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Research Article

Fermented Rainbow Trout Production Using *Lactobacillus sakei* and *Saccharomyces cerevisiae*: Effects on Microbiological, Biochemical, and Sensory Quality

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ABSTRACT

The aim of this study was to evaluate the microbiological, biochemical and sensory properties of fermented rainbow trout (*Oncorhynchus mykiss*) using *Lactobacillus sakei* ATCC® 15521™ (L), *Saccharomyces cerevisiae* ATCC® 9080™ (S) and their combination (M) as starter cultures and without starter culture as spontaneous fermentation (F). Rainbow trout slices (94% w/w) were mixed with curing ingredients—sucrose (2% w/w), salt (3% w/w), garlic powder (0.3% w/w), ginger powder (0.3% w/w), and sweet red pepper powder (0.3% w/w)—and kept at 4 °C for 2 days. After the cured trout were dried at 60 °C for 3 hours, starter cultures were evenly injected into the trout slices at a rate of 1% (1 mL starter culture: 100 g cured fish). The fermentation process was conducted at 24 °C for 14 days. During fermentation of the trout, the recommended 6 log CFU/g value for LAB, which is beneficial for gastrointestinal health, was reached on different days depending on the group: on day 12 in the F group (6.07 ± 0.25 log cfu/g), on day 4 in the S group (6.25 ± 0.11 log cfu/g), and on day 2 in the M group (6.52 ± 0.09 log cfu/g). Additionally, the highest value in the L group was 7.21±0.11 log cfu/g on day 2, indicating rapid fermentation. *Enterobacteriaceae* counts remained low across all groups, with values dropping to <1 log cfu/g by the end of fermentation. The results showed that the pH of fresh fish, initially 6.70, decreased significantly to 4.01, 4.18, and 4.09 in L, S, and M groups, respectively, by the end of fermentation. Nutritional analysis revealed higher protein content and reduced lipid oxidation levels in the M group, which also exhibited the lowest levels of total volatile basic nitrogen and thiobarbituric acid, indicating improved freshness and oxidative stability. Sensory evaluation identified the M group as the most preferred due to its balanced acidity, texture, and flavor. These findings suggest the potential of combining *L. sakei* and *S. cerevisiae* to produce high-quality fermented trout with enhanced safety, nutritional value, and consumer acceptance. This study represents the first effort to produce the fermentation process specifically for rainbow trout, marking a contribution to the field of fermented fish production.

Keywords: Fermentation, Starter culture, Lactic acid bacteria, Yeast, Microbial safety, Biochemical properties, Sensory characteristics, Fermented fish

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INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is one of the inexpensive, abundant, and widely consumed freshwater fish species due to its high nutritional value, mild flavor, and versatility in processing (Wilburn & Ryan, 2017; Escamilla-Rosales et al., 2024). However, freshwater fish, unlike marine fish, are marketed primarily for fresh consumption (Zang et al., 2020; Tavares et al., 2021). Traditional fish processing methods such as smoking, salting, and drying of freshwater fish often involve high salt concentrations, which may also limit their nutrition-

al benefits upon consumption (Belleggia & Osimani, 2023). The use of fermentation in processing these fish species appears to be a promising approach. Fermenting fish for human consumption has several benefits (Gelman et al., 2000; Paludan-Muller et al., 2002). It is a low-cost method to develop new fermented fish products that reduce fishy odors, increase the nutritional value and organoleptic qualities of fish, and increase consumption by providing long-term preservation of rainbow trout (Kılınç & Çaklı, 2021; Cai et al., 2024).

The use of LAB and yeasts together in fish fermentation contributes to the microbial safety (barrier technology) and sensory quality of fermented fish products. Therefore, significant research is still needed to better understand the optimal conditions for fish fermentation and the interactions between microorganisms (Wang et al., 2020; Chan et al., 2023). LAB ensure product safety by reducing pH through sugar fermentation, while yeasts affect aroma and texture properties (Glover et al., 2009; Carvalho et al., 2020). The proteolytic and lipolytic activities of these microorganisms contribute to the degradation of proteins and lipids, forming flavor compounds that increase the overall acceptability of the final product (Zang et al., 2018; Laranjo et al., 2019). However, little is known about the microbiological and biochemical changes that occur in freshwater fish fermentation inoculated with the combination of *Lactobacillus sakei* and *Saccharomyces cerevisiae*.

