Karadeniz Fen Bilimleri Dergisi, 15(2), 865-880, 2025. DOI: 10.31466/kfbd.1654864

KFBD

Karadeniz Fen Bilimleri Dergisi

The Black Sea Journal of Sciences

ISSN (Online): 2564-7377 https://dergipark.org.tr/tr/pub/kfbd

Araştırma Makalesi / Research Article

Effects of Starter Cultures on the Physicochemical and Nutritional Characteristics of Fermented Fish Sausages

Fatma ÖZTÜRK[1], Hatice GÜNDÜZ[2*], Fettah GÜNDÜZ[3]

Abstract

This study investigated the effects of amine-negative starter cultures, *Lactobacillus sakei* subsp. *sakei*, *Staphylococcus xylosus*, and *Pediococcus pentosaceus*, both individually and in combination, on the nutritional composition, texture, color, and amino acid profile of fish sausages during ripening and storage. The moisture content differences were significant during fermentation but not during storage (P> 0.05). Sausages with *S. xylosus* had the highest ash content, which increased by the end of storage. Color measurements showed declines inrevealed decreases in the *L** and *b** values, whereas the *a** values varied with bacterial addition. Texture analysis revealed no significant differences in hardness between the groups. Amino acid analysis demonstrated a notable increase in total free amino acids postfermentation, particularly in sausages inoculated with mixed cultures (P< 0.05). Significant increases in flavor-related amino acids such as alanine, valine, and leucine were observed in sausages with *P. pentosaceus* and mixed cultures. The results highlight the potential of *S. xylosus*, *P. pentosaceus*, and mixed cultures to improve fish sausage quality, stability, and flavor development during storage.

Keywords: Fish sausage, Microbial fermentation, Organoleptic attributes, Biochemical properties.

Starter Kültürlerin Fermente Balık Sucukların Fizikokimyasal ve Besinsel Özellikleri Üzerine Etkileri

Öz

Bu çalışmada, amin negatif starter kültürler olan *Lactobacillus sakei* subsp. sakei, *Staphylococcus xylosus* ve *Pediococcus pentosaceus*'un, olgunlaşma ve depolama süresince balık sucuklarının besin bileşimi, tekstür, renk ve amino asit profili üzerindeki etkileri ayrı ayrı ve birlikte incelenmiştir. Fermantasyon sırasında nem içeriğinde anlamlı farklılıklar gözlenmiş ancak depolama sürecinde fark tespit edilmemiştir (P> 0.05). En yüksek kül içeriği *S. xylosus* içeren sucuklarda bulunmuş ve bu içerik depolama sonunda artmıştır. Renk ölçümleri, *L** ve *b** değerlerinde azalma olduğunu, *a** değerlerinin ise değişkenlik gösterdiğini ortaya koymuştur. Tekstür analizinde gruplar arasında sertlik açısından fark bulunmamıştır. Amino asit analizinde, fermantasyondan sonra toplam serbest amino asit içeriğinde anlamlı bir artış gözlenmiş ve en yüksek seviyeler karışık kültürlerle aşılanmış sucuklarda kaydedilmiştir (P< 0.05). Özellikle lezzet gelişiminde önemli amino asitlerin *P. pentosaceus* ve karışık kültürlerde daha yüksek olduğu tespit edilmiştir. Sonuçlar, *S. xylosus*, *P. pentosaceus* ve karışık kültürlerin, kalite, stabilite ve lezzet geliştirme açısından balık sucuklarında kullanımı için uygun olduğunu göstermektedir.

Anahtar Kelimeler: Balık sucuğu, Mikrobiyal fermentasyon, Organoleptik özellikler, Biyokimyasal özellikler.

[1,2,3]Department of Fisheries and Fish Processing Technology, Faculty of Fisheries, Izmir Katip Celebi University, Izmir, Turkey, fatma.ozturk@ikcu.edu.tr hatice.gunduz1@ikcu.edu.tr fettahgunduz@gmail.com

[*]Sorumlu Yazar/Corresponding Author

Geliş/Received: 10.03.2025 **Kabul/Accepted:** 27.04.2025 **Yayın/Published:** 15.06.2025

Öztürk, F., Gündüz, H., Gündüz, F. (2025). Effects of Starter Cultures on the Physicochemical and Nutritional Characteristics of Fermented Fish Sausages. *Karadeniz Fen Bilimleri Dergisi*, 15(2), 865-880.

1. Introduction

Fish are a rich source of easily digestible protein with high biological value and can provide the body with vitamins, minerals and essential fatty acids (Arslan et al., 2001, Sadeghi et al., 2021). The popularity of seafood as a foodstuff is increasing, largely because of its perceived health benefits and flavorful nature. The fish industry has responded to this trend by developing a range of processed or minced fish products, including fish burgers, fish fingers, and fish sausages. These products offer a convenient alternative while providing nutritional advantages. Fish sausage, in particular, involves mixing fish meat with additives and stuffing it into appropriate casings (Rahman et al., 2007). In response to the growing consumer demand for low-fat meat products, interest in the use of microbial fermentation to produce fish sausage is increasing (Hu et al., 2007).

