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BIOLOGICAL AGENTS AS AN ALTERNATIVE TO CHEMICAL PRESERVATIVES IN THE SEAFOOD PROCESSING INDUSTRY, A REVIEW

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ABSTRACT

The present research investigated the possibility of replacing probiotic bacteria instead of chemical preservatives in the seafood processing industry. The data were used from articles published in the databases including Science Direct, Pub Med, Scopus Springer Link, and Scopus. The search was carried out using the keywords fish fillet, beneficial non-dairy foods, increasing shelf life, biological protection, probiotic food, microbial treatment, and Lactobacillus bacteria. A biological preservation technique was used to maintain the quality of hake, spanish mackerel, mackerel, sea bass, tilapia, swordfish, rainbow trout, and ribbon fillets. Swordfish coated with *L. paracasei* IMPC 2.1 was acceptable for 3 months. Followed by spanish mackerel and rainbow trout fillets coated with *L. casei* and *L. plantarum* subsp. *plantarum* ranked second and third for 30 and 21 days, respectively. The minimum shelf life of fillets was 48 hours (Tilapia coated with *L. plantarum* 1.19). In freezing, *L. brevis* could maintain the quality of rainbow trout fillet for 6 months. The present study suggests Lactobacillus strains, especially *L. paracasei* IMPC 2.1, as a preservative to preserve the quality of fish fillets. However, the use of this species in the seafood processing industry requires wider research to investigate its effect on other aquatic species.

Keywords: Biological Protection, Fish Fillet, Increasing Shelf Life, Microbial Treatment, Probiotic Bacteria

1. INTRODUCTION

Consumers' increasing interest in nutritional aspects and attention to food quality have helped to increase the consumption of fish and products derived from it. Like many animal foods, fish meat represents a highly perishable matrix that is challenged by rapid microbial growth. It has been reported that refrigerated fish have a limited shelf life and tend to spoil due to microbial growth and enzymatic reactions. Spoilage can affect processing and limit product storage, in addition, it may lead to health problems and economic losses Seifzadeh (2021). The fish spoilage process is very complicated due to the metabolic activity of native microorganisms and some physicochemical interactions. Although the initial loss of freshness of fish is usually due to the action of internal enzymes and chemical reactions, major spoilage occurs due to microbial growth and metabolism. Therefore, it would be interesting to find an effective and alternative approach that delays spoilage and increases the shelf life of fish without any toxic effects. Also, the growing demand for increasing the quality, ease of use, safety, fresh appearance, and shelf life of fish products led to the provision of nonthermal preservation technologies such as natural biological protection. Lactic acid bacteria have been widely used in this field. Recently, lactic acid bacteria have been proposed as an alternative method for biopreservation. Biopreservation is an advanced method to increase the shelf life and safety of food and its nutritional value through the injection of probiotic bacteria that can prevent the growth of undesirable bacteria in food Iorizzo (2022), De Rezende et al. (2022). Several species of lactic acid bacteria including Lactobacillus, Leuconostoc, Pediococcus, Lactococcus, Streptococcus, and *P. lactici*, have been listed as a safe food by the United States Food and Drug Administration. Various studies have shown the presence of food-associated bacteria such as *P. pentasaceus* in the gut of healthy humans Terpou et al. (2019), Pereira et al. (2022a).

Many preservation techniques have been developed to improve microbial safety and extend the shelf life of aquatic products. Despite the benefits of chemical preservatives, food producers and consumers tend to reduce the use of artificial chemicals in food preservation. Recently, special attention has been paid to the use of lactic acid bacteria and their metabolites as natural food preservation agents Kumari (2020). Several studies have been conducted on preventing spoilage of fish to increase shelf life. These studies include the use of natural antioxidants such as rosemary, green tea extract, and tea polyphenol coating, as well as physical treatments such as irradiation, vacuum packaging, and modified atmosphere packaging. Similarly, some artificial preservatives, such as chelating agents and chemical preservatives, have also been used to extend the shelf life of seafood. However, they are strictly prohibited due to the toxic and health problems associated with them. As a result, there is a growing interest in using natural agents as biological preservatives that can increase shelf life and minimize the negative impact on nutritional and sensory properties Seifzadeh et al. (2019), Dixit et al. (2016).

In the field of aquatic products, a large number of studies have focused on the use of lactic acid bacteria to control pathogenic organisms in semi-preserved products. However, studies on the use of lactic acid bacteria to preserve the freshness of fish fillets are relatively few. Now, functional manufacturers are exploring them as part of the daily diet to provide proven probiotic strains. Therefore, they can increase the added value of these products in addition to the shelf life Onyenweaku et al. (2016), Pereira et al. (2022b).

Based on the antibacterial effect of lactic acid bacteria, various studies have been conducted to increase the shelf life through the control of microbial spoilage of some foods. In the past, chemicals were traditionally used to inhibit microorganisms that cause food spoilage and increase the shelf life of food products in the refrigerator. This issue increased the concern of consumers about the health risks associated with some artificial preservatives. Therefore, researchers suggested the use of natural biological protective substances such as lactic acid bacteria to inhibit the activity of pathogenic microorganisms with food spoilage Kobyliak et al. (2016).

Probiotics are living and specific microorganisms that, when consumed by humans and animals, exert beneficial effects on the health of the host by affecting the microbial flora of the body. Many benefits of using probiotics are maintaining and restoring the natural intestinal microbiota, preventing and reducing changes in the digestive system, inhibiting the growth of pathogenic microorganisms, strengthening the intestinal mucosal barrier, antimicrobial and anticancer activity; Modulating the immune system, reducing blood cholesterol levels, antioxidant activity, improving calcium absorption, increasing the synthesis of vitamins, antifungal effect; reduction of gastrointestinal infections and ulcers caused by *Helicobacter pylori*, treatment of diarrhoea caused by travel to tropical areas and treatment with antibiotics, control of colitis caused by rotavirus and *Clostridium difficile,* improving the nutritional quality of food, improving lactose digestion, Modulation of stress, weight loss and improvement of anxiety symptoms Giribaldi et al. (2019), Rong et al. (2015). Therefore, the present study was conducted to investigate the effect of probiotic lactic acid bacteria on the microbial, chemical, sensory characteristics, and shelf life of fish fillets.

2. METHODS

The present research was conducted by reviewing published studies on the effects of probiotic Lactobacillus bacteria on fish fillets in Iran and other countries. The data of some published articles from databases including Science Direct, Pub Med, Scopus Springer Link, and Scopus were used regarding increasing the shelf life of fish fillets. The search was carried out using the keywords fish fillet, beneficial non-dairy foods, increased shelf life, biological protection, probiotic food, probiotic bacteria, microbial treatment, coating, lactobacillus bacteria, and active coating during the years 2012 - 2023. Lactobacillus probiotics include *L. paracasei* IMPC 2.1, *L. plantarum* 1.19, *L. plantarum* SKD4, *P. stilesii* SKD11, *L. sakei* ATCC 15521, *L. plantarum* JCM 1149 , *L. acidophