Evaluation of applying *Lactobacillus plantarum* and *Lactobacillus sakei* starters on chemical and microbial properties of "Mahyaveh" fermented fish sauce

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Abstract

Mahyaveh, a traditional fermented fish product consumed as condiments in the southern part of Iran. In this study, the effects of the *Lactobacillus plantarum* and *Lactobacillus* as starters culture on the chemical and microbial properties of fermented sauce were studied during a 45-days interval. The studied parameters were pH, acidity, crude protein, TVB-N, salt content, biogenic amines (i.e. Histamine and tyramine), halophilic bacteria, LAB, mold and yeast counts. The results showed that pH values and crude protein content were significantly different among the studied groups ($p<0.05$). The interaction between time and applying starters on significant changes in acidity was observed. The highest amount of TVB-N was measured for the treated group. The amount of histamine and the salt content of the samples decreased over time. There was also a significant difference in the tyramine levels of the samples ($p<0.05$). The highest count of Halophiles, Bacillus, Mold and Yeast were observed in the control group. The cfu of LAB increased during the time of fermentation process. It can be concluded that the inoculated sauce with *L. plantarum* and *L. starters* was more acceptable in terms of chemical and microbial properties compared to the control group.

Keywords: Mahyaveh sauce, Fermentation, Starters, *Lactobacillus plantarum*, *Lactobacillus sakei*

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Introduction

Fish sauce is a traditionally fermented product characterized by its salty brown liquid form, with a history of production that dates back to ancient civilizations across various regions, including Greece, Italy, and Southeast Asia (notably Thailand, Malaysia, the Philippines, Japan, and China) (Mooraki and Sedaghati, 2019; Zang *et al*., 2019). Mahyaveh represents a specific type of traditional fermented fish sauce, predominantly produced in the southern regions of Iran, particularly in Hormozgan province. The formulation of this sauce is influenced by familial traditions, the availability of raw materials, consumer preferences, and climatic conditions, leading to variations in both the production process and the ingredients used over time and across different geographical areas. Typically, Mahyaveh is made using various species of sardines or anchovies, along with salt, water, and Eruca sativa (Zarei *et al*., 2012; Nazari *et al*., 2021; Moghadam *et al.,* 2019). The quality of the final product is affected by numerous factors, including the species of fish utilized, the quality of salt, the fish-to-salt ratio, any additional ingredients, and the conditions under which fermentation occurs (Lopetcharat and Park, 2002; Cai *et al*., 2024).

Fermented fish sauce serves dual purposes as both a condiment and a main dish, recognized for its nutritional benefits, including a rich amino acid profile that is particularly high in lysine, along with being a significant source of vitamins and minerals. However, its consumption is not without limitations, primarily due to its elevated salt content, the potential for high levels of biogenic amines produced during fermentation, and the prevalent bacterial flora (Zarei *et al*., 2012; Mooraki and Sedaghati, 2019). The presence of biogenic amines, which arise from the decarboxylation of amino acids, can result in scombroid poisoning among consumers. Of the eight principal amines associated with this condition, histamine, putrescine, and cadaverine are the most prevalent (Zamman *et al*., 2010; Mohebbi *et al*., 2014; Ding and Li, 2024). The primary factor contributing to the increased histamine levels in fish sauce is the prevalence of Gram-negative bacteria and their decarboxylase activity during the initial processing stages. In light of this, the incorporation of lactic acid bacteria as a starter culture in the production of various fish sauces has been adopted (Zhang *et al*., 2022). Additionally, research by Muñoz-Atienza *et al*. (2011) has shown that this method can effectively regulate and reduce histamine concentrations. The European Union (EU) has established a maximum residue limit (MRL) for histamine in fish at 100 mg/kg, whereas the United States has set this limit at 50 mg/kg (Zhou *et al.*, 2020). According to the US Food and Drug Administration (USFDA) and the Food Safety Commission (FSC), the maximum histamine limits are 50 mg/kg and 200 mg/kg, respectively. Nevertheless, numerous samples have exhibited elevated histamine levels, with Tsai *et al.* (2006) reporting concentrations of

394, 263, and 382 mg/kg in fish sauce, fish paste, and shrimp paste, respectively. Naila *et al.* (2011) identified elevated histamine concentrations (5487 mg/kg) in 28 samples of Rihaakura. According to the European Commission Regulation (no 1441/2007), the maximum allowable histamine level in fishery products is established at 200 mg/kg, whereas for fish sauce produced through the fermentation of fishery products, the permissible concentration is 400 mg/kg (Commission Regulation, 2007; Buczkowska *et al*., 2024). Zarei *et al*. (2012) conducted an analysis of Mahyaveh, identifying histamine and spermidine as the predominant biogenic amines. The objective of the current study was to regulate the levels of biogenic amines through the application of *Lactobacillus plantarum* and *Lactobacillus sakei* as starter cultures, while also assessing the chemical and microbial quality of the treated sauces.