Some fermented fish products are very popular in eastern countries and also in some parts of western countries. In recent years, the use of pure or mixed bacterial cultures to produce fermented products has become increasingly popular (Siddiqui et al., 2023). Previous research has demonstrated the potential of using starter cultures in fermentation in fish processing and highlighted its impact on food safety, sensory properties and overall quality improvement. Bernbom et al. (2009) studied the fermentation of trout (*Salmo trutta*) with salt and boiled rice, showing that only the addition of at least 6% garlic combined with *L. plantarum* 509 effectively inhibited *Salmonella*, improving food safety. Zhu et al. (2019) investigated the fermentation of sturgeon (*Acipenser sinensis*) with *S. cerevisiae*, finding that it enhanced flavor, aroma, texture, and color, while increasing free amino acids, fatty acids, and peptides. Li & Xu (2021) examined the role of *L. plantarum* in fish paste fermentation, reporting that it inhibited the lipolysis and flavor formation caused by *S. cerevisiae*, affecting the biochemical properties of the product. An et al. (2022) evaluated the impact of *L. plantarum*, *Pediococcus acidilactici*, and *P. pentosaceus* on Zhayu, a traditional Chinese fermented fish, demonstrating that lactic acid bacteria improved food safety and quality by lowering pH and TVB-N levels. Zeng et al. (2013) analyzed the effects of autochthonous starter cultures (*L. plantarum*, *Staphylococcus xylosus*, and *S. cerevisiae*) on the microbiological and physicochemical properties of Suan yu, a low-salt fermented fish made from common carp (*Cyprinus carpio*). Zang et al. (2018) investigated the microbial changes during the fermentation of Suan yu, showing that a 1:1:1 mixture of *L. plantarum* 120, *S. xylosus* 135, and *S. cerevisiae* 31, inoculated at 1% concentration and fermented at 25°C, influenced the microbial flora throughout the process. Despite the increasing interest in fish fermentation, research on the production and quality of fermented rainbow trout using specific starter cultures has not been encountered. The aim of this study was to produce fermented trout with *L. sakei* ATCC® 15521™ (L), *S.cerevisiae* ATCC® 9080™ (S), their combination (M), and a spontaneously fermented control group (F) and to monitor their quality throughout fermentation.

MATERIALS AND METHODS

Preparation of the starter cultures

Twice-subcultured starter cultures, *Lactobacillus sakei* ATCC® 15521™ was incubated in Man Rogosa and Sharpe (MRS, Merck, Germany) broth, and *Saccharomyces cerevisiae* ATCC® 9080™ was incubated in Yeasts and Molds (YM, DifcoTM, France) broth at 37°C for 24 hours for use in trout fermentation. After incubation, cell pellets were harvested by centrifugation at 10,000 x g for 10 minutes at 4°C and washed twice with sterile saline (0.9% NaCl). The pellets were resuspended in the same saline solution, and the cell concentrations were adjusted to 8-9 log cfu/g for *L. sakei* and 5-6 log cfu/g for *S. cerevisiae* by measuring the optical density (OD) at 600 nm. A mixed culture of *L. sakei* and *S. cerevisiae* in a 1:1 (v/v) ratio was used for inoculation. The starter cultures were stored at -80°C with 30% sterile glycerol solution.

Preparation of fermented rainbow trout

The fermented trout was prepared according to the suan-yu fermented carp fish production technique described by Zeng et al. (2013), with modifications based on preliminary studies of the fermentation process on sliced trout. The Black Sea rainbow trout used in the study were sourced fresh from Isparta Fish Market (July 2024), cleaned, and aseptically sliced into pieces weighing 100 ± 10 g at the Food Processing Laboratory of the Eğirdir Fisheries Faculty. A total of 8 fish, each weighing 1.5 ± 0.5 kg, were used, resulting in approximately 12 kg of fish. Rainbow trout slices (94% w/w) and curing ingredients—sucrose (2% w/w), salt (3% w/w), garlic powder (0.3% w/w), ginger powder (0.3% w/w), and sweet red pepper powder (0.3% w/w)—were kept at 4 °C for 2 days. Then cured samples were dried in a digital oven (Crystal, CKD 044 E, Germany) at 60 °C for 3 hours. After drying for fermentation, 1% (1 mL starter culture: 100 g cured fish) of the inoculation solution was homogeneously applied to the trout slices (Sürengil, 2024). The trout slices were divided into four groups, each containing 25 slices. These slices were tightly wrapped in stretch film and packed in polystyrene containers for fermentation, with two slices per package. The containers were sealed to prevent contamination and moisture loss during the fermentation process. Fermentation was conducted in an incubator (NÜVE ES110, Türkiye) at 24 ± 2°C for 14 days. Analyses were performed on all groups at day 0 (before inoculation), after curing, and on days 2, 4, 6, 8, 10, 12, and 14. Additionally, analyses were performed on fresh fish (T), cured fish (C), and fermented trout groups: without bacteria (F); with *L. sakei* starter culture (L); with *S. cerevisiae* starter culture (S); and with a combination of *L. sakei* and *S. cerevisiae* starter cultures (M).

Microbiological analyses

Microbiological analyses were performed in the Food and Processing Laboratory of Isparta University of Applied Sciences, Eğirdir Fisheries Faculty (AOAC 2000). In these analyses, 10 g of fish sample was transferred to a sterile bag under aseptic condi-

tions and homogenized with 90 ml of sterile peptone water (Maximum recovery diluent, MRD, Merk, Germany) solution in Stomacher (Seward 400, London, UK) for 1 minute. The microbial counts were determined using the pour plate method: LAB on De Man, Rogosa and Sharpe (MRS, Merk, Germany) agar medium at 96 hours at 30°C; Yeast-mold (YM) on Yeast Extract Glucose Chloramphenicol (YGC, Merk, Germany) agar medium at 24 hours at 30 °C; and Enterobacteriaceae (ENT) on Violet Red Bile Dextrose (VRBD, Merk, Germany) Agar medium at 24 hours for 30 °C. The analysis results were expressed as colony-forming units per gram (log CFU/g).

