth, and *S. xylosum* was inoculated in Brain Heart Infusion (BHI, Merck) Broth and incubated at 37 °C for 48 h. The cells were collected by centrifugation (3000×g, 10 min), washed twice with saline solution (0.85% NaCl), and resuspended. These suspensions were used as starter cultures.

Preparation of fish sausages

The rainbow trout fillets used in sausage production were purchased from a fish processing plant in Turkey. Fish meat (80%) and tail fat (20%) were passed through a 3 mm diameter disc and turned into minced meat. The mince was mixed with red pepper (7 g/kg), garlic (10 g/kg), black pepper (5 g/kg), cumin (9 g/kg), allspice (2.5 g/kg), sucrose (4 g/kg), and NaNO₂ (0.15 g/kg) (Kamiloglu et al., 2019). This mixture was then divided into six batches. Four batches were prepared with 1.5% salt and inoculated with different starter cultures, including *L. sakei* subsp. *sakei*, *S. xylosum*, *P. pentosaceus*, and a mixed culture containing all three strains (*L. sakei* subsp. *sakei*, *S. xylosum*, and *P. pentosaceus* at a 1:1:1 ratio).

Two batches without any starter culture, containing 1.5% salt or 2.5% salt (traditional) as a control, were prepared. Each starter culture was inoculated at a final concentration of 7 log CFU/g of sausage batter (Figure 1). These sausage batters with added bacterial cultures were stuffed into casings (35 mm diameter), with approximately 100 g of the final mass of each sausage. The sausage samples were fermented in an automatically adjustable air-conditioned room under conditions of 92% relative humidity for 1 day at 24 °C (0 days), 90% relative humidity for 2 days at 20 °C (1–3 days), and 88% relative humidity for 3 days at 18 °C (4–6 days). By reducing the relative humidity and temperature of the environment during the fermentation and ripening stages of sausage, the drying process is carried out homogeneously so as not to cause surface drying of the product, and the flavor, odor, and texture of the final product are improved (Fernández et al., 2000; Ordóñez et al., 1999). In addition, by controlling the partial humidity during fermentation, the onset of drying, the development of excess yeast or mold on the surface, and the formation of a hard outer layer are prevented (Varnam and Sutherland, 1995).

Analyses were carried out on the 0th, 3rd, and 6th days of fermentation. Two batches were prepared for each treatment, and two replicates were collected from each batch at each time point. Therefore, the analyses were performed in quadruplicate.

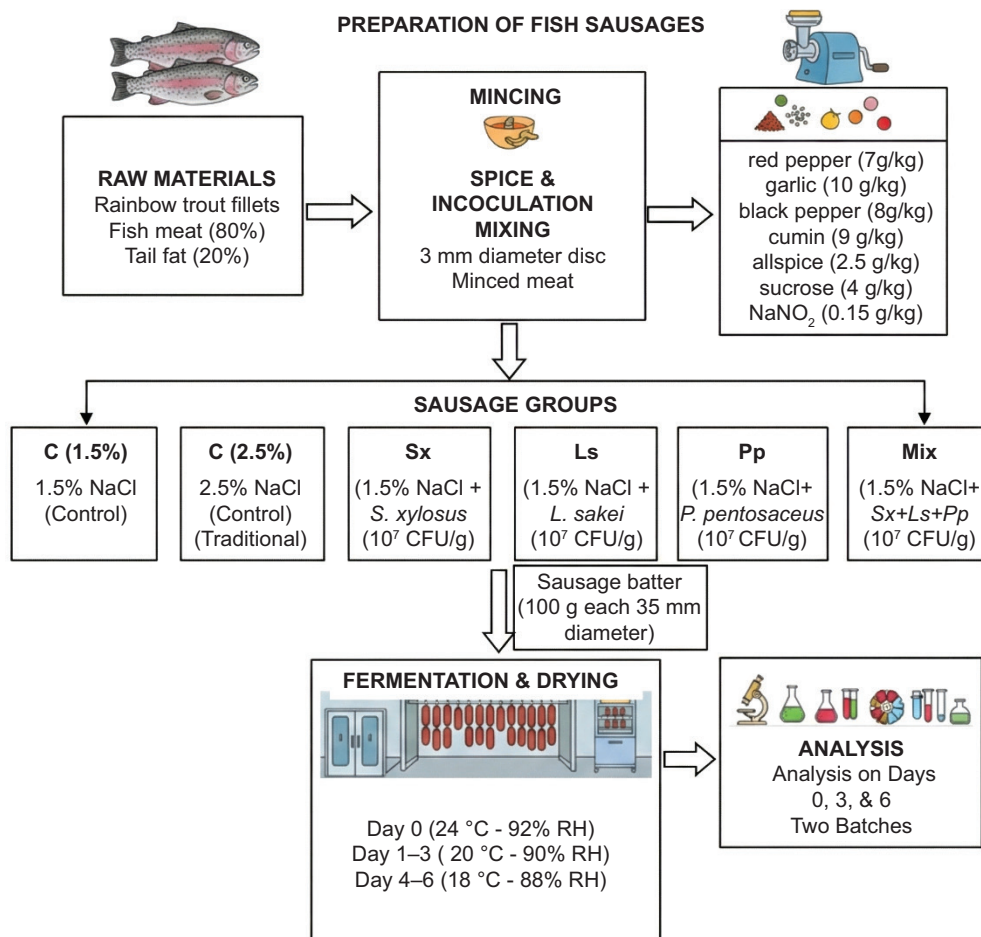


Figure 1. Schematic representation of the preparation and fermentation process of reduced-salt fermented fish sausages.

Determination of pH and thiobarbituric acid reactive substances

The chemical changes occurring during the ripening and storage of all fermented fish sausage samples were assessed using pH and thiobarbituric acid reactive substances (TBARS) analyses. For pH measurements, 10 g of sample was homogenized in 100 mL of distilled water, and the filtrate was analyzed using a digital pH meter (Hanna, HI11312, UK) according to AOAC (1998) and Guran *et al.* (2015). TBARS values were determined following the method of James (1995). All analyses were performed in duplicate.