Material and methods

Sauce preparation

A total of 500 grams of sun-dried and ground sardinella sp. were subjected to roasting and subsequently combined with 25 grams of coarse salt. This mixture was then stored at a temperature of 25°C for a fermentation period of 30 days. On the 30th day, the mixture underwent filtration, after which a blend of spices—including 250 grams of coriander seeds, 500 grams of Eruca sativa, 125 grams of fennel, and 250 grams of barley—was ground into a powder, mixed in, and diluted with hot water. The resulting preparation was then allowed to ferment for an additional 15 days in a clay pot. The study involved two experimental groups: the control group (C) and the treatment group (T), which contained a mixture of two starter cultures, specifically 10^8 log/ml of *Lactobacillus plantarum* and 10^8 log/ml of *Lactobacillus sakei*. These groups were monitored over a 60-day period, with evaluations conducted on days 30, 45, and 60.

Microbial tests

A total of 10 grams of samples were subjected to homogenization with 90 milliliters of sterile saline under aseptic conditions for a duration of 60 seconds, followed by the preparation of serial dilutions of fish sauce. The enumeration of various microbial populations, including Bacillus species, lactic acid bacteria, Enterobacteriaceae, halophilic bacteria, molds, and yeasts, was conducted using specific culture media: Tryptic Soy Agar (TSA), de Man Rogosa and Sharpe (MRS), Violet Red Bile Glucose Agar (VRBG), Tryptic Soy Agar supplemented with 10% salt (TSA), and Potato Dextrose Agar (PDA), respectively. The TSA and VRBG plates were incubated in a standard incubator at 35ºC for 36 hours, while the MRS plates were placed in a CO2 incubator at 30ºC for a period of 48 hours. For the enumeration of halophilic bacteria, the plates were incubated at 35ºC for 10 days. The results were expressed as log CFU/g of the samples, as referenced by Feldsine *et al.* (2002).

Chemical analysis

The evaluation of salt concentration and pH levels in the samples was performed through the titration technique as specified by AOAC (2000). The quantification of total volatile basic nitrogen (TVB.N) was achieved via a method combining distillation and titration, utilizing a Kjeldahl apparatus, in accordance with AOAC (2000) guidelines. The analysis of biogenic amines, particularly Histamine and Tyramine, was carried out using high-performance liquid chromatography (HPLC) with a Shimadzu 10A-VP system equipped with a UV-visible detector set to 254 nm. A C18RS 250 column was utilized, with the mobile phase comprising methanol and water in a 70:30 v/v ratio, at a flow rate of 1 ml/min, adhering to the sample preparation protocols established by Zarei *et al.* (2012).

Statistical analysis

The experimental findings were analyzed using the Kolmogorov-Smirnov test, with a significance level set at $p<0.05$, to assess the normality of the data distribution. Subsequently, the normally distributed data were subjected to one-way ANOVA, also at $p<0.05$, followed by LSD post hoc analysis, utilizing IBM SPSS version 26.

Results

Bacillus bacteria population

Despite the fact that Bacillus bacteria are not particularly tolerant to saline environments, their numbers have progressively risen over the 45-day fermentation period in the control samples, whereas their growth was notably limited in the treated samples. The recent investigation indicated that the Bacillus population ranged from 2.48 to 2.94 log cfu/ml (Table 1).

Table1: Bacteria level over the 45 days' fermentation process.