Fermented sausages are important meat products, accounting for the majority of global food production. Their special characteristics include improved shelf-life, increased nutritional value due to reduced nitrosamines, and improved odor and flavor as a result of bacterial metabolites during fermentation (Sadeghi et al., 2021).

During fermentation, enzymatic and microbial activities lead to the breakdown of proteins, lipids, and carbohydrates, resulting in the formation of organic acids, amino acids, small peptides, and other compounds. These substances contribute to nutritional enhancement, flavor enrichment, and the distinct soft and fluffy texture characteristic of fermented fish products (An et al., 2022).

There are numerous benefits to fermenting fish for human consumption. First, fermentation serves as an affordable and practical method for preserving fish muscle without refrigeration. Second, it enhances the sensory properties of fish. Finally, it can increase the nutritional value and digestibility of the raw material (Hu et al., 2007).

Fermented meats and fish are highly popular in many Oriental and some Western countries. Recently, interest in the use of pure bacterial cultures to create fish-like products has increased (Hu et al., 2008). Lactic acid bacteria increase product safety by lowering the pH through sugar fermentation, whereas coagulase-negative staphylococci impact other technological characteristics of fermented meat products (Hu et al., 2007).

Various researchers have highlighted that the use of starter cultures in fish meat can significantly increase the quality of the final product (Yin and Jiang, 2001; Hu et al., 2007; An et al., 2008; Xu et al., 2010a; Xu et al., 2010b; Zaman et al., 2011; Zeng et al., 2013a; Zeng et al., 2013b Nie et al., 2014a; Nie et al., 2014b; Sadeghi et al., 2021; An et al., 2022). *L. sakei* and *P. pentosaceus*, both lactic acid bacteria, are recognized for their rapid acidification capacity and antimicrobial properties, whereas *S. xylosus* plays a key role in flavor development and nitrate reduction (Lauková 2011; Zheng et al., 2025).

This study aims to investigate the impacts of three amine-negative starter cultures, *Lactobacillus sakei* subsp. *sakei*, *Staphylococcus xylosus*, and *Pediococcus pentosaceus,* both individually and in mixed cultures, on the nutritional composition, textural properties, and color characteristics of fish sausages during ripening and storage. By addressing gaps in the literature, this research provides detailed insights into how these specific starter cultures affect fish sausage quality. In this context, this study examines the effects of various factors on the moisture content; protein, fat, ash, and amino acid contents; and color attributes of a product, with the goal of enhancing the quality. Moreover, this study aims to provide practical implications for the commercial production of fish sausages, with the potential to increase product stability and safety.

2. Materials and Methods

2.1. Preparation of Starter Cultures

Three strains of lactic acid bacteria were used as starter cultures: *Lactobacillus sakei* subsp. *sakei* ATCC 15521, *Pediococcus pentosaceus* ATCC 33316, and *Staphylococcus xylosus* ATCC 29971. Freeze-dried cultures of *L. sakei* and *P. pentosaceus* were rehydrated and inoculated into De Man Rogosa Sharpe (MRS, Merck) Broth, while *S. xylosus* was grown in Heart Infusion Broth. All the cultures were incubated at 37 °C for 48 hours. After incubation, the cells were harvested via centrifugation at 3000 × g for 10 minutes, washed twice with a saline solution (0.85% NaCl), and resuspended in fresh saline. These cell suspensions were then used as starter cultures.

2.2. Preparation of Fish Sausages

Rainbow trout (*Oncorhynchus mykiss*) fillets were used in the production of sausages. The fish meat, comprising 80%, and tail oil, comprising 20%, were ground through a 3 mm disc to produce minced meat.

The preparation of sausages is carried out following the formulation specified by Kamiloğlu et al. (2019). This mince was combined with various seasonings, including 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 $NaNO_2$ (0.15 g/kg). The prepared mixture was divided into five portions: four were inoculated with different starter cultures*, Lactobacillus sakei, Staphylococcus xylosus, and Pediococcus pentosaceus*, and a mixed culture (*L. sakei, S. xylosus, and P. pentosaceus* at a 1:1:1 ratio), whereas the remaining batch, which served as a control, contained 1.5% NaCl without any starter cultures. In bacterial cultures, 1 mL (10^{10} CFU/mL) was inoculated into 1 kg of sausage dough. In this way, a final concentration of

7 log CFU/g was achieved in the sausage batter. (Table 1). The batters with bacterial cultures were then stuffed into casings with a diameter of 35 mm, each weighing approximately 100 g. The sausages were ripened in a climate-controlled chamber with 92% relative humidity for 1 day at 24 °C, 90% relative humidity for 2 days at 20 °C, and 88% relative humidity for 3 days at 18 °C. As sausage is a fermented raw meat product, the fermentation and maturation stages play a crucial role. According to the TS 1070 Turkish Sausage Standard (TSE, 2002), the final product should contain no more than 40% moisture, no more than 5% salt, and should have a pH value between 4.7 and 5.5 at the end of the maturation period. In all sausage groups produced in this study, these parameters moisture content, salt concentration, and pH were found to meet the specified standards by the sixth day of maturation. Following the ripening phase, the sausages were stored at 4 °C. This study was carried out in 2 replications. Analyses were performed on days 0, 3, 6, 15, and 25.