Determination of biogenic amines

The sample (5 g) was extracted with 15 mL of 0.4 M perchloric acid (HClO₄) and centrifuged, and the supernatant was filtered through a 0.45 µm membrane filter. The derivatization procedure was carried out with dansyl chloride at 40 °C for 45 min. After

incubation in the dark, acetonitrile was added to obtain a final volume of 5 mL. The solution was then filtered through a 0.45 µm membrane filter prior to injection (Topuz *et al.*, 2021).

Chromatographic conditions

Analyses were performed using a Thermo Scientific Ultra-performance liquid chromatography (UPLC-Photodiode array detector) and Hypersil Gold C18 column. The injection volume was 20 µL, and detection was carried out at 254 nm. A gradient elution system consisting of acetonitrile (eluent A) and 0.1 M ammonium acetate (eluent B) was applied as follows: 0 min, A 65%/B 35%; 15 min, A 95%/B 5%; 20 min, A 95%/B 5%; 25 min, A 65%/B 35%. Retention times for the analytes ranged from 7.72 to 17.96 min. Calibration curves were established over 0–50 mg/kg with *R*² values between 0.99986 and 0.99997 for all biogenic amines. The method showed good sensitivity, with limit of detection (LOD) and limit of quantification values of 0.39–0.79 mg/kg and 1.18–2.40 mg/kg, respectively.

Microbiological analysis

Microbiological analysis was conducted to determine the counts of Enterobacteriaceae, total yeast and molds, and lactic acid bacteria. A sausage sample weighing 10 g was aseptically added to 90 mL of sterilized maximum recovery diluent and homogenized. Serial decimal dilutions (10^{-1} to 10^{-6}) were prepared, and 0.1 mL of the appropriate dilutions was spread onto selective agar plates. Potato dextrose agar (Merck) was used at 30 °C for 4–5 days for total yeast and mold counts. Enterobacteriaceae were enumerated using violet red bile glucose (Merck) agar incubated at 37 °C for 24 h. Lactic acid bacteria counts were determined on MRS agar incubated at 37 °C for 72 h. After incubation, colonies were counted and expressed as log CFU/g (FDA, 1998).

Microbial counts were assessed at three time points (Days 0, 3, and 6) to monitor microbial dynamics throughout fermentation and to evaluate the product's final microbial safety. Lactic acid bacteria, Enterobacteriaceae, and yeast and mold counts were determined at each stage. This enabled assessment not only of fermentation efficacy but also of the hygienic and safety parameters of the final product.

Sensory evaluation

After fermentation was complete, the sausage samples were sliced into 0.5-cm-thick pieces, and each sensory sample consisted of three half-slices. Prior to sensory evaluation, the sliced samples were cooked under standardized conditions and then presented to the panelists. The samples were labeled with three-digit random codes, and the presentation order was fully randomized for each panelist to eliminate potential order effects. The sensory panel consisted of 10 trained panelists with experience in evaluating fermented meat and fish products. All sensory tests were conducted in a well-lit, odor-free laboratory under controlled temperature conditions. Written and verbal instructions regarding the evaluation procedures were provided to the panelists. Participants were informed about the purpose of the study, the procedures to be followed, and possible allergens, and written informed consent was obtained from each panelist. They were clearly notified that they could withdraw from the study at any time, discontinue tasting if they experienced any discomfort, and that all personal data would be kept confidential.

The sensory attributes evaluated included appearance, odor, color, flavor, chewiness, and overall acceptability, using a nine-point hedonic scale (1 = “dislike extremely” to 9 = “like extremely”). A control sample (1.5% and 2.5% salt, without starter culture) was included as a reference

(Hu *et al.*, 2007). Each panelist evaluated all samples, and panelists were considered as fixed effects in the statistical model. The sensory data were reported as mean \pm standard error, and differences among groups were analyzed using ANOVA and Duncan's multiple range test, with a significance level set at $p < 0.05$.

Ethical considerations

The study was conducted in accordance with the guidelines established for sensory studies by the Ethics Committee of the Faculty of Science and Engineering Research at İzmir Katip Çelebi University and received ethical approval (Decision No: 2025/09-05). The entire process was conducted in accordance with the core ethical principles of voluntariness, safety, and transparency, and the safety and comfort of the panelists were prioritized throughout the study.

Statistical analysis

The data were analyzed using one-way ANOVA in SPSS 22, and mean comparisons were performed using Duncan's multiple-range test. Statistical analyses were conducted both within the same day and across sampling days. A significance level of $p < 0.05$ was adopted for all statistical evaluations. In addition, Pearson correlation analysis was performed to determine the relationships between microbiological parameters and biogenic amine levels. Correlation matrices and significance values ($p < 0.05$) were used to evaluate the strength and direction of associations among variables.

Results and Discussion

pH and TBARS

The results of pH and TBARS analyses during fermentation and storage are presented in Figure 2.

The pH of fermented foods is a crucial factor in determining their safety. This is because a low pH effectively inhibits the growth of spoilage bacteria and maintains product safety (An *et al.*, 2022). The highest pH value in the fish sausages was detected at 0 days in all the groups ($p < 0.05$). The initial pH ranged from 6.82 to 6.96 and decreased to 4.94–5.20 at 6 days, at which point fermentation was complete ($p < 0.05$). The pH decreased from 6.96 to 4.94 in the *L. sakei* subsp. *sakei* inoculated sausages at 6 days. During the first 3 days of fermentation, pH values in inoculated samples decreased rapidly, whereas those in control samples decreased more slowly ($p < 0.05$). Our findings were in line with those reported by Sun *et al.* (2016). The decrease in pH of the inoculated sausages can be attributed to the presence of