Biocochemical and proximal analyses

Moisture determination was performed by placing approximately 0.5 g of sample in a moisture measuring device (AND MX-50, Japan). The moisture content was calculated as a percentage of the weight loss. The total ash content (%) was determined by burning 2 g of the sample in a muffle furnace (Ash Furnace Nüve TS 500) at approximately 500°C for 4 hours. Fat content determination; a 5 g sample was first dried in an oven (WiseVen Witeg, Germany) at 105°C, and then crude fat was extracted using the Soxhlet method with diethyl ether. The amount of fat (%) in each the sample was calculated. Protein content determination; the Velp UD-20 protein pre-combustion unit was used according to the Kjeldahl method (Nx6.25) with the Velp UDK 142 protein distillation unit. As the exact fermentation time was not determined, the proximate analyses were performed on the 2nd day, when the first bacteria were inoculated. pH analysis; 10 g of the samples were weighed and 10 ml of distilled water was added and homogened in the homogenizer. The pH meter (Hanna HI221) probe was immersed in the prepared sample, and the reading was recorded (AOAC, 1990). Lactic acid value; a 10 g sample was taken, homogenized with 100 ml of pure water, and then titrated with 0.1 N NaOH to pH 8. The % lactic acid was determined based on the amount of NaOH consumed (Acton and Keller, 1974).

Determination of thiobarbituric acid (TBA) and total volatile basic nitrogen (TVB-N)

TBA analysis was performed according to the method by Gassem (2019). Ten grams of the sample were homogenized with 50 mL of distilled water. The pH was adjusted to 1.5 by adding 2.5 mL of 4 N HCl. Five milliliters of the distillate solution were boiled with 5 mL of TBA reagent (0.2883 g thiobarbituric acid in 100 mL of 90% acetic acid) for 35 minutes. The absorbance was measured at 535 nm using a spectrophotometer (PG INSTRUMENTS- T80+ UV/VIS), and MDA concentration was calculated as mg MDA per kg by multiplying the absorbance by 7.8.

TVB-N analysis was performed according to the method by Antonocoupoulos and Vyncke (1989). The sample was homogenized by adding 1-2 g of MgO to 10 g of the sample in Kjeldahl tubes, and the solution was distilled with 3% boric acid using the Velp UDK 142 protein distillation unit. The final distillate was titrated with 0.1 N HCl and the consumption was calculated based on the amount of acid used.

Sensory evaluation

Fermented fish produced was submitted for sensory evaluation to analyze whether the control sample (F) and the samples with mixed starter cultures (L, S and M) differed. A panel of 20 individuals, consisting of faculty members and graduate students with experience in seafood consumption at Isparta University of Applied Sciences, was selected, and the panelists were informed about the product. Fermented products at room temperature were coded with three-digit random numbers; they were evaluated for appearance, color, odor, taste, saltiness, texture and overall taste. A nine-point hedonic scale for evaluation; in which 1 point equals very dislike, 5 points equals moderate neither like nor dislike, and 9 points equals extremely liked. The sensory evaluation results were calculated by taking the mean and standard deviation of the panelists' scores (Zeng et al., 2014).

Statistical analysis

All analyses were conducted in three parallel groups in fermented fish groups, and one-way analysis of variance (ANOVA) was performed with the SPSS (version 22.0, IBM, USA) statistical package program, and the differences were determined with the Duncan Multiple Comparison Test. Values are expressed as "mean ± standard deviation".

RESULTS AND DISCUSSION

Microbiological results

Fermentation significantly influenced LAB development in different groups. While LAB was not detected in the naturally fermented F and L groups on the first day after heat treatment, the S group (inoculated with S. cerevisiae) had 5.78 ± 0.01 log CFU/g, and the M group had 4.82 ± 0.11 log CFU/g ($P<0.05$). LAB counts were performed before inoculation to ensure consistency and observe fermentation-related changes. The LAB presence in the S group is attributed to the natural microbiota of the fish and spontaneous fermentation, though its increase remained lower than in the inoculated groups. A synergistic effect between LAB and yeast was observed, but dominant species exhibited more pronounced growth, highlighting the role of starter cultures.

LAB counts in fermented fish with mixed cultures increased significantly throughout fermentation ($P<0.05$, Table 1). The initial LAB count was 2.39 ± 0.20 log CFU/g in fresh fish, 3.85 ± 0.27 log CFU/g in cured trout, and 3.16 ± 0.53 log CFU/g on day 0 after heat treatment. These values were obtained from preliminary analyses conducted prior to the fermentation process. LAB growth in naturally fermented trout was slower in the early stages than in starter culture-inoculated products ($P<0.05$), emphasizing the impact of controlled fermentation on microbial dynamics. The 6 log value, which is recommended for beneficial effects on gastrointestinal health (Hemarajata and Versalovic, 2013), was reached on different days depending on the group. Notably, in the L group, the highest value of 7.21 ± 0.11 log CFU/g was reached on the 2nd day, while in the M group, the highest value of 6.52 ± 0.09 log CFU/g was also observed on the 2nd day, indicating rapid growth at early stages of fermentation. Zeng et al. (2013) reported that the LAB count in carp suan-yu fermented fish inoculated with different mixed starter cultures was significantly different from the control group and gradually increased over 14 days, reaching values between 8.59 and 8.73 log CFU/g. However, the control group reached 8.31 log CFU/g with a slower increase by the 21st day, and the LAB count remained stable during

Table 1. Microbiological results in the fermented trout during fermentation (Log cfu/g).