Name	**45th day**		**30th day**	
Type	**T**	**C**	**T**	**C**
Bacillus	2.481 ± 0.021^{c}	2.944 ± 0.049^{a}	2.531 ± 0.045^{c}	2.740 ± 0.52^{b}
Lactic acid	4.955 ± 0.040^{a}	3.86 ± 0.035^{c}	4.802 ± 0.059^{b}	3.635 ± 0.055^{c}
Enterobacteriaceae	3.597 ± 0.045^{b}	4.539 ± 0.231^{a}	3.575 ± 0.059^{b}	4.221 ± 0.1078^{b}
Halophile	3.233 ± 0.026^{c}	4.856 ± 0.0205^{a}	3.526 ± 0.0442^{c}	4.518 ± 0.112^{b}
Mold and yeast	1.970 ± 0.036^{b}	2.091 ± 0.027^{a}	1.95 ± 0.024^{b}	2.041 ± 0.029^{a}

*The small letters show the significant difference among the experimental groups ($p<0.05$).

The findings demonstrate a significant increase in the Bacillus population within the control groups throughout the testing period. Specifically, the population on the 45th day in the control group was significantly higher than that on the 30th day ($p<0.05$). Moreover, Table 1 indicated that the Bacillus population in the treated group on the 45th day was lower than that on the 30th day; however, this reduction was not statistically significant ($p>0.05$). On the 30th day, the Bacillus population in the treated group was also lower than in the

control group, and by the 45th day, the treated group exhibited a significantly reduced population compared to the control group (p<0.05). These results suggest that the Bacillus population in the treated samples is inferior to that in the control samples, with the presence of *Lactobacillus plantarum* and *Lactobacillus sakei* starters inhibiting the growth of Bacillus bacteria relative to the control samples.

Enterobacteriaceae population

Enterobacteriaceae are a group of bacteria commonly located within the digestive systems of humans and animals, as well as being prevalent in various environmental settings, where they are classified as saprophytes. This investigation revealed that the population of Enterobacteriaceae ranged from 3.57 to 4.53 log cfu/ml. As indicated in Table 1, the treated group exhibited a notable reduction in Enterobacteriaceae population on the 30th day when compared to the control group (p<0.05). This trend was similarly observed on the 45th day across the studied groups. Notably, in the control groups lacking starter cultures (*Lactobacillus plantarum* and *Lactobacillus sakei*), there was a significant increase in Enterobacteriaceae populations as the fermentation period of the sauce extended (p<0.05). Conversely, the treated groups that included starter cultures did not demonstrate a significant rise in Enterobacteriaceae populations (p>0.05). Overall, it can be inferred that the incorporation of starter cultures has led to a significant reduction in the population of Enterobacteriaceae (p<0.05). The research revealed that the control group exhibited the greatest quantity of Enterobacteriaceae colonies on the 45th day, while the treated sample demonstrated the least number of colonies on the 30th day. Additionally, the control sample recorded the highest count of Bacillus colonies on the 45th day, whereas the treated sample also showed the lowest count on the same day.

Halophilic bacteria population

The data presented in Table 1 indicates that the population of halophilic bacteria varied between 3.23 and 4.85 log cfu/ml. The findings demonstrated that the treated samples exhibited the lowest population of halophilic bacteria on the 45th day, while the control samples showed the highest population on the same day. An assessment conducted on the 30th day revealed that the treated samples contained fewer halophilic bacteria compared to the control samples, with this difference being statistically significant (p<0.05). A similar trend was observed on the 45th day, where the treated samples continued to show a lower population relative to the control samples. It is important to note that the population of halophilic bacteria in the control samples experienced a significant increase over time (p<0.05), whereas the treated samples exhibited a negligible decrease (p>0.05). These results suggest that the incorporation of starter cultures, specifically *Lactobacillus plantarum*

and *Lactobacillus sakei*, has led to a significant reduction in the population of halophilic bacteria ($p<0.05$).

Mold and yeasts population

The present investigation revealed that the populations of molds and yeasts ranged from 1.95 to 2.09 log cfu/mL (Table 1). The highest and lowest concentrations of fungi were recorded in the control samples on the 45th day and in the treated samples on the 30th day of fermentation, respectively. The findings indicate that the fungal population in the control samples exhibited a negligible decrease over time ($p> 0.05$). Interestingly, a similar trend of insignificant increase was noted in the treated samples throughout the study period. On the 30th day, the fungal population in the treated samples was lower than that in the control samples, and this disparity was statistically significant on the 45th day ($p<0.05$). Overall, the incorporation of starter cultures effectively diminishes the populations of molds and yeasts, thereby inhibiting their growth, particularly in the presence of *Lactobacillus plantarum* and *Lactobacillus sakei.*

Lactic acid bacteria

Lactic acid bacteria are crucial for the sensory attributes of fermented fish sauces, specifically Mahyaveh. Their primary function involves the fermentation of available carbohydrates and the subsequent reduction of the pH in the fish sauce. The interplay of low pH, organic acids—particularly lactic acid—and salt serves as a key factor in the preservation and stability of fish fermentation products. In this study, the population of lactic acid bacteria ranged from 3.63 to 4.95 log cfu/ml (Table 1). The highest and lowest populations were observed in treated samples on the 45th day and control samples on the 30th day, respectively. Notably, the lactic acid bacteria population in treated samples exhibited a significant increase during fermentation when compared to control samples ($p<0.05$). On the 30th day, treated samples demonstrated a higher population of lactic acid bacteria than the control group, a trend that continued on day 45 ($p<0.05$). These findings indicate that the lactic acid bacteria population in treated samples significantly increased over time, attributed to the presence of *Lactobacillus plantarum* and *Lactobacillus sakei* as starter cultures ($p<0.05$).

pH values

Figure 1 illustrates that the control samples exhibited the highest pH value at 5.14 on the 30th day, while the treated samples recorded the lowest pH value of 5.06 on the 45th day. The analysis indicated that the variations in pH levels of the control samples throughout the fermentation period were statistically significant ($p<0.05$). A comparative analysis of the pH values between the control and treated samples on the 30th and 45th days revealed a significant decrease in the pH of the treated samples relative to the control samples ($p<0.05$). Consequently, it can be inferred that the duration of fermentation had a significant impact on the pH values of Mahyaveh samples ($p<0.05$).

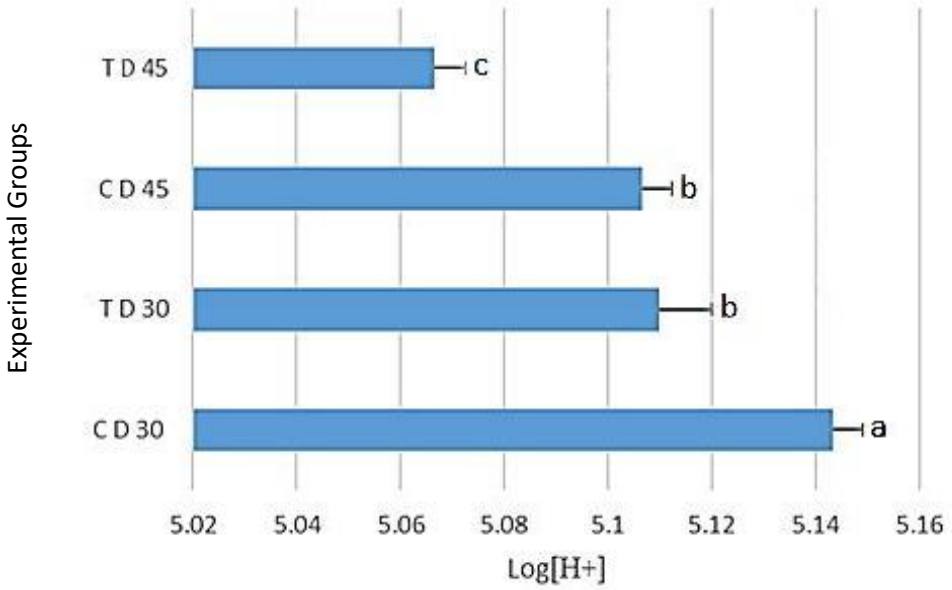

Figure1: the changes of PH amount in Mahyaveh sauce over the 30- and 45-days' storage. *The small letters show the significant difference among the experimental groups(p<0.05)

NaCl content

Fish sauces are typically characterized by their elevated salt content, which limits their consumption to smaller amounts. In the analyzed samples, the salt concentrations ranged from a minimum of 14.3% to a maximum of 18.8%. Data presented in Figure 2 indicate that the control samples exhibited a greater salt concentration on both the 30th and 45th days, with the observed differences being statistically significant (p<0.05).