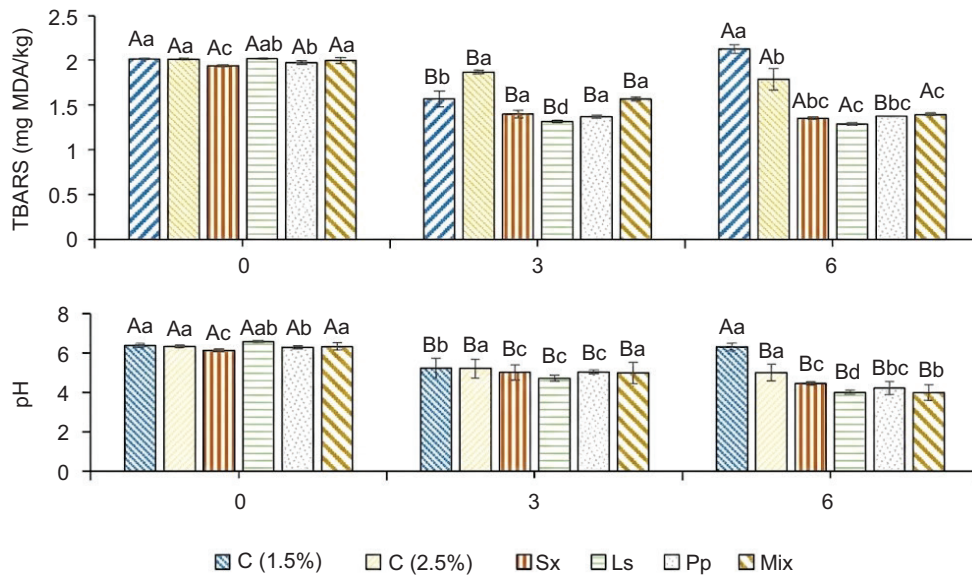


Figure 2. Changes in pH and TBARS in fish sausages fermented with/without starter culture. *C (1.5%): sausage without starter culture containing 1.5% salt; C (2.5%): sausage without starter culture containing 2.5% salt; Sx: sausage with *S. xyloso*; Ls: sausage with *L. sakei* subsp. *sakei*; Pp: sausage with *P. pentosaceus*; Mix: sausage with mixed starter culture (*S. xyloso*, *L. sakei* subsp. *sakei*, *P. pentosaceus*). **Values represent mean \pm standard error of two replications (n:2). Different lowercase letters (a, b, c) indicate significant differences among groups within the same period, and uppercase letters (A, B, C) denote differences among periods within the same group ($p < 0.05$). Statistical analyses were performed using one-way ANOVA followed by DUNCAN post hoc test.

starter culture bacteria, which metabolize carbohydrates with the help of lactic acid bacteria and coagulase-negative staphylococci, ultimately leading to the formation of lactic acid (Sun *et al.*, 2016). Studies by Zhang *et al.* (2013a), Zeng *et al.* (2013b), An *et al.* (2022), Fang *et al.* (2023) and Mu *et al.* (2023) demonstrated that pH values decreased when lactic acid bacteria were included in the fermentation process, compared with fermentation without bacteria.

Lipid hydrolysis and oxidation in muscle and adipose tissues are key processes that contribute to the development of fermented flavor. However, excessive lipid oxidation is a major cause of quality deterioration in fermented meat products, leading to discoloration, rancidity, nutrient loss, and protein oxidation (Chen *et al.*, 2017). In this study, TBARS values were used to evaluate the extent of lipid oxidation and thereby assess the oxidative stability of the reduced-salt fermented rainbow trout sausages. TBARS analysis reflects the accumulation of malondialdehyde (MDA), a secondary lipid oxidation product commonly used as an indicator of oxidative damage (Kanjjan *et al.*, 2021; Sun *et al.*, 2016).

A TBARS value of 5 mg MDA/kg is widely considered the upper acceptability threshold for lipid oxidation in

fish and fish-based products, beyond which noticeable rancid odors and off-flavors occur, leading to consumer rejection (Hu *et al.*, 2008). In the present study, initial TBARS values ranged from 0.29 to 0.77 mg MDA/kg, indicating low levels of lipid oxidation at the beginning of fermentation. Throughout the fermentation period, sausages were inoculated with mixed starter cultures, *P. pentosaceus*, and *Lactobacillus sakei* subsp. *sakei* exhibited significantly lower TBARS values compared to the control ($p < 0.05$). On the 6th day, corresponding to the end of fermentation, the control sample (C, 1.5%) displayed the highest TBARS value (1.97 mg MDA/kg), whereas sausages inoculated with mixed starter cultures had the lowest value (0.87 mg MDA/kg; $p < 0.05$).

Importantly, all measured TBARS values remained substantially below the 5 mg MDA/kg acceptability threshold, demonstrating that none of the samples approached a level at which rancidity or sensory rejection would be expected. This outcome confirms that the fermentation process and the applied starter cultures not only suppressed lipid oxidation but also ensured the production of oxidatively stable and sensorially acceptable fermented sausages, even under reduced-salt conditions.

These findings are in line with previous studies reporting that specific starter cultures reduce the oxidation of unsaturated fatty acids (Hu *et al.*, 2007a, 2007b; Zeng *et al.*, 2013a). The reduced TBARS values in inoculated samples can be attributed both to the antioxidative activity of lactic acid bacteria and the modulatory effect of lowered salt concentration. TBARS formation is known to be affected by multiple factors, including raw material composition, antioxidant addition, fermentation microbiota, processing conditions, storage environment, and oxygen exposure (Sun *et al.*, 2019; Zeng *et al.*, 2013a). Overall, the results clearly indicate that bacterial fermentation is an effective strategy to limit lipid oxidation and maintain acceptable quality in reduced-salt fermented sausages.

Microbiological growth

Counts of lactic acid bacteria, Enterobacteriaceae, and yeast are presented in Figure 3. The initial counts of Enterobacteriaceae in the fish sausage samples ranged from 3.16 to 3.74 log CFU/g ($p > 0.05$). The increase in the number of Enterobacteriaceae during the fermentation

stage was significantly lower in the sausage samples inoculated with starter cultures than in the control samples C (1.5%) and C (2.5%) ($p < 0.05$). The number of Enterobacteriaceae decreased when the salt concentration increased.