Table 1. The experimental groups and compositions of sausages

C (1.5%)	1.5% NaCl (Control)
Sx	1.5% NaCl + *S. xylosus* (10^7 CFU/g)
Ls	1.5% NaCl + *L. sakei* sups *sakei* (10^7 CFU/g)
Pp	1.5% NaCl + *P. pentosaceus* (10^7 CFU/g)
Mix	1.5% NaCl + (*L. sakei*, *P. pentosaceus*, *S. xylosus*) (1:1:1-10^7 CFU/g)

2.3. Determination of Chemical Composition

The moisture, protein and crude fat contents of the fish were measured via the oven method (AOAC, 1984), the Kjeldahl method (AOAC, 1984) and the Bligh and Dyer method (1959), respectively. The ash content of the samples was determined by incineration in a muffle furnace at 550 °C for 24 hours (Ismail, 2017).

2.4. Color Evaluation

The color of the samples was assessed on a flat surface via a colorimeter (Color Muse, Variable Inc., Tennessee, USA). The colorimeter was configured to take absolute measurements in the L*, a*, b* mode (CIE 1976). Hunter L* (indicating lightness), a* (indicating redness), and b* (indicating yellowness) values were used to monitor color changes during fermentation. To account for color variation, the average of three readings from the surface of each sample was used (Hu et al., 2007).

2.5. Texture Analysis

Shear measurements were performed to determine the hardness and toughness of the fish sausages. The samples were analyzed with a TA HD Plus texture analyzer (Stable Micro Systems Ltd., UK) via a Warner bratzler cutting probe. Analyses were performed under the following conditions:

Pre Test speed: 2.00 mm/sec
Test speed: 2.00 mm/sec
Post Test speed: 10.00 mm/sec
Distance: 30.000 mm
Trigger force: 20.0 g

2.6. Determination of Amino Acids

The free amino acid composition of the samples was analyzed using High Performance Liquid Chromatography (HPLC), following the methods described by Aristoy and Toldra (1991) and Antoine et al. (1999). Initially, the samples were homogenized, weighed to 5 g, and hydrolyzed with 6 N HCl for 24 hours at 110 °C. Single-detector UV and Zorbax Eclipse-AAA 4.6 × 150 mm, 3.5 µm HPLC were employed to determine the free amino acid composition. FMOC (9-fluorenylmethyl chloroformate) with OPA (ortho-phthalaldehyde) was used as the derivatization agent, and 0.4 N borate (pH 10.2) served as the buffer solution for amino acids.

2.7. Statistical Analysis

The obtained data were analyzed using Analysis of Variance (ANOVA). The results were further evaluated with the Duncan Multiple Comparison Test. The SPSS statistical software package was used to determine if there were any significant differences between the application groups (IBM SPSS, 2012).

3. Results and Discussion

3.1. Chemical Compositions

Moisture, protein, fat and ash analyses were performed to evaluate the nutritional properties of the fish sausages. The values obtained during fermentation and storage are shown in Figure 1.