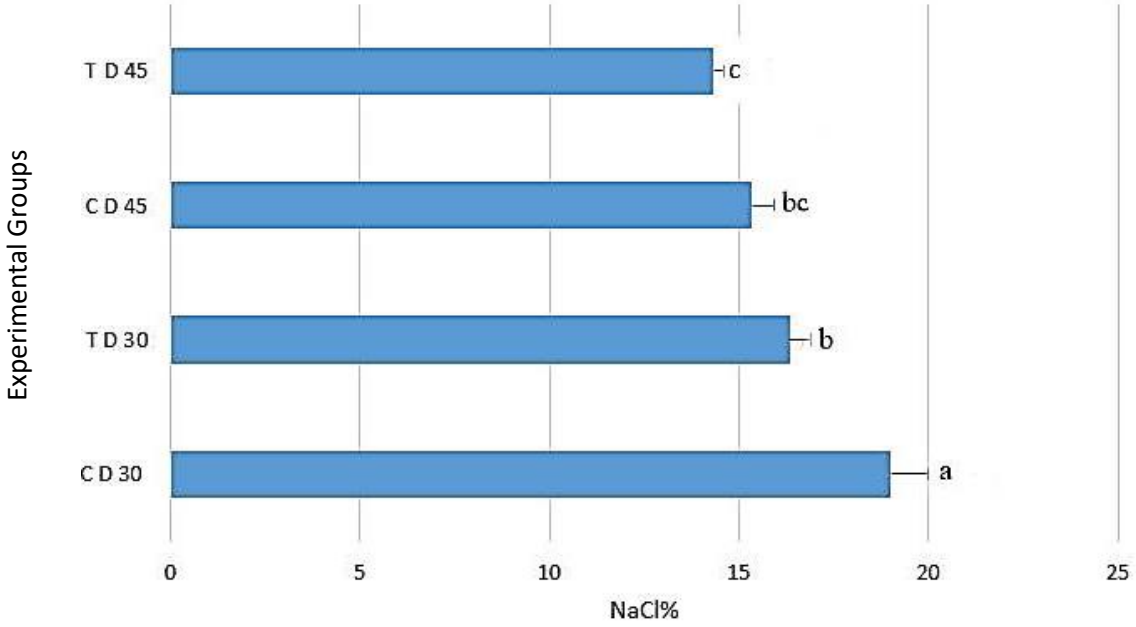

Figure 2: The Percentage changes of Nacl in Mahyaveh sauce over the 30 and 45 days' storag. *The small letters show the significant difference among the experimental groups(p<0.05).

TVB-N

As illustrated in Figure 3, the control samples exhibited the highest TVB-N concentration on the 45th day, while the treated samples showed the lowest levels on the 30th day. The findings indicate a significant increase in the concentration of volatile nitrogen compounds (TVB-N) in both control and treated samples throughout the fermentation period (p<0.05). Notably, the TVB-N levels were consistently lower in the treated samples compared to the control samples on both testing days. This suggests that the starter culture had a substantial impact on the TVB-N levels in the treated samples (p<0.05), attributed to the inclusion of *Lactobacillus plantarum* and *Lactobacillus sakei* as starter cultures (p<0.05).