DAY		0	2	4	6	8	10	12	14
LAB	F	3.16±0.53^{aA}	4.17±0.21^{aAB}	4.33±0.42^{aB}	5.01±0.12^{aB}	5.21±0.25^{aB}	5.34±0.15^{adB}	6.07±0.25^{aB}	6.01±0.21^{aB}
	L	3.16±0.53^{aA}	7.21±0.11^{dB}	8.56±0.04^{dC}	8.87±0.02^{cC}	9.12±0.35^{cdC}	9.34±0.66^{cC}	9.35±0.75^{cC}	9.37±0.05^{cC}
	S	3.16±0.53^{aA}	5.14±0.12^{bB}	6.25±0.11^{bC}	6.67±0.52^{bC}	7.11±0.24^{bCD}	7.87±0.01^{bD}	7.79±0.25^{bD}	7.61±0.02^{bC}
	M	3.16±0.53^{aA}	6.52±0.09^{bB}	7.75±0.17^{cC}	8.99±0.13^{cCD}	9.24±0.08^{cE}	9.27±0.04^{cE}	9.12±0.42^{cE}	9.07±0.11^{cE}
YM	F	<1	3.60±0.42^{aA}	4.73±0.02^{bB}	4.86±0.17^{bB}	5.11±0.13^{bB}	5.10±0.05^{bB}	5.15±0.42^{bB}	5.03±0.07^{bB}
	L	<1	3.67±0.12^{aA}	3.85±0.31^{aA}	3.72±0.03^{aA}	3.88±0.17^{aA}	4.19±0.13^{aA}	4.12±0.42^{aA}	4.21±0.06^{aA}
	S	<1	6.45±0.18^{cA}	6.58±0.15^{cA}	6.92±0.14^{cA}	6.98±0.32^{cA}	6.93±0.15^{cA}	6.91±0.31^{cA}	6.87±0.07^{cA}
	M	<1	5.85±0.37^{bA}	5.90±0.11^{bcA}	6.15±0.15^{cA}	6.82±0.01^{cA}	6.86±0.11^{cA}	6.85±0.21^{cA}	6.52±0.33^{cA}
ENT	F	2.29±0.05^{aA}	2.51±0.04^{aA}	2.42±0.3^{aA}	2.31±0.22^{aA}	2.30±0.2^{aA}	2.28±0.17^{aA}	<1	<1
	L	2.29±0.05^{aA}	2.17±0.03^{aA}	<1	<1	<1	<1	<1	<1
	S	2.29±0.05^{aA}	2.13±0.03^{aA}	2.12±0.2^{aA}	<1	<1	<1	<1	<1
	M	2.29±0.05^{aA}	<1	<1	<1	<1	<1	<1	<1

F: naturally fermented trout, L: *L. sakei* starter culture added, S: *S. cerevisiae* starter culture added, M: *L. sakei* and *S. cerevisiae* starter cultures added
Values with different uppercase letters in the same row indicate significant differences between days (P<0.05).
Values with different lowercase letters in the same column indicate significant differences between groups (P<0.05).

subsequent ripening. Similar to the current study, the LAB counts in samples inoculated with starter cultures were significantly different from those in the control groups (P < 0.05). This indicates that L. sakei adapted well to the fish during fermentation and became the dominant flora.

Yeast and mold (YM) counts varied significantly among the fermentation groups (P < 0.05, Table 1). While YM was detected in the F and L groups 3.60±0.42 and 3.67±0.12 on the 2th days, it was 6.45±0.18 log CFU/g in the S group, which received *S. cerevisiae*, and 5.85±0.37 log CFU/g in the M group. In the naturally fermented F group, a gradual increase was observed, reaching 5.15 ± 0.42 log CFU/g by day 12. The L group exhibited the lowest YM counts throughout fermentation, with values remaining below 4.21 ± 0.06 log CFU/g. In contrast, the S and M groups, which contained *S. cerevisiae*, showed significantly higher yeast counts from the early stages. The S group reached its highest value (6.98 ± 0.32 log CFU/g) on day 8, while the M group followed a similar trend, peaking at 6.86 ± 0.11 log CFU/g on day 10. These results indicate that the addition of *S. cerevisiae* significantly influenced yeast proliferation, whereas the *L. sakei*-inoculated L group exhibited lower YM growth, likely due to LAB-driven competition or inhibitory effects. These results showed that LAB were the dominant microorganisms during early fermentation due to the intense fermentation activity in fish meat. *L. sakei* exhibited strong competitive power, growing rapidly during trout fermentation. In rainbow trout fermented with mixed cultures, *S. cerevisiae* and *L. sakei* demonstrated strong competitiveness, inhibiting the growth of other microorganisms, including yeast, through the production of organic acids and bacteriocins (P > 0.05). Similarly, Liu et al. (2021) observed that YM did not grow in the initial stages of fermentation in suan-yu products prepared without the addition of *L. plantarum* B7 and LAB. After 7 days, the pH of the fermentation stabilized, and acid-resistant yeasts reached a maximum value of 4 log CFU/g. Kılınç and Sürengil (2016) found that LAB was the dominant flora compared to YM during fermented pastrami production in whiting. LAB counts increased throughout the storage period, suppressing *Enterobacteriaceae* counts at the beginning of storage.

The *Enterobacteriaceae* (ENT) values are shown in Table 1. After heat treatment, the ENT count was 2.29 ± 0.05 log CFU/g on the first day. There was no significant difference between the groups on the first day (P > 0.05). The ENT counts were significantly lower in starter culture-inoculated groups during fermentation compared to the naturally fermented group (P < 0.05). During fermentation, ENT counts exhibited a significant decrease in starter culture-inoculated groups compared to the naturally fermented F group (P < 0.05). In the F group, ENT counts gradually declined, becoming undetectable (<1 log CFU/g) by day 12. In contrast, the L group showed a rapid reduction, with ENT dropping below detectable levels by day 4. The S group reached undetectable levels by day 6, and the M group showed a rapid decrease, becoming undetectable as early as day 2, as indicated in the table 1. The observed reductions in ENT levels align with previous findings on the antimicrobial effects of LAB. The acidification caused by LAB activity likely inhibited pathogenic and spoilage microorganisms, creating unfavorable conditions for ENT survival. Adab et al. (2018) emphasized the role of LAB in inhibiting enterobacteria through organic acid production, a mechanism that contributes to the stability of fermented meat and fish products. Likewise, Anihouvi et al. (2007) reported significant decreases in ENT counts in fermented fish inoculated with mixed starter cultures, reinforcing the competitive advantage of LAB over enterobacteria. Additionally, Yang et al. (2012) and Darbandi et al. (2022) highlighted the role of LAB-produced bacteriocins in suppressing ENT and other foodborne pathogens. The combination of *L. sakei* and *S. cerevisiae* (M group) demonstrated the most rapid suppression of ENT, suggesting a synergistic effect between these cultures in improving microbial safety. The results of this study confirm that controlled fermentation using starter cultures enhances food safety by effectively suppressing ENT growth in fermented fish products.

Chemical results

The pH and titratable acidity (TA) values of the fresh rainbow trout used in the study on the first day were determined as 6.70 ± 0.06 and 0.17 ± 0.01, respectively, while the values for the cured trout were 7.10 ± 0.08 and 0.21 ± 0.05. In the fermented products,

the pH values of the natural and starter culture-applied samples were observed to be lower than those detected in the control group during fermentation, as shown in Figure 1. While the pH of fresh fish meat was 6.70, the pH values of the L, S, and M groups decreased to 4.01, 4.18, and 4.09, respectively, within 14 days. However, the pH of the control group remained at values between 5.37 and 5.08 until the 10th day of fermentation, eventually decreasing to 4.52. These shows that the pH values are significantly different ($P < 0.05$) compared to products prepared with mixed starter cultures.

In the fermented products, the TA values of the natural and starter culture-applied samples were observed to be higher than those detected in the control group during fermentation, as shown in Figure 2. While the TA of fermented products increased significantly ($P < 0.05$), the F, L, S, and M values increased from 0.17 to 1.05, 3.07, 2.18, and 3.13, respectively, during 14 days of fermentation and storage. The TA values increased in parallel with the LAB count throughout the fermentation periods ($P < 0.05$) but remained almost unchanged at the end of storage, i.e., during ripening ($P > 0.05$). Although there was no statistically significant change in the F group samples, gradual and slow increases were observed throughout storage ($P > 0.05$). However, the TA of the control group was lower than that of the products prepared with starter cultures ($P < 0.05$). Significant differences were observed in product M compared with other groups ($P < 0.05$), although no significant change occurred after the 4th day ($P > 0.05$). Similarly, no significant changes were observed in the L group from day 4 or in the S group from the 8th day ($P > 0.05$). In previous studies conducted on fermented fish, a similar result was obtained in Suanyu products, and it was reported that the pH value in fermented fish using starter culture decreased below 5 after 1 week (Zang et al., 2018; Lv et al., 2022). The decrease in pH is attributed to the increase in organic acids in meat due to LAB development during fermentation (Hwanhlem et al., 2011). It has also been stated that the rapid decrease in pH to 4.5 within the first 48 hours of fermentation is essential for preventing the development of pathogens and harmful bacteria, ensuring the safety, texture, and flavor development of fermented products (Kılınç, 2004; Panda et al., 2011; Benkerroum, 2013). Acid changes and the density and increase of various organic acids (mostly lactic acids) are crucial for the fermentation of fish (Kuley et al., 2020). The gradual decrease in pH in all products during fish fermentation, particularly the rapid decrease in pH and the increase in acid value in the groups where *L. sakei* was applied as a starter culture, indicate that trout meat adapted well to the lactic acid environment. The significantly higher lactic acid concentrations in the starter culture-inoculated groups further support this finding.

Nutritional component analyses of natural and starter culture-applied samples of fermented products are shown in Table 2. The nutrient compositions of fresh rainbow trout and fermented products differed statistically significantly in terms of protein, moisture, fat and ash contents ($P < 0.05$). The presence of high protein content and low moisture in all fermented fish indicates that fermented fish can be consumed as a nutritious and safe product. The results reveal that all fermented fish products, whether naturally fermented or inoculated with starter cultures, showed notable changes compared to fresh trout, indicating the significant influence of fermentation processes on the nutritional profile of the fish ($P < 0.05$). The addition of starter cultures further enhanced protein content, with the L group and M group showing higher protein levels (25.03 ± 0.45 g/100 g and 26.18 ± 0.48 g/100 g, respectively) compared to the naturally fermented group (F), which had a protein content of 20.08 ± 0.51 g/100 g. The synergistic effect of the combined LAB and yeast cultures in the M group may have accelerated the breakdown of proteins, leading to more efficient protein degradation and an overall increase in protein content. The L group (*L. sakei*) also showed a higher fat content (3.00 ± 0.05 g/100 g) compared to the naturally fermented group (2.90 ± 0.03 g/100 g), though it