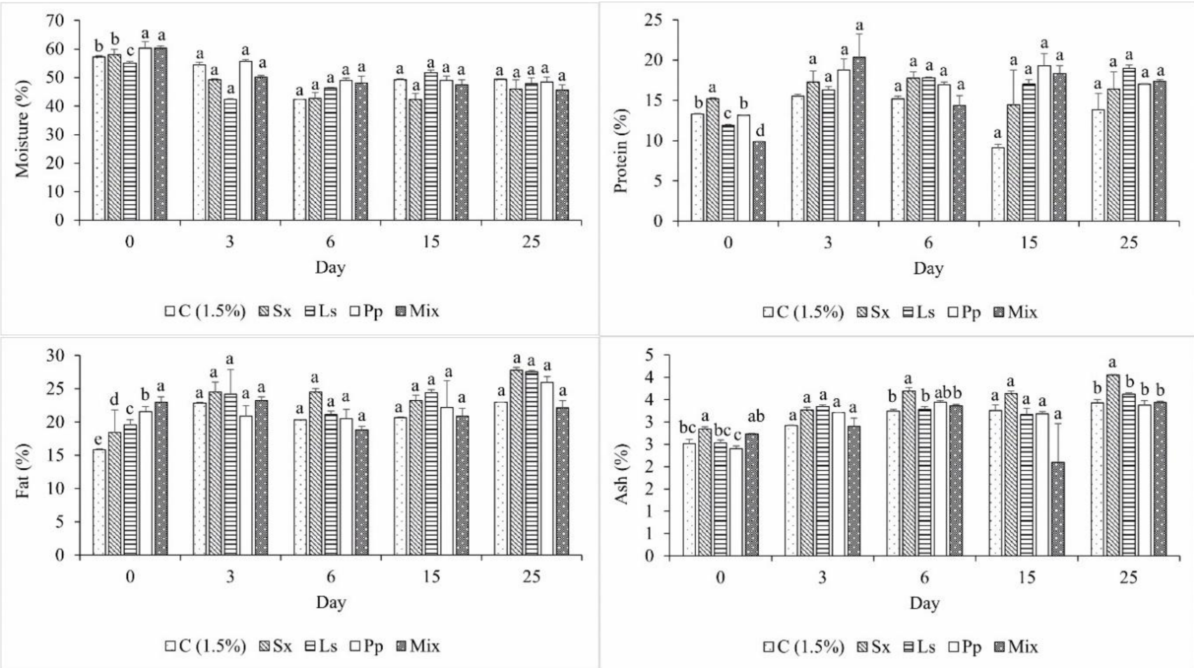

Figure 1. Chemical composition of the fermented fish sausage groups

There was a statistically significant increase in the moisture content of the groups during storage after fermentation (*P< 0.05*). Although differences were observed in the moisture contents of the groups at the beginning of storage and during the fermentation process (*P< 0.05*), no significant difference was detected between the moisture contents of the groups in the analyses performed on the 15th and 25th days of storage after fermentation was completed (*P> 0.05*).

Fermentation enhances the functional value of food by inducing chemical changes in organic substrates such as carbohydrates, proteins, and fats through the enzymatic activities of microorganisms (Afifah et al., 2023). The initial moisture content of the fish sausages produced with different starter cultures ranged from 55.04--60.31% (*P< 0.05*). However, there was a significant decrease in the moisture content of the experimental groups at the fermentation stage (*P< 0.05*). The observed decrease in moisture content as fermentation progressed may be due to an increase in dry matter content as a result of microbial cell proliferation (Obadina et al., 2013). A decrease in the moisture content of fermented mackerel sausages has also been reported (Afifah et al., 2023). Zeng et al. (2013a) reported similar results for fermented fish parts. The decrease in moisture level may be due to the added raw materials as well as the evaporation that occurs during the fermentation stage. Before fermentation, some water molecules form hydrates with other molecules (oxygen, nitrogen, carbohydrates, protein and other organic compounds) so that the water becomes free water. During fermentation, free water evaporates to a considerable extent because of enzyme activity, breaking the bonds of water into free water (Afifah et al., 2023).

On the first day, a statistically significant difference was detected in the protein content (9.84--15.18%) of the fish sausage groups (*P< 0.05*), but this difference was not detected during the fermentation and storage periods (*P> 0.05*). The changes in the protein content of the fish sausage samples over the analysis periods (except for *L. sakei*) were not statistically significant (*P> 0.05*).

The increase in protein levels in the groups is thought to be due to the increase in the total nitrogen amount and lactic acid bacteria (LAB) population in the fermented sausages. The increase in nitrogen is due to LAB proteases, which actively degrade proteins into amino acids and peptides, resulting in increases in nitrogen and protein contents (Afifah et al., 2023). In our study, no significant difference was detected between the protein values of all the sausage groups with and without starter cultures. Similar results were obtained with fermented fish pieces using different starter cultures (Zeng et al., 2013a).

While there was a statistically significant difference in the fat content of the fish sausage samples on day 0 (*P< 0.05*), no significant differences were detected in any of the groups except for the *L. sakei* culture group on the other days of analysis.

While the fat content of the fish sausage samples ranged from 15.82--22.96% at the beginning of fermentation, it ranged from 18.80--24.50% at the end of fermentation (6^{th} day). The increase in fat content is due to the secondary lipolytic activity of LAB, which actively releases fatty acids from fat molecules during fermentation (Afifah et al., 2023). In our study, the increase in fat content was not statistically significant except for sausage samples containing *P. pentosaceus* and mixed culture during the 6 days of fermentation (*P> 0.05*). Hu et al. (2008) reported that there was no significant difference in the fat content of fish sausages fermented with starter cultures. Zeng et al. (2013a) reported that the fat content of fermented fish pieces from different starter cultures was greater in a group containing mixed cultures (*P. pentosaceus, S. xylosus, and S. cerevisiae*) than in other groups.

The ash content indicates the presence of mineral substances in food (Yorukoglu and Dayısoylu, 2016). Among all the groups, the highest ash content was found in the group containing *S. xylosus* on the 0^{th}, 6^{th} and 25^{th} days of storage (*P< 0.05*). The ash content in all the groups was greater on the 25^{th} day of storage than at the beginning of storage (*P< 0.05*).

Zeng et al. (2013a) and Afifah et al. (2023) reported an increase in ash content in fermented fish products during fermentation. Zeng et al. (2013a) reported no significant difference between the ash contents of fermented fish pieces prepared by using different starter cultures, whereas in our study, the ash contents of the sausage groups containing 1.5% salt, *L. sakei* and mixed culture at the end of fermentation (6^{th} day) were significantly lower than those of the other groups (*P< 0.05*). In another study on fermented fish, the ash value of the experimental group without starter culture was lower than that of the experimental groups with starter culture (Zeng et al., 2013b).

The chemical composition may be influenced by the sources of raw materials, the types of ingredients used, the extent of water removal before processing, and the water loss that occurs during fermentation (Zeng et al., 2013a).

3.2. Color Properties

The color (*L**, *a**, and *b**) values of the fish sausage samples were analyzed during fermentation and storage. Color is one of the most important quality indicators in foods. According to the International Commission on Illumination (CIE), in the *L**, *a**, *b** system, *L** ranges from 0 (black, dull) to 100 (white, bright); *a** represents (+) red or (-) green; and *b** represents (+) yellow or (-) blue (Ormancı, 2013).

The changes in the brightness (*L**) values of the fish sausage samples during fermentation and storage are shown in Table 2. In this study, the *L** values of all the fish sausage groups at the beginning of storage varied between 48.37 and 57.53 ($P > 0.05$). The *L** values were between black and white on average. At the end of storage, the *L** value varied between 32.73 and 37.35 ($P > 0.05$). Analyses conducted during fermentation and storage revealed that the addition of different lactic acid bacteria had no effect on the *L** values of the fish sausage samples ($P > 0.05$). However, the *L** values measured in all the groups at the beginning of storage were lower at the end of storage as a result of the dulling of the fish sausages ($P < 0.05$). This decrease is related to the thickening of the samples due to weight and water loss (Sadeghi et al., 2021).

Table 2. Changes in the *L** values of the fish sausage samples during fermentation and storage

Day	C (1.5%)	Sx	Ls	Pp	Mix
Fermentation					
0	53.87±4.31^{Aa}	48.70±2.76^{Ab}	54.70±5.22^{Aa}	48.37±1.54^{Aa}	57.53±3.5^{Aa}
3	45.23±5,60^{Ab}	44.37±4.08^{Ac}	43.55±5.60^{Ab}	37.18±2.25^{Ab}	41.85±3.95^{Ac}
6	55.17±3.60^{Aa}	55.45±1.69^{Aa}	55.92±6.46^{Aa}	52.15±1.89^{Aa}	48.28±5.84^{Ab}
Storage					
15	53.72±4.27^{Aa}	50.07±2.36^{Ab}	51.78±7.8^{Aab}	48.98±6.39^{Aa}	54.97±4.79^{Aa}
25	37.35±3.00^{Ac}	34.95±3.13^{Ad}	32.73±3.63^{Ac}	37.12±2.82^{Ab}	35.95±1.32^{Ac}

Mean (n=2) ± std.error a, b, c (↓); There is a statistically significant difference between days with different letters in the same column ($P > 0.05$)

A, B, C (→): There is a statistically significant difference between days with different letters in the same row. ($P > 0.05$)

C: sausage without starter culture containing 1.5% salt; Sx: sausage with *S. xylosus*; Ls, sausage with *L. sakei*; Pp, sausage with *P. pentosaceus*; Mix, sausage with mixed starter culture (*S. xylosus*, *L. sakei*, *P. pentosaceus*)

The changes in the *a** values of the fish sausage samples during fermentation and storage are given in Table 3. In this study, the *a** values of the fish sausage samples at the beginning of storage varied between 12.03 and 13.07 (*P> 0.05*). At the end of the fermentation process (6th day), the highest and lowest *a** values were 14.52--8.43 for the control and *L. sakei* subsp. *sakei* groups, respectively. On the 25th day, the last day of storage, the value was found to vary between 6.05 and 10.87 (*P> 0.05*). While no difference was observed in the values of the groups before fermentation, the *a** value of the *L. sakei* subsp. *sakei* group was different from those of the S. xylosus and control groups after fermentation.

Table 3. Changes in the *a** values of the fish sausage samples during fermentation and storage

Day	C (1.5%)	Sx	Ls	Pp	Mix
Fermentation					
0	12.60±0.75^{Aa}	12.97±0.77^{Aa}	13.07±1.43^{Aa}	12.03±2.21^{Aa}	12.20±1.53^{Aa}
3	11.70±2.20^{ABa}	12.55±1.47^{Aa}	11.12±1.57^{ABab}	7.72±1.93^{Ca}	9.65±2.20^{BCa}
6	14.52±1.64^{Aa}	13.23±2.82^{ABa}	8.43±2.74^{Cbc}	9.55±3.4^{BCa}	9.97±4.42^{BCa}
Storage					
15	12.58±3.32^{Aa}	8.88±3.42^{Aa}	8.30±3.74^{Abc}	8.08±0.86^{Aa}	8.38±4.22^{Aa}
25	7.78±1.83^{Ab}	10.87±2.18^{Aa}	6.05±1.05^{Ac}	9.02±4.06^{Aa}	9.80±3.55^{Aa}