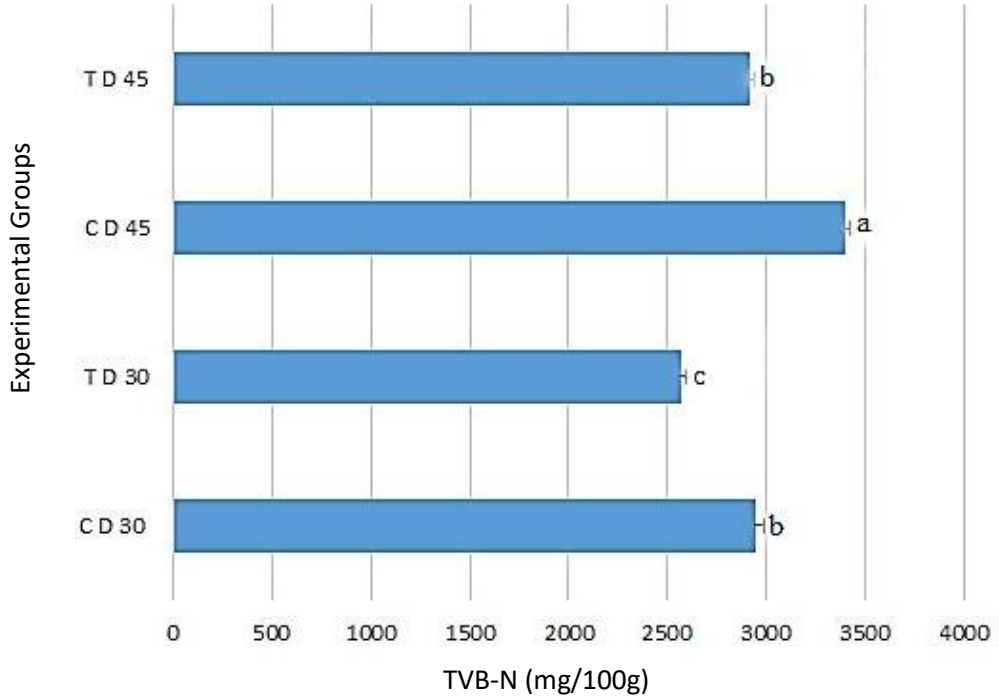

Figure 3: The Percentage changes of TVB-N in Mahyaveh sauce over the 30- and 45-days' storage. *The small letters show the significant difference among the experimental groups (p<0.05).

Histamine content

As indicated in Table 2, the highest and lowest concentrations of histamine were observed in control samples on the 30th day and in treated samples on the 45th day of fermentation. Overall, there was a notable reduction in histamine levels over time in both control and treated samples (p<0.05). This suggests that fermentation duration significantly influenced the histamine content in Mahyaveh samples (p<0.05). On both the 30th and 45th days, histamine levels in treated samples were lower than those in control samples, indicating that the starter culture had a significant impact on histamine concentrations in the treated samples (p<0.05), attributed to the presence of *Lactobacillus plantarum* and *Lactobacillus sakei* as starter cultures (p<0.05).

Table 2: Histamine and Tyramine level over the 45 days' fermentation process.

Name	60th day		45th day		30th day	
Type	**T**	**C**	**T**	**C**	**T**	**C**
Histamine	32.86 ±0.585^{e}	38.566 ±0.602^{b}	33.333 ±0.577^{d}	39.733 ±0.568^{b}	37.666 ±1.0577^{c}	46.333 ±0.577^{a}
Tyramine			13.293 ±0.490^{c}	14.306 ±0.272^{b}	14.283 ±0.431^{b}	16.01 ±0.356^{a}

*The small letters show the significant difference among the experimental groups (p<0.05).

Tyramine content

The highest and lowest concentrations of tyramine were observed in control samples on the 30th day and in treated samples on the 45th day of fermentation (Table 2). Overall, there was a notable reduction in tyramine levels over time in both control and treated samples (p<0.05). This suggests that the duration of fermentation significantly influenced the tyramine content in mahyaveh samples (p<0.05). On both the 30th and 45th days, tyramine levels in treated samples were found to be lower than those in control samples. Consequently, the findings indicate that the starter culture had a significant impact on the tyramine levels in treated samples (p<0.05), attributed to the presence of *Lactobacillus plantarum* and *Lactobacillus sakei* as starter cultures (p<0.05).

Discussion

The fisheries sector plays a crucial role in the development of the national economy, holding substantial importance for food security and the thriving marine economy. According to data from the Food and Agriculture Organization, global fisheries and aquaculture achieved a total production of 184.1 million tons in 2022 (Cai *et al*., 2024). Over the past two decades, there has been a marked increase in seafood consumption among the Iranian populace. Although seafood provides significant nutritional benefits, it is highly susceptible to rapid spoilage due to its nearly neutral pH, elevated water activity, and the presence of autolytic enzymes. These factors foster an environment that is favorable for microbial proliferation and oxidative deterioration. As a result, various methods are being implemented to reduce spoilage and inhibit the development of secondary toxic metabolites (Ahmed, 2022). A multitude of quality control producers prioritize the evaluation of chemical indices, particularly focusing on toxic metabolites such as biogenic amines. This emphasis is supported by various international guidelines, including those from the Food and Agriculture Organization (FAO), Codex Alimentarius, European Commission regulations, and the United States Food and Drug Administration (USFDA). Biogenic amines, which are low molecular weight bases resulting from the decarboxylation of amino acids, serve as critical indicators for assessing the freshness of seafood products (Hamada-Sato *et al.,* 2005; Sedaghati and Mooraki, 2019; Xu *et al*., 2020; Dind and Li, 2024). Throughout the