The results show that the growth of Enterobacteriaceae can be suppressed by the use of a starter culture in reduced-salt fermented fish sausage. These results are consistent with those of Zhong-Yi *et al.* (2010), who reported that the Enterobacteriaceae counts in fermented bighead carp surimi with starter culture were lower than those in fermented bighead carp surimi without starter culture during 24 h of fermentation. Additionally, our results were similar to those reported by Yin and Jiang (2001), Hu *et al.* (2008), Xu *et al.* (2010), Zeng *et al.* (2013a, 2013b), Zhang *et al.* (2013), Nie *et al.* (2014b), and Xu *et al.* (2023) who reported that starter culture is effective in limiting the number of Enterobacteriaceae in fish sausages and fermented fish products. Additionally, Enterobacteriaceae are microorganisms that are usually associated with amine production. At the beginning of fermentation, the lactic acid bacteria count of the fish sausages without starter culture ranged from 3.40 to

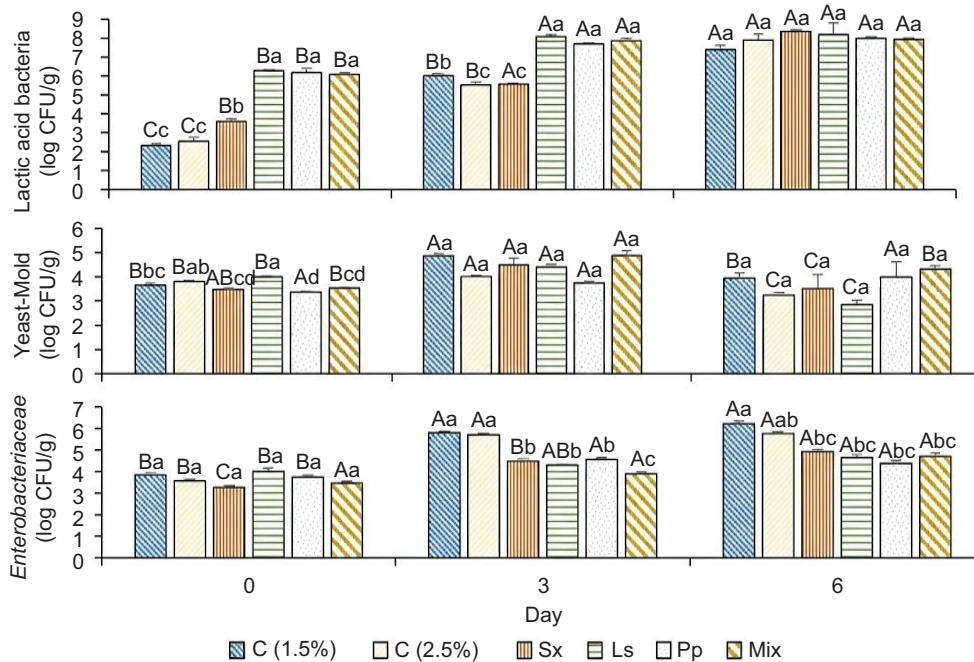


Figure 3. Microbiological changes in fish sausages fermented with/without starter culture. *C (1.5%): Sausage without starter culture containing 1.5% salt; C (2.5%): Sausage without starter culture containing 2.5% salt; Sx: Sausage with *S. xylosum*; Ls: Sausage with *L. sakei* subsp. *sakei*; Pp: Sausage with *P. pentosaceus*; Mix: Sausage with mixed starter culture (*S. xylosum*, *L. sakei* subsp. *sakei*, *P. pentosaceus*). ** Values represent mean \pm standard error of two replications (n:2). Different lowercase letters (a,b,c) indicate significant differences among groups within the same period, and uppercase letters (A,B,C) denote differences among periods within the same group ($p < 0.05$). Statistical analyses were performed using one-way ANOVA followed by DUNCAN post hoc test.

3.55 log CFU/g, and the counts of the fish sausages with starter culture ranged from 4.56 to 6.84 log CFU/g. This result was in agreement with the results of Hu *et al.* (2007, 2008), Zhong-Yi *et al.* (2010), Zeng *et al.* (2013a, 2013b), Zhang *et al.* (2013), and Nie *et al.* (2014a) who reported that the lactic acid bacteria counts of fermented fish sausages with starter culture were initially significantly greater than those of the control groups ($p < 0.05$). In our study, lactic acid bacteria counts increased as fermentation progressed ($p < 0.05$). This increasing trend was similar to studies conducted on fish sausage, fermented fish pieces, and surimi produced with fermentation technology (Hu *et al.*, 2007a; Zeng *et al.*, 2013a; Zhang *et al.*, 2013; Nie *et al.*, 2014a). During fermentation, the counts of lactic acid bacteria increased and reached 8.18–8.48 log CFU/g in the sausages with starter culture and 7.75–8.15 log CFU/g in the control groups on the 6th day. Lactic acid bacteria rapidly increase in number and become the predominant microorganisms in fermented products (Nie *et al.*, 2014b; Zhou *et al.*, 2023; Mu *et al.*, 2023; Mao *et al.*, 2024). This situation was also observed in our study. The hygienic aspect of the product is improved by lactic acid bacteria, which produce lactic acid and bacteriocin, enhance color and flavor, accelerate ripening, and inhibit the growth of pathogenic and spoilage bacteria, thus preserving the product's quality.

Yeasts play an important role in developing fermented products' aroma, taste, and color (Mugula *et al.*, 2003). In our study, the counts of yeast and mold increased in the first 3 days of fermentation in sausages with starter culture and without starter culture. The counts of yeast and mold decreased after the 3rd day; thereafter, the counts were 2.39–3.57 log CFU/g in the control groups and ranged from 2.15 to 3.56 log CFU/g in the sausages with starter cultures on the 6th day. Similar results were reported by Zhang *et al.* (2013). It is thought that the acidic environment created by lactic acid bacteria during fermentation did not cause a significant decrease in the growth of yeasts, which is due to the high tolerance of yeasts to acid and salt, as stated by (Saitthong *et al.*, 2010; Zhang *et al.*, 2013).