Mean (n=2) ± std.error a, b, c (↓); There is a statistically significant difference between days with different letters in the same column (*P> 0.05*)
A, B, C (→): There is a statistically significant difference between days with different letters in the same row. (*P> 0.05*)
C: sausage without starter culture containing 1.5% salt; Sx: sausage with *S. xylosus*; Ls, sausage with *L. sakei* Pp, sausage with *P. pentosaceus*; Mix, sausage with mixed starter culture (*S. xylosus, L. sakei, P. pentosaceus*)

The *a** value determined at the beginning of storage was lower at the end of storage on the 25th day, and the redness decreased (*P> 0.05*); however, this decrease in redness was found to be significant only in the fish sausage groups containing 1.5% salt and *L. sakei* (*P< 0.05*). Similar results were reported by Hu et al. (2008), who reported that the *a** value of samples containing starter cultures was significantly lower than that of samples without starter cultures. The decrease in the *a** value and the increase in the *L** value could be attributed to pigment denaturation and the exposure of muscle proteins under acidic conditions. The coagulation of these proteins may be linked to surface water release, which can cause light scattering. Fish muscles contain sarcoplasmic proteins, including chromoproteins such as myoglobin. Myoglobin is particularly sensitive to pH and temperature changes, making it one of the most labile proteins in these contexts (Hu et al., 2007).

The changes in the *b** values of the fish sausage samples during fermentation and storage are given in Table 4. At the beginning of storage, the *b** value detected at positive values (yellow) varied between 25.13 and 29.70, but this change was not statistically significant (*P> 0.05*). On the 25th day, the last day of storage, the *b** value was found to vary between 17.08 and 19.87 (*P> 0.05*). Similar to the *L** and *a** values, the addition of different lactic acid bacteria had no effect on the *b** values of the fish sausage samples during fermentation and storage (*P> 0.05*). The *b** value determined at the beginning of storage was lower in the fish sausages at the end of storage (*P< 0.05*). An increase in yellow color may have occurred in the oxidized fish sausage samples. However, in our study, the addition of salt to the fish sausage samples and the addition of protective lactic acid bacteria delayed oxidation, and no increase in the yellow color *b** value was observed.

Table 4. Changes in the *b** values of the fish sausage samples during fermentation and storage

Day	C (1.5%)	Sx	Ls	Pp	Mix
Fermentation					
0	28.50±1.49^{Aa}	28.17±3.51^{Aa}	29.50±2.87^{Aa}	25.13±0.95^{Aa}	29.70±3.72^{Aa}
3	21.78±3.31^{Acd}	22.03±1.72^{Ab}	23.38±2.73^{Ab}	18.48±1.78^{Ab}	21.02±4.39^{Aa}
6	24.27±1.48^{Abc}	26.58±0.82^{Aa}	26.67±3.28^{Aab}	25.68±2.17^{Aa}	28.78±12.31^{Aa}
Storage					
15	25.45±1.50^{Aab}	23.22±1.78^{Ab}	22.75±3.52^{Ab}	23.70±2.45^{Aa}	23.82±4.05^{Aa}
25	19.87±2.05^{Ad}	17.88±1.69^{Ac}	17.57±2.45^{Ac}	18.70±1.32^{Ab}	17.08±1.80^{Aa}

Mean (n=2) ± std.error a, b, c (↓); There is a statistically significant difference between days with different letters in the same column (*P> 0.05*)
A, B, C (→): There is a statistically significant difference between days with different letters in the same row. (*P> 0.05*)
C: sausage without starter culture containing 1.5% salt; Sx: sausage with *S. xylosus*; Ls, sausage with *L. sakei*; Pp, sausage with *P. pentosaceus;* Mix, sausage with mixed starter culture (*S. xylosus, L. sakei, P. pentosaceus*)

3.3. Free Amino Acids

The contents of free amino acids in the fish sausages are shown in Table 5. Compared with the samples before fermentation, the fermented sausages presented a noticeable increase in total free amino acid levels (*P< 0.05*).

Several studies agree that the free amino acid content of fermented products generated via starter culture increases after fermentation (Hu et al., 2007; Zhang et al., 2013; Nie et al., 2014b; Liu et al., 2021). Similarly, in our study, the amino acid contents of the Sx, Ls, Pp, and mixed culture groups were greater than those of the control group. Free amino acid release is caused by the proteolytic activity of endogenous and microbial enzymes (Zhang et al., 2013; Tagawa et al., 2023). However, Zaman et al. (2011) reported that the addition of starter culture did not influence the production of amino acids.