fermentation process, proteins undergo gradual degradation, leading to the liberation of free amino acids. These amino acids serve as important precursors for the formation of appealing flavors and may also play a role in the synthesis of biogenic amines. This transformation occurs when the enzyme amino acid decarboxylase converts amino acids into various biogenic amines. Additionally, as previously noted, biogenic amines can also be produced from aldehydes and ketones via the enzymatic activity of aminotransferases (Xu *et al.,* 2020; Cai *et al*., 2024). Histamine, an important nitrogenous compound, has been recognized by both the European Union and the United States for its toxicological effects. The maximum allowable concentration for fishery products from species with high histidine content is established at 100 mg/kg, while a limit of 400 mg/kg is designated for fishery products that have undergone enzymatic maturation in brine. Furthermore, a threshold of 50 mg/kg is set for other applications. In comparison, tyramine is acknowledged to possess a toxic range of 100-300 mg/kg (Biji *et al.,* 2016). The Food and Drug Administration (FDA) has determined a threshold for histamine levels in fish species such as Scombridae, Clupeidae, Engraulidae, Pomatomidae, Scombresosidae, among others, establishing it at 50 mg/kg (Ding and Li, 2024). Concentrations exceeding 500 to 1000 mg/kg are deemed potentially harmful to human health. Regarding tyramine safety limits, the recommended upper reference values range from 100 to 800 mg/kg. It is crucial to recognize that permissible limits for biogenic amines differ among various countries and regulatory bodies (EEC, 1991; FDA, 2001; SABS, 2001; AFSC, 2001, FAO/WHO, 2013; GB 5009.208–2016, 2016: FSSAI 1–10 (2), 2017). The substantial activity of bacteria in fermented products poses a challenge for both consumers and producers regarding the formation of biogenic amines, necessitating effective control measures. The use of negative amine-producing starter cultures has been suggested as a method to inhibit the formation of biogenic amines (Lee *et al*., 2016; Sedaghati and Mooraki, 2019; Gawad *et al*., 2022).

The findings of the current study indicated a significant decrease in pH levels in the inoculated sauce containing *L. sakei and L. plantarum* over a 45-day storage period, in contrast to the control group. This decline may be attributed to the production of lactic acid by the bacteria present in the treated sauce, alongside the accumulation of alkaline compounds such as ammonia and TMAO in the control group. Similarly, Gao *et al*. (2014) observed a comparable pattern in the production of dry sausage utilizing starter cultures of *Staphylococcus carnosus* and *S. xylosus*. Additionally, Ba *et al.* (2016) demonstrated that the starter culture (SA7), which includes *Staphylococcus carnosus* and *Lactobacillus sakei*, was more effective in producing high-quality products with lower concentrations of biogenic amines.

The total volatile basic nitrogen (TVB-N) levels increased in both experimental groups; however, the rise was less pronounced in the inoculated sauce. This phenomenon may be attributed to the proteolytic activity of bacterial enzymes, such as amine oxidase, produced by lactic acid bacteria (LABs) (Mooraki and Sedaghati, 2019; Belleggia and Osimani, 2023). Additionally, the role of salt must be taken into account, as it can act as both a promoter and an inhibitor of proteolytic enzymes. In this study, the concentration of NaCl decreased over time, with a more significant reduction observed in the treated group compared to the control.

The assessment of microbial characteristics revealed a notable presence of Bacillus bacteria in the fermented sauce, which exhibited a decreasing trend throughout the fermentation process. Thapa *et al.* (2006) conducted a study on microbial diversity in Ngari, Hentak, and Tungtap, fermented fish products from northeastern India, and found that the population of Bacillus bacteria closely mirrored the findings of this investigation, with values ranging from 2.69 to 1.89 log cfu/ml. Similarly, Taheri *et al.* (2014) examined the bacterial population in Iranian fish sauce (Mahyaveh) and reported a significant reduction in Bacillus bacteria during fermentation, aligning with the declining trend observed in the current study.

The investigation into microbial characteristics revealed that the population of halophilic bacteria in fermented fish sauce was notably significant, exhibiting a declining trend in the treated samples throughout the fermentation process. Mahyaveh sauce, characterized by its elevated salt concentration, demonstrates that high salinity profoundly influences both microbial growth and the fermentation rate, ultimately enhancing the product's quality and safety. Initially, a variety of non-halophilic bacteria are present during the early fermentation stages; however, the high salt concentration in this sauce inhibits their growth. Consequently, as fermentation progresses, these non-salt-tolerant bacteria are supplanted by halophilic and salt-resistant bacterial populations.