At the final stage (day 6), lactic acid bacteria counts ranged from 8.18 to 8.48 log CFU/g in the inoculated groups, whereas Enterobacteriaceae counts were significantly lower than in the control. These results confirm that the product was microbiologically stable and safe at the end of the fermentation period.

Changes in the biogenic amine content

Biogenic amines are compounds formed as a result of the decarboxylation of free amino acids found in foods. The amount of biogenic amines in fresh fish is generally

low, and their presence is often associated with spoilage. For this reason, histamine and other biogenic amines are used as quality indicators and microbial spoilage indices, especially in fish and fish products (Aras Hisar *et al.*, 2004; Özbay-Doğu and Sariçoban, 2015; Zhang *et al.*, 2013).

Histamine, cadaverine, putrescine, spermidine, and spermine contents are presented in Table 1. According to Zhang *et al.* (2013), the initial levels of biogenic amines in fermented fish sausages were relatively low, ranging from 18.07 to 18.23 mg/kg for tyramine, 12.77 to 12.89 mg/kg for spermidine, and 5.20 to 5.24 mg/kg for histamine, while putrescine and cadaverine were not detected. In contrast, the initial concentrations in our study were 20.3 mg/kg for putrescine, 17.0 mg/kg for cadaverine, 33.5 mg/kg for spermidine, and 27.3 mg/kg for spermine. Tyramine, histamine, and 2-phenylethylamine were not detected at the beginning and were therefore reported as <LOD (tyramine: LOD = 0.3927 mg/kg; histamine: LOD = 0.3896 mg/kg; 2-phenylethylamine: LOD = 0.7491 mg/kg) (Table 1). These findings indicate that the fish material used in our study met the expected quality standards.

Histamine is the most toxic amine and is responsible for the health risks associated with consuming fish. Different factors promote histamine accumulation in fermented products, including potential toxicity; increased levels of other biogenic amines, such as putrescine, cadaverine, and tyramine; and decarboxylase activities of Enterobacteriaceae, lactic acid bacteria, *Pseudomonas*, *Micrococcus*, and *Staphylococcus* (Shalaby, 1996; Hu *et al.*, 2007b; Zeng *et al.*, 2013b). In this study, although histamine was not detected in fish sausages at the beginning of fermentation, its levels changed during the process depending on the starter culture used. Histamine concentrations significantly increased ($p < 0.05$) in sausages inoculated with *L. sakei* subsp. *sakei*. Similar increases were also observed in the C (1.5%) and *S. xyloso* groups. However, histamine formation decreased with the higher salt concentration in C (2.5%) ($p < 0.05$). In contrast, histamine production was markedly reduced in sausages inoculated with mixed starter cultures, and it was not detected in samples inoculated with *P. pentosaceus*, where the values were <LOD (LOD for histamine = 0.3896 mg/kg) ($p < 0.05$). Biogenic amines that were not detected were reported as <LOD based on the analytical detection limits established for each compound. Similarly, Leuschner *et al.* (1998) and Li *et al.* (2024) reported that the *Pediococcus* genus has the highest potential for histamine degradation among lactic acid bacteria. This effect is likely associated with the strain's biochemical characteristics, such as its decarboxylase-negative phenotype and potential amino oxidase activity, which contribute to the inhibition or degradation of biogenic amines during fermentation.

Table 1 Changes in histamine, cadaverine, putrescine, spermidine, and spermine contents in fish sausages fermented with/without starter culture (mg/kg). The results are the average of two replications.

	Day	C (1.5%)	C (2.5%)	Sx	Ls	Pp	Mix
Histamine (mg/kg)	0	nd	nd	nd	nd	nd	nd
	3	21.50±1.50 ^{Ba}	nd	20.40±0.40 ^{Bb}	30.30±0.30 ^{Aa}	nd	6.40±1.20 ^{Ca}
	6	23.10±0.70 ^{Ba}	12.80±1.00 ^{Ca}	24.70±0.80 ^{Ba}	34.40±1.60 ^{Aa}	nd	9.00±1.20 ^{Da}
Cadaverine (mg/kg)	0	17.00±1.80 ^{Ab}	17.00±1.80 ^{Aa}	17.0±1.80 ^{Ab}	17.00±1.80 ^{Ab}	17.00±1.80 ^{Aa}	17.00±1.80 ^{Ab}
	3	22.50±0.70 ^{Cb}	10.60±0.60 ^{Db}	49.40±0.90 ^{Aa}	21.30±0.50 ^{Cb}	nd	35.90±0.30 ^{Ba}
	6	35.50±1.30 ^{Da}	nd	53.80±0.20 ^{Aa}	44.10±0.80 ^{Ba}	nd	39.40±0.60 ^{Ca}
Putrescine (mg/kg)	0	20.30±0.50 ^{Ac}	20.30±0.50 ^{Aa}	20.30±0.50 ^{Ab}	20.30±0.50 ^{Ab}	20.30±0.50 ^{Aa}	20.30±0.50 ^{Ac}
	3	23.10±0.00 ^{Bb}	16.10±0.20 ^{Db}	21.80±0.30 ^{Ca}	24.50±0.20 ^{Aa}	nd	21.50±0.20 ^{Cb}
	6	25.10±0.10 ^{Aa}	nd	15.20±0.30 ^{Dc}	17.90±0.20 ^{Cc}	nd	24.50±0.20 ^{Ba}
Spermidine (mg/kg)	0	33.50±1.70 ^{Ab}	33.50±1.70 ^{Ab}	33.50±1.70 ^{Ab}	33.50±1.70 ^{Ab}	33.50±1.70 ^{Aa}	33.50±1.70 ^{Ac}
	3	31.50±1.50 ^{Bb}	30.00±1.00 ^{Bb}	31.10±1.20 ^{Bb}	39.90±1.40 ^{Ab}	nd	41.90±1.30 ^{Ab}
	6	51.50±0.90 ^{Ba}	57.90±2.10 ^{Aa}	58.30±2.50 ^{Aa}	56.20±1.50 ^{ABa}	nd	54.3±1.70 ^{ABa}
Spermine (mg/kg)	0	27.3±1.40 ^{Ab}	27.30±1.40 ^{Ab}	27.30±1.40 ^{Ab}	27.30±1.40 ^{Ab}	27.30±1.40 ^{Aa}	27.3±1.40 ^{Ab}
	3	30.60±0.70 ^{Cb}	29.00±0.50 ^{Cb}	36.80±0.90 ^{ABa}	38.80±0.30 ^{Aa}	nd	34.9±1.00 ^{Bb}
	6	44.50±1.30 ^{Ba}	39.50±1.90 ^{BCa}	37.60±0.30 ^{Ca}	38.80±2.00 ^{BCa}	nd	61.5±2.60 ^{Aa}