Table 5. Changes in the free amino acid contents of the fish sausages fermented with/without starter culture (g/100 g)

Free amino acid	Initial sausage	Fermented sauge-25. day				
		C (1.5%)	Sx	Ls	Pp	Mix
Aspartic acid	26.73±0.1^{c}	54.08±0.18^{a}	0.83±0.03^{e}	1.28±0.09^{e}	2.05±0.13^{d}	49.87±0.2^{b}
Threonine	1.86±0.06^{e}	6.59±0.42^{d}	11.02±0.6^{ab}	8.82±0.27^{c}	11.70±0.3^{a}	10.12±0.4^{bc}
Serine	0.51±0.23^{e}	3.17±0.05^{d}	12.60±0.4^{a}	8.14±0.24^{c}	10.91±0.4^{b}	10.61±0.2^{b}
Glutamic acid	5.08± 0.22^{d}	8.85±0.22^{c}	24.31±0.2^{a}	24.11±0.32^{a}	16.6±0.18^{b}	8.59±0.11^{c}
Glycine	19.98±0.21^{a}	4.19±0.08^{e}	4.49±0.17^{e}	5.40±0.1^{d}	8.29±0.25^{b}	6.39±0.19^{c}
Alanine	5.90±0.14^{e}	25.78±0.42^{b}	22.6±0.35^{c}	20.46±0.27^{d}	26.4±0.2^{ab}	27.26±0.1^{a}
Cystine	0.01±0.00^{d}	0.02±0.00^{d}	33.21±0.4^{c}	32.98±0.22^{c}	38.53±0.3^{a}	36.71±0.2^{b}
Valine	1.90±0.16^{d}	14.10±0.13^{b}	13.89±0.3^{bc}	12.97±0.14^{c}	16.94±0.5^{a}	16.19±0.4^{a}
Methi- onine	1.36±0.16^{d}	7.44±0.12^{c}	9.51±0.27^{b}	7.87±0.2^{c}	10.51±0.1^{a}	10.49±0.1^{a}
Isoleucine	0.71±0.17^{e}	7.70±0.04^{d}	11.29±0.1^{b}	9.98±0.2^{c}	12.13±0.2^{a}	11.00±0.0^{b}
Leucine	1.84±0.14^{e}	18.95±0.08^{d}	23.16±0.2^{c}	19.46±0.3^{d}	25.26±0.2^{a}	23.92±0.0^{b}
Tyrosine	0.20±0.01^{d}	0.11±0.02^{e}	1.09±0.03^{a}	0.94±0.01^{b}	0.46±0.03^{c}	0.48±0.04^{c}
Phenyla lanine	1.89±0.16^{e}	8.64±0.10^{d}	10.78±0.2^{b}	10.00±0.1^{c}	12.29±0.1^{a}	11.97±0.1^{a}
Histidine	0.05±0.01^{c}	5.84±0.32^{b}	7.07±0.47^{b}	6.98±0.3^{b}	8.64±0.32^{a}	8.86±0.47^{a}
Lysine	0.37±0.16^{e}	21.13±0.10^{a}	11.51±0.3c^{d}	11.13±0.2^{d}	13.72±0.3^{b}	12.38±0.3^{c}
Arginine	0.01±0.00^{c}	0.23±0.02^{a}	0.24±0.01 ^{a}	0.13±0.02^{b}	0.19±0.02^{a}	0.19±0.01^{a}
Total	68.41±1.99^{e}	186.82±2.4^{d}	197.6±3.9^{c}	180.73±3.2^{d}	214.6±3.6^{b}	254.03±3.6^{a}

Mean (n=2) ± std.error a, b, c (↓); There is a statistically significant difference between days with different letters in the same column (*P> 0.05*)
A, B, C (→): There is a statistically significant difference between days with different letters in the same row. (*P> 0.05*)
C: sausage without starter culture containing 1.5% salt; Sx: sausage with *S. xylosus*; Ls, sausage with *L. sakei*; Pp, sausage with *P. pentosaceus*; Mix, sausage with mixed starter culture (*S. xylosus, L. sakei, P. pentosaceus*)

Free amino acids are strongly related to the characteristic taste development of fermented products, and amino acids such as alanine, aspartic acid, glutamic acid, leucine, methionine, serine, valine, and threonine are known as sweetening amino acids (Hu et al., 2007). In this study, the contents of sweetening amino acids and amino acids such as arginine, histidine, phenylalanine, isoleucine, and lysine increased compared with those before fermentation. Nie, Lin, et al. (2014) reported that high concentrations of valine and leucine were found in inoculated sausages. In our study, high concentrations of alanine, valine, leucine, threonine, and methionine were detected in sausages inoculated with *Pediococcus pentosaceus* and mixed cultures. The role of these amino acids as precursors for flavor compounds in fermented foods is well established (Hu et al., 2007; Nie et al., 2014a; Yuan et al., 2023).

The addition of *P. pentosaceus* and mixed culture mixture (*L. sakei, S. xylosus,* and *P. pentosaceus*) clearly affected the release of free amino acids in fermented fish sausage (*P< 0.05*). Compared with the control groups, the starter-inoculated groups presented greater contents of threonine, alanine, cysteine, valine, methionine, isoleucine, leucine, histidine, and arginine but lower contents of aspartic acid (*P< 0.05*). This result was in agreement with the results of Nie et al. (2014a). These findings are consistent with the changes in a-amino nitrogen content (Nie et al., 2014a).