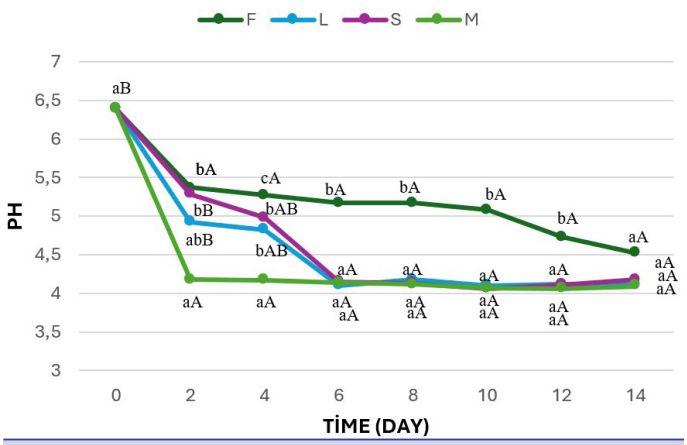


Figure 1. The pH in fermented trout during fermentation.
F: naturally fermented trout, L: with *L. sakei* starter culture added, S: with *S. cerevisiae* starter culture added, M: with *L. sakei* and *S. cerevisiae* starter cultures added
Values with different uppercase letters in the same row indicate significant differences between days (P<0.05).
Values with different lowercase letters in the same column indicate significant differences between groups (P<0.05).

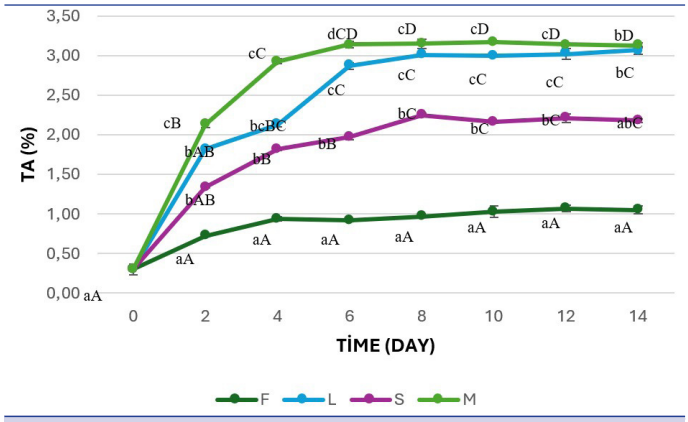


Figure 2. The titratable acidity in fermented trout during fermentation.
F: naturally fermented trout, L: with L. sakei starter culture added, S: with S. cerevisiae starter culture added, M: with L. sakei and S. cerevisiae starter cultures added
Values with different uppercase letters in the same row indicate significant differences between days (P<0.05).
Values with different lowercase letters in the same column indicate significant differences between groups (P<0.05).
Values with different lowercase letters in the same column indicate significant differences between groups (P<0.05).

Table 2. Nutritional component of fermented trout (g/100 g).

	Protein	Fat	Moisture	Ash
T	19.39 ± 0.35[a]	2.05 ± 0.04[a]	76.36 ± 0.15[a]	2.63 ± 0.01[a]
F	20.08 ± 0.51[b]	2.90 ± 0.03[bc]	69.0 ± 0.11[b]	3.09 ± 0.04[b]
L	25.03 ± 0.45[cd]	3.00 ± 0.05[c]	68.6 ± 0.15[b]	3.10 ± 0.08[b]
S	24.68 ± 0.76[c]	2.47 ± 0.12[b]	68.9 ± 0.17[b]	3.01 ± 0.02[b]
M	26.18 ± 0.48[d]	3.96 ± 0.07[d]	68.7 ± 0.21[b]	3.11 ± 0.01[b]

T: fresh rainbow trout, F: naturally fermented trout, L: *L. sakei* starter culture added, S: *S. cerevisiae* starter culture added, M: *L. sakei* and *S. cerevisiae* starter cultures added
Values with different uppercase letters in the same row indicate significant differences between days (P<0.05).
Values with different lowercase letters in the same column indicate significant differences between groups (P<0.05).

was slightly lower than the M group. The S group (*S. cerevisiae*) showed a moderate increase in fat content (2.47 ± 0.12 g/100 g), which could indicate that yeast alone has a less pronounced effect on lipid metabolism in fermented fish. The L, S, and M groups showed similar moisture levels (68.6 ± 0.15 g/100 g to 69.0 ± 0.11 g/100 g), suggesting that the addition of starter cultures (whether LAB or yeast) had similar effects on moisture retention during fermentation. This is consistent with the idea that fermentation not only reduces moisture but also modifies the texture and enhances the overall flavor profile of the fish. Ash content, which is an indicator of the mineral content, was highest in the M group (3.11 ± 0.01 g/100 g) and lowest in the T group (2.63 ± 0.01 g/100 g). The increase in ash content during fermentation is expected, as minerals from the fish tissue become more concentrated due to the loss of moisture. The addition of starter cultures, particularly the combination of LAB and yeast in the M group, may have contributed to enhanced mineral retention by affecting the solubility of mineral compounds during fermentation. This is in line with findings from other studies, where the fermentation process improved the mineral content of fermented products by reducing water content and concentrating mineral elements. Xu et al. (2010) reported an increase in fish meat protein values in fermented silver carp sausage using *Pediococcus pentosaceus* during a 48-hour fermentation period. The gradual increase in protein is attributed to the degradation of salt-soluble and water-soluble proteins as pH decreases. Wang et al. (2017) found that adding LAB could increase acid activity in fish meat, thereby promoting a decrease in moisture and a parallel increase in lipid content and protein degradation. Thus, fermentation of fish meat with starter cultures causes significant changes in nutritional content due to the rapid proliferation of LAB and a rapid decrease in pH ($P < 0.05$).