*a, b, c (↓); Within a column for each biogenic amine, means with different superscript lowercase letters are significantly different ($p < 0.05$).

A, B, C (→) Within a row, means with different superscript uppercase letters are significantly different ($p < 0.05$).

**C (1.5%): sausage without starter culture containing 1.5% salt; C (2.5%): sausage without starter culture containing 2.5% salt; Sx: sausage with *S. xyloso*; Ls: sausage with *L. sakei* subsp. *sakei*; Pp: sausage with *P. pentosaceus*; Mix: Sausage with mixed starter culture (*S. xyloso*, *L. sakei* subsp. *sakei*, *P. pentosaceus*) (mean ± standard deviation); nd: not detected (<LOD, below the method detection limit).

The use of mixed starter cultures in fermented products has been reported to significantly decrease histamine levels by Majjala *et al.* (1995b), Hu *et al.* (2007a), Zaman *et al.* (2011), and Sun *et al.* (2016, 2019). Similarly, our study revealed that mixed cultures can reduce histamine production ($p < 0.05$). Nout (1994) stated that the histamine content in sausages processed according to “good manufacturing practices” should be in the range of 50–100 mg/kg. Fardiaz and Markakis (1979) reported that the maximum amine amount in fermented fish paste was 64 mg/100 g, and the main amine found was histamine. In this study, the histamine concentration in sausages inoculated with mixed culture was found to be significantly lower than the toxic level throughout fermentation (0–9 mg/kg), and it was determined that histamine production decreased by 39% compared with that in C (1.5%) with the use of mixed starter culture ($p < 0.05$). Importantly, the histamine levels detected in all starter culture–inoculated sausages remained far below the FDA hazard action level of 50 mg/kg, indicating that the products pose no histamine-related health risk. Similarly, Mah and Hwang (2009) and Zhang *et al.* (2013) reported that the histamine content in fermented fish products is much lower than its upper limit in foods (100 mg/kg). According to Yongsawatdigul *et al.* (2004), the histamine level reflects the hygienic quality of the raw material and/or production process. Zeng *et al.* (2013b) and Nie *et al.*

(2014b) reported that there was no significant change in the histamine content in fermented sausages, regardless of starter inoculation. It is rare to find bacteria in meat that have the ability to decarboxylate histidine (Paulsen and Bauer, 1997), and the absence of this biogenic amine in these products can be attributed to the good hygiene practices used in handling raw meat (González-Fernández *et al.*, 2003; Suzzi and Gardini, 2003). Biogenic amine formation differs depending on the meat species. This difference is due to differences in pH and muscle fiber length; short fibers are more readily degraded by proteolytic enzymes, resulting in faster softening of the tissue (Aktop and Şanlıbaba, 2018). In addition, the presence of histidine and bacteria with the histidine decarboxylase enzyme in fish may be responsible for the high histamine content in fish sausages (Serdaroğlu and Deniz, 2001; Vinci and Antonelli, 2002). Biogenic amine production is a characteristic of certain microorganisms, such as Micrococcaceae, Enterobacteriaceae, Pseudomonas, and lactic acid bacteria (Halász *et al.*, 1994).

Aliphatic diamines such as putrescine and cadaverine, together with histamine, are commonly used to assess hygiene levels in food sources such as meat and fish (Bover-Cid *et al.*, 2009). The main amines found in fermented sausages are generally putrescine and cadaverine (Latorre-Moratalla *et al.*, 2008). In our study, the

cadaverine concentration increased from an initial level of 17 mg/kg (day 0) to 35.5, 0.00, 53.8, 44.10, 0.00, and 39.4 mg/kg on day 6 for the C (1.5%) and C (2.5%) control groups and sausages inoculated with *S. xylosum*, *L. sakei* subsp. *sakei*, *P. pentosaceus*, and mixed cultures, respectively ($p < 0.05$) (Table 1). Cadaverine formation was significantly reduced in the C (2.5%) control group, and it was not detected in samples inoculated with *P. pentosaceus*, which were reported as <LOD (LOD = 0.4623 mg/kg) ($p < 0.05$). Similarly, putrescine levels decreased from 20.3 mg/kg (day 0) to 25.1, 0.00, 15.2, 17.9, 0.00, and 24.5 mg/kg on day 6 for the C (1.5%) and C (2.5%) control groups and sausages inoculated with *S. xylosum*, *L. sakei* subsp. *sakei*, *P. pentosaceus*, and mixed cultures, respectively ($p < 0.05$). During the first 6 days of fermentation, putrescine formation was significantly reduced in the C (2.5%) control samples and in sausages inoculated with *S. xylosum* or *L. sakei* subsp. *sakei*, while it was not detected in *P. pentosaceus* inoculated samples, where values were recorded as <LOD (LOD = 0.4317 mg/kg). These findings are consistent with previous studies reporting that the use of starter cultures can effectively suppress the formation of putrescine and cadaverine in fermented fish products (Hua et al., 2021; Maijala et al., 1995a; Nie et al., 2014; Hernández-Jover et al., 1997; Zaman et al., 2011; Zeng et al., 2013a, 2013b). Despite differences in the specific cultures used, similar outcomes have been achieved, demonstrating that starter cultures play a crucial role in preventing putrescine and cadaverine formation. Hu et al. (2007b) and Sun et al. (2019) reported that when mixed starter cultures were used, putrescine and cadaverine concentrations gradually increased during storage, and this increase was significantly lower than that in the control group. In contrast, in our study, the use of mixed culture caused an increase in putrescine and cadaverine concentrations. The reason for this may be that the bacterial species used had an inhibitory effect on each other. Such findings suggest that mixed starter cultures may exhibit strain-dependent synergistic or antagonistic interactions that can alter their decarboxylase activity profiles. Therefore, future research should explore these interspecies interactions in greater depth to identify culture combinations that minimize biogenic amine formation. Toksoy and Beyatlı (1999) reported that, in selecting mixed starter cultures for the production of fermented meat products, the cultures should have a symbiotic relationship.