3.4. Texture Properties

Figure 2 shows the results of the texture analysis of the fish sausage samples during fermentation and storage.

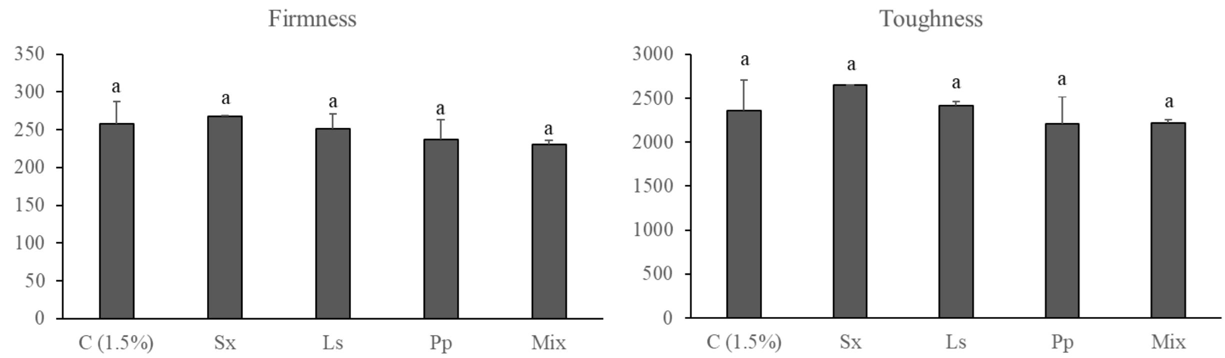

Figure 2. Results of texture analysis of the fish sausage samples

At the end of storage, the firmness values of the experimental groups varied between 230.70 and 267.51 (*P> 0.05*), but there was no significant difference between the control and bacteria-treated groups. Similarly, there was no significant difference between the hardness values of the experimental groups (*P> 0.05*).

Among texture properties, firmness and toughness are important properties for consumers because they determine the acceptability of a product's texture (Chambers and Bowers, 1993). Many minced food products, such as meatballs, sausages and sausages, have a cutoff point at which the texture is unacceptable (Yu and Yeang, 1993). In our study, according to the results of the shear test, the firmness value was lower in the experimental group containing mixed culture than in the other groups. The highest firmness value was determined in sausage samples containing *S. xylosus*. However, the difference between the firmness values of the groups was not significant (*P> 0.05*).

The toughness value is the ability of the food to resist any applied force. The toughness value obtained from shear force analysis is the sufficient force required to compress a sample (Roldán et al., 2013). In this study, the toughness values of the sausage samples ranged from 2209.55--2732.42. The lowest toughness value was determined in sausage samples containing *P. pentosaceus,* and the highest value was determined in sausage samples containing *S. xylosus*. The increase in toughness is due to denaturation of collagen in the meat or a change in the myofibrillar structure of the meat (Bıyıklı et al., 2020).

4. Conclusions

This study evaluated the effects of three amine-negative starter cultures, *L. sakei* subsp. *sakei*, *S. xylosus*, and *P. pentosaceus,* both individually and in combination, on the nutritional composition, textural properties, color characteristics, and amino acid profile of fish sausages during ripening and storage. The results demonstrated that while the moisture content decreased significantly during fermentation, it increased during storage, with no significant differences observed in moisture content on days 15 and 25. The protein and fat contents remained stable throughout the study, with only initial differences noted. The highest ash content was found in sausages containing *S. xylosus*.

Amino acid analysis revealed a significant increase in total free amino acid content after fermentation, particularly in sausages inoculated with mixed cultures ($P< 0.05$). The presence of essential amino acids such as alanine, valine, leucine, threonine, and methionine was notably greater in sausages inoculated with *P. pentosaceus* and mixed cultures, contributing to enhanced flavor profiles.

On the basis of these findings, *S. xylosus*, *P. pentosaceus* and mixed cultures have emerged as the most beneficial starter cultures because of their positive effects on ash content, overall stability during storage, and enhanced amino acid profile. This study contributes to the development of higher-quality fish sausages, offering practical insights for improving product attributes and advancing the commercial production of fish sausages.

Acknowledgments

This study was supported by Izmir Katip Celebi University Scientific Research Project Coordination (Project number: 2021-GAP-SUÜF-0003).

Authors' Contributions

Study conception and design: FÖ, HG; data collection: FÖ, HG, FG; analysis and interpretation of results: FÖ, HG, FG; draft manuscript preparation: FÖ, HG,. All authors reviewed and approved the final version of the manuscript.

Statement of Conflicts of Interest

The authors declare that there are no conflicts of interest.

Statement of Research and Publication Ethics

The authors declare that this study complies with Research and Publication Ethics.

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