In the fermented products, total volatile basic nitrogen (TVB-N) values of natural and starter culture-applied samples were observed to be higher in the control group compared to the starter culture-added groups during fermentation, as shown in Figure 3. In all starter culture-inoculated groups, TVB-N increased to 21.93, 19.91, and 17.13 mg/100 g in the L, S, and M groups, respectively, at the end of storage. According to the quality classification based on chemical criteria, samples with TVB-N values below 25 mg/100 g are considered very good, those with values around 30 mg/100 g are classified as good, samples with values between 30–35 mg/100 g are considered marketable, while those exceeding 35 mg/100 g are regarded as spoiled (Huss, 1988; Varlık et al., 1993). The TVB-N of naturally fermented trout increased from

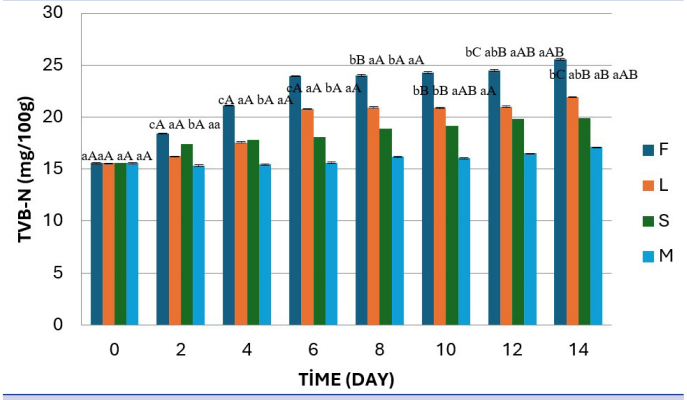


Figure 3. The TVB-N (mg/100g) in fermented trout during fermentation.
F: naturally fermented trout, L: with L. sakei starter culture added, S: with S. cerevisiae starter culture added, M: with L. sakei and S. cerevisiae starter cultures added
Values with different uppercase letters in the same row indicate significant differences between days (P<0.05).
Values with different lowercase letters in the same column indicate significant differences between groups (P<0.05).

15.58 ± 0.06 mg/100 g to 25.57 mg/100 g, remaining within the acceptable freshness range. Although it reached the freshness standard (25 mg/100 g–30 mg/100 g), this TVB-N remained below the national standard (35 mg/100 g). TVB-N is a commonly used index for evaluating fish freshness (Oyelese et al., 2013), and it increases steadily during fermentation due to the production of alkaline, nitrogen-containing substances by enzymatic and microbial decomposition of fish (Weiner et al., 2015). These compounds also contribute to the characteristic flavor of fermented fish (Debevere and Boskou, 1996). Preventing the increase in TVB-N and improving the safety of fermented fish can be achieved using mixed cultures that facilitate carbohydrate breakdown. TVB-N contents in group L and S samples increased gradually during fermentation, but the TVB-N content of naturally fermented trout was significantly higher than that of products fermented with starter culture ($P < 0.05$). Hu et al. (2008) reported similar findings, noting that LAB and *S. cerevisiae* applications slowed TVB-N accumulation by producing lactic acid and bacteriocins, consistent with our study.

In the fermented products, thiobarbituric acid (TBA) values of the natural and starter culture-applied samples were observed to be higher in the control group compared to the starter culture-add-

ed groups during fermentation, as shown in Figure 4. TBA values are often used as an indicator of lipid oxidation in foods (Wang et al., 2016). According to Sikorski et al. (1990), the TBA value of fish products should be <5 mg/kg. In this study, all products remained below this limit. The growth rate of TBA content in naturally fermented trout was higher than that in trout fermented with starter culture ($P < 0.05$). The TBA content of naturally fermented trout was significantly higher ($P < 0.05$) than that of trout inoculated with starter culture. Bao et al. (2018) reported initial TBA contents of natural, L, W, and LW samples ranging from 0.03 to 0.72 mg MDA/kg. By the end of a 5-day fermentation period, the highest TBA value reached 1.93 mg MDA/kg in the natural group, while the LAB group exhibited the lowest TBA value at 1.21 mg MDA/kg. LAB has been reported to have antioxidant effects on unsaturated fatty acids, suppressing the increase in TBARS in fermented fish (Zeng et al., 2013) and dry sausages (Sun et al., 2016). Consistent with these findings, starter cultures in this study effectively prevented oxidation of unsaturated fatty acids, demonstrating strong antioxidant activity (Zeng et al., 2014).