Spermine and spermidine can react with nitrite in foods to form carcinogenic nitrosamines (Shalaby, 1996; Zaman et al., 2011; Ekici and Khalid Omer, 2020). The spermidine concentration was changed from the initial value of 33.5 mg/kg (day 0) to 51.5, 57.9, 58.3, 56.2, and 54.3 mg/kg on day 6 for the C (1.5%) and

C (2.5%) control sausages and sausages inoculated with *S. xylosum*, *L. sakei* subsp. *sakei*, and mixed cultures, respectively ($p < 0.05$) (Table 1). Similarly, the concentration of spermine also increased from 27.3 mg/kg (day 0) to 44.5, 39.50, 37.6, 38.8, and 61.5 mg/kg on day 6 for the C (1.5%) and C (2.5%) control sausages and sausages inoculated with *S. xylosum*, *L. sakei* subsp. *sakei*, and mixed culture, respectively ($p < 0.05$) (Table 1).

A correlation analysis integrating microbiological counts and biogenic amine levels revealed several significant associations. Enterobacteriaceae displayed a strong positive correlation with spermine, whereas yeasts and molds showed a moderate relationship with cadaverine. LAB counts were positively associated with spermidine levels. Furthermore, putrescine, cadaverine, and spermidine clustered closely together, suggesting shared biochemical pathways. Notably, samples inoculated with starter cultures, particularly *P. pentosaceus*, exhibited markedly reduced levels of histamine, putrescine, and cadaverine, confirming the inhibitory role of starter cultures in biogenic amine formation.

The use of *P. pentosaceus* culture largely inhibited the production of both spermine and spermidine ($p < 0.05$). Li et al. (2021) reported that *P. pentosaceus* can remove spermine and spermidine from fermented fish sausage. Many studies have shown that the concentrations of spermine and spermidine in fermented fish products are low and that the addition of starter culture does not cause a significant change in the concentrations of these biogenic amines (González-Fernández et al., 2003; Hu et al., 2007b; Nie et al., 2014b; Rabie et al., 2009; Zeng et al., 2013b). These amines are not formed by enzymatic decarboxylation by microorganisms but are already present in the raw materials as physiological micro-components. Additionally, since polyamines can be consumed by microorganisms as a source of nitrogen, a slight decrease in their concentrations may be observed (Bardócz, 1995; Bover-Cid et al., 2000; González-Fernández et al., 2003; Hu et al., 2007a; Hernández-Jover et al., 1997; Wang et al., 2020). In contrast, our study revealed that the spermine and spermidine concentrations increased significantly in the other groups, except for the sausage inoculated with *P. pentosaceus*. Similarly, Mah et al. (2002) reported that the spermine and spermidine contents increased during the ripening and storage periods in Korean salted fermented fish products (Myeolchi-jeot), and Maijala et al. (1995a) and Zhong-Yi et al. (2010) reported similar increases in fermented products. These increases may be explained by the concentration effect resulting from water loss during the ripening process (Hernández-Jover et al., 1997).

Sensory evaluation

The results of the sensory evaluation of the fish sausage samples with and without starter culture are presented in Figure 4. Among the control groups, the sample containing 2.5% salt received higher scores in color, odor, flavor, and overall acceptability than the control sample with 1.5% salt. This finding indicates that increased salt concentration can partially improve sensory attributes, particularly by masking the intense fishy odor. However, the scores of both control groups remained significantly lower than those of the starter-culture-inoculated samples ($p < 0.05$).

The sausage samples supplemented with *P. pentosaceus* and the mixed starter culture (*S. xyloso*, *L. sakei* subsp. *sakei*, and *P. pentosaceus*) received the highest appreciation scores in terms of color, odor, flavor, and overall acceptability. No significant differences were observed among the starter culture inoculated groups ($p > 0.05$), suggesting that all tested starter cultures were effective in enhancing the sensory quality of fish sausages. The addition of starter cultures did not produce a significant

difference in appearance or chewiness but resulted in clear improvements in color, odor, flavor, and overall acceptability.

These results are consistent with the findings of (Hu *et al.*, 2007a) and (Zeng *et al.*, 2013a), who reported that fermented fish products inoculated with starter cultures were superior to control groups in terms of appearance, taste, texture, flavor, and overall acceptability, whereas the odor of the fish was more intense in the controls. The lactic acid produced by starter cultures not only imparts a characteristic lactic acid flavor by masking or suppressing undesirable fish odors but also enhances firmness and mouthfeel due to the acid-induced denaturation of muscle proteins (Xu *et al.*, 2010). Similarly, Kanjan *et al.* (2021) reported that fish sauce prepared with starter culture was more desirable than control samples, a difference attributed to the formation of volatile compounds and amino acid hydrolysis by the starter cultures.