A comparable investigation conducted by Zarei *et al.* (2012) examined the chemical and microbial properties of Mahyaveh, a traditional Iranian fish sauce. Their findings indicated that the average concentration of halophilic bacterial colonies was recorded at 3.66±2.24 log cfu/ml, aligning closely with the halophilic bacterial populations identified in a more recent study. Additionally, Ranjbar *et al.* (2017) reported on the chemical and microbial characteristics of Mahyaveh sauce from Zarrin Dasht, Iran, revealing that the mean levels of halophilic bacteria, such as Staphylococcus, were approximately 3.52±1.08 log cfu/ml, which corresponded with the halophilic bacterial averages observed in the current research. Furthermore, Fukui *et al.* (2012) noted a reduction in the average number of halophilic bacteria throughout a six-week fermentation

period, with this decreasing trend persisting as fermentation progressed, mirroring the decline in halophilic populations observed in the samples analyzed in the present study.

Research on enterobacteriaceae bacteria indicates that the indigenous bacteria of the gastrointestinal tract exhibited a decline in treated samples when compared to the control group. The inclusion of *Lactobacillus plantarum* and *Lactobacillus sakei* as starter cultures positively influenced the reduction of the enterobacteriaceae population (Nomoto, 2005). In a 21-day investigation, Santo *et al.* (2005) examined the impact of varying concentrations of sodium chloride and glucose on sardine fermented fish sauce produced by *Lactobacillus sakei*, finding that the enterobacteriaceae population was below 3 log cfu/ml, which is lower than the minimum threshold established in prior research. Lactic acid bacteria naturally produce bacteriocins that play a significant role in the bio-preservation of food. These substances act as a protective mechanism, demonstrating antagonistic, inhibitory, and antimicrobial characteristics that target pathogens and microorganisms responsible for food spoilage (Ayivi *et al.,* 2020). Zarei *et al.* (2012) conducted a study examining the chemical and microbial characteristics of Mahyaveh, revealing that the population of enterobacteriaceae colonies was approximately 3.41±2.03 log cfu/ml, a figure that aligns with the minimum levels identified in recent investigations.

An investigation into the fungal community associated with Mahyaveh fish sauce revealed that the inclusion of *Lactobacillus plantarum* and *Lactobacillus sakei* as starter cultures significantly contributed to the reduction of fungal populations in the samples subjected to treatment. Taheri *et al.* (2014) noted an increase in mold and yeast colony counts over a 45-day fermentation period at ambient temperatures, with Saccharomyces identified as the dominant species. In contrast, the findings from the current study indicate a decline in mold and yeast colony populations during fermentation. Furthermore, recent research conducted by Ranjbar *et al.* (2017) on the chemical and microbial characteristics of Mahyaveh fish sauce in Zarrin Dasht, Iran, reported a colony population of 2.27±0.71 log cfu/ml, which aligns with the results of the present investigation.

The analysis of lactic acid bacterial populations in Mahyaveh fish sauce samples indicates that both treated and control samples exhibited an increase in lactic acid populations throughout the fermentation process. In a microbial investigation of fish sauce, Zaman *et al.* (2010, 2011) identified the presence of lactic acid bacteria, corroborating the findings of the current study. Additionally, research conducted by Sanni *et al.* (2002) on the fermented products Momoni and Bakasang reported lactic acid bacterial populations ranging from 4.8 to 6.15 log cfu/ml, aligning with the minimum levels observed in the present research.

Conclusion

The findings of this research indicated that Mahyaveh sauce inoculated with *Lactobacillus plantarum* and *Lactobacillus sakei* exhibited superior acceptability in terms of chemical and microbial parameters compared to the control group. These two starter cultures play a crucial role in inhibiting the proliferation of undesirable microorganisms and facilitating the acidification process of Mahyaveh. The treated samples demonstrated a notable reduction in histamine levels, which correlated with a decrease in the total viable bacterial count and an increase in the viability of the total lactic acid bacteria (LAB) count. From a human safety perspective, the incorporation of LAB treatments to mitigate histamine levels is essential for the production of Mahyaveh.

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