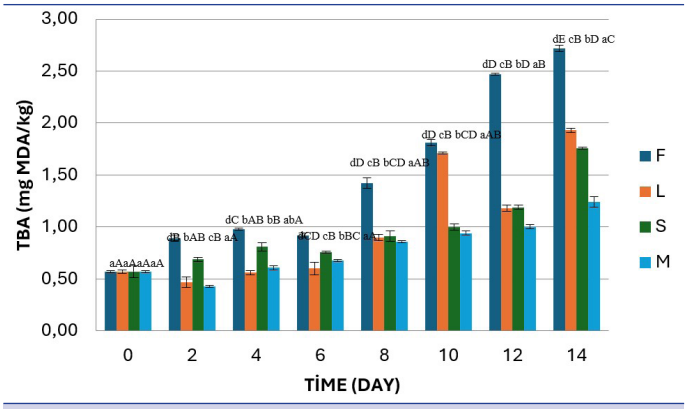


Figure 4. The TBA (mg MDA/kg) in fermented trout during fermentation.

F: naturally fermented trout, L: with L. sakei starter culture added, S: with S. cerevisiae starter culture added, M: with L. sakei and S. cerevisiae starter cultures added
Values with different uppercase letters in the same row indicate significant differences between days (P<0.05).
Values with different lowercase letters in the same column indicate significant differences between groups (P<0.05).

Sensory evaluation

The sensory properties of fermented trout are shown in Figure 5. It was determined that the appearance and color sensory properties of the L and F groups were similar to each other (P>0.05), while the S group was the most liked in terms of these criteria (P<0.05). The sensory properties of the M and S groups were statistically similar (P>0.05), indicating positive sensory effects in the presence of S. cerevisiae in the product. Controlled humidity adjustment as a result of controlled heat treatment before fermentation created a similar sensory salinity effect for all groups; therefore, no significant difference was observed between them (P>0.05).

The most liked group in terms of taste, texture, and overall appreciation, which was significantly different from the other groups, was the product with mixed starter culture (P<0.05). These findings are consistent with the expected results that using starter culture in fermented products can enhance sensory quality (Abdel-Naeem et al., 2021; Christ-Ribeiro et al., 2021). This improvement is likely due to the faster pH decrease, which may accelerate product ripening and improve taste and appearance. Liu et al. (2021) reported that the high-salt (15-20%) Suan-yu products they produced contained high-quality proteins and had an intense acidic taste without a fishy smell or taste, further supporting the benefits of this method of production.

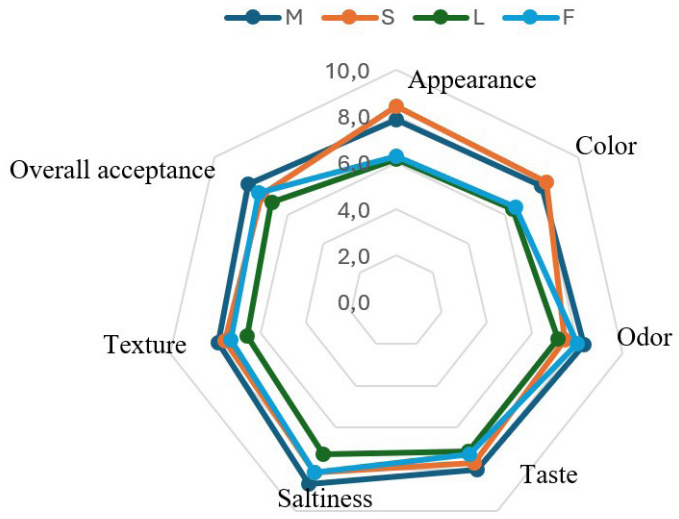


Figure 5. Sensory properties of fermented trout.

F: naturally fermented trout, L: with L. sakei starter culture added, S: with S. cerevisiae starter culture added, M: with L. sakei and S. cerevisiae starter cultures added
Values with different uppercase letters in the same row indicate significant differences between days (P<0.05).
Values with different lowercase letters in the same column indicate significant differences between groups (P<0.05).

CONCLUSION

This study effectively demonstrated the synergistic contributions of *L. sakei* and *S. cerevisiae* in enhancing the sensory, nutritional, and microbiological qualities of fermented trout. The findings underline their roles in providing appropriate acidity, salinity, and stability at low temperatures, while also contributing to higher fat and protein content, as well as enhanced antioxidative and antimicrobial effects. The achievement of the recommended 6 log value for probiotics critical for gastrointestinal health at different stages of fermentation highlights the efficiency of the starter cultures, particularly in groups L and M, which achieved this threshold as early as the second day. They was achieved on the 12th day in the F group (6.07 ± 0.25 log cfu/g), on the 4th day in the S group (6.25 ± 0.11 log cfu/g), on the 2nd day in the M group (6.52 ± 0.09 log cfu/g), and on the 2nd day in the L group (7.21 ± 0.11 log cfu/g).

The study also confirmed the ability of starter cultures to mitigate lipid oxidation (TBA) and total volatile base nitrogen (TVB-N) levels by producing lactic acid and bacteriocins. These properties emphasize the potential of using mixed starter cultures to ensure the safety, quality, and shelf life of fermented fish products.

The conclusion that fermented trout can be successfully prepared and consumed under low-salinity and room-temperature conditions is significant for broadening the scope of fish fermentation techniques and consumer accessibility. Fermented trout can be successfully products prepared and consumed by slicing 1.5–2 kg trout into 100–150 g portions when stored at low salinity and room temperature, based on their technological and sensory properties.

Conflict of Interests: The authors declare that there is no potential conflict of interest.

Ethics Committee Approval: Ethics approval was not required for this study.

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