In addition, the higher acidity, lower salinity, lipid oxidation, and intensified hydrolysis of muscle proteins

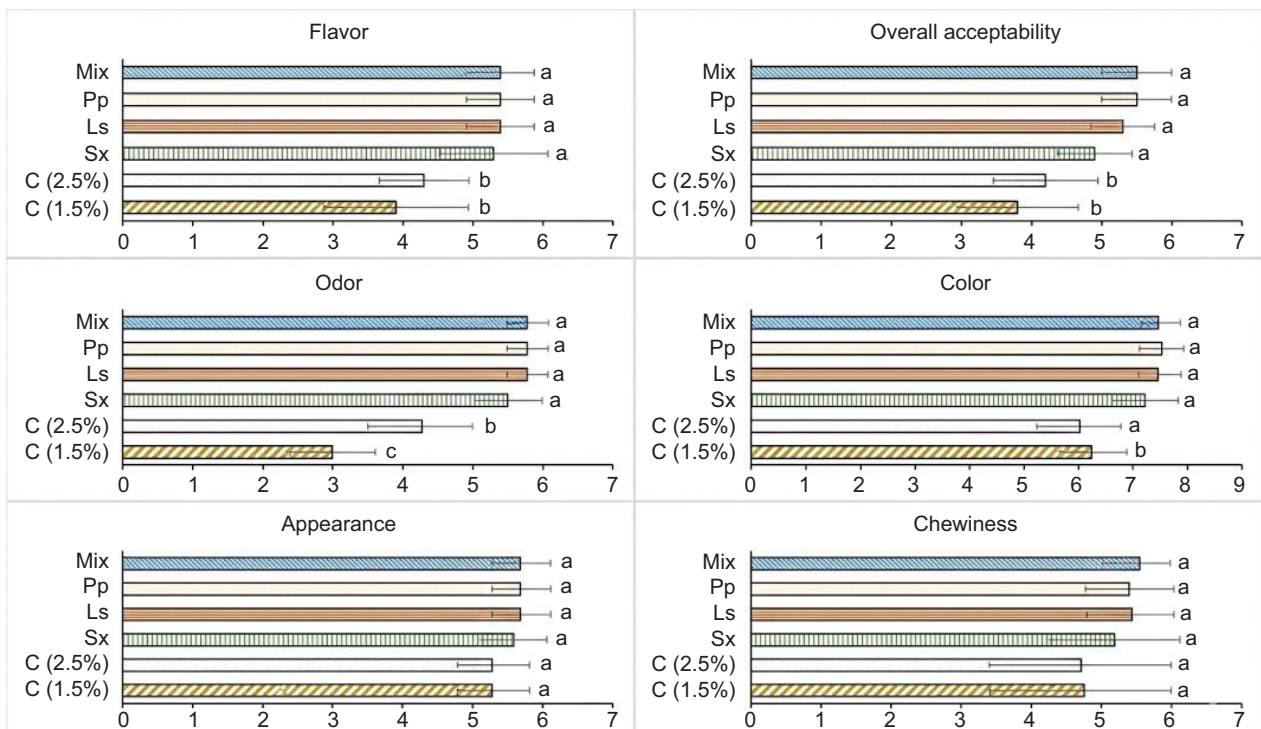


Figure 4. Sensory evaluation of the fish sausages with/without starter culture. C (1.5%): Sausage without starter culture containing 1.5% salt; C (2.5%): sausage without starter culture containing 2.5% salt; Sx: sausage with *S. xyloso*; Ls: Sausage with *L. sakei* subsp. *sakei*; Pp: Sausage with *P. pentosaceus*; Mix: sausage with mixed starter culture (*S. xyloso*, *L. sakei* subsp. *sakei*, *P. pentosaceus*). *Values represent mean \pm standard error (n:10). Different lowercase letters (a, b, c) indicate significant differences among groups ($p < 0.05$). Statistical analyses were performed using one-way ANOVA followed by DUNCAN post hoc test.

associated with starter culture inoculation contribute to the unique flavor profile of fermented fish products (Zeng *et al.*, 2013a). Taken together, the findings of the present study confirm that while increasing salt concentration (2.5%) can moderately enhance the sensory attributes of fish sausages compared to 1.5% salt, the addition of starter cultures has a much more pronounced positive impact on overall product quality. In conclusion, this study demonstrated that the use of starter cultures improved the sensory quality of low-salt fish sausages, particularly enhancing aroma and overall acceptability. Although differences in some sensory parameters were not statistically significant, the incorporation of starter cultures contributed positively to overall consumer acceptance. Nevertheless, the interpretation of these sensory results should account for several limitations, particularly the limited number of panelists and the subjective nature of sensory perception, which may constrain the broader applicability of the conclusions.

The differences in some sensory parameters were not statistically significant; however, the incorporation of starter cultures provided a positive contribution in terms of overall consumer acceptance. Additional studies involving a larger sensory panel could be conducted to further assess consumer preferences.

Conclusions

Compared with the control sausages, the reduced-salt fermented fish sausages inoculated with starter cultures exhibited lower pH and TBARS values, as well as reduced biogenic amine formation, indicating enhanced product safety. The use of mixed cultures led to a marked decrease in histamine accumulation, and sausages inoculated with *P. pentosaceus* showed significantly lower levels of histamine, putrescine, cadaverine, spermine, and spermidine. Although high salt levels in fermented products can suppress microbial growth, reducing overall salt intake remains important for public health. Overall, the findings demonstrate that starter cultures have strong potential for improving the safety and quality of reduced-salt fermented fish sausages. Sensory evaluation further confirmed that samples inoculated with *P. pentosaceus* and mixed cultures exhibited superior aroma and overall acceptability compared with controls. These results highlight that high-quality, safe, reduced-salt fermented fish sausages can be produced using appropriate starter cultures.

Despite the study's valuable findings, several aspects warrant further investigation. Future research addressing pathogen screening, the effects of water activity (aw), and product behavior over extended storage periods would provide a more comprehensive

understanding of product stability and safety. Such work would strengthen the interpretation and practical applicability of the present results.

Mandatory Disclosure on Use of Artificial Intelligence

The authors declare that no AI-assisted tools were used in the preparation of this manuscript. All references have been manually verified for accuracy and relevance.

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Author Contributions

All authors contributed equally to this article.

Conflict of Interest

The authors declare that they have no competing interests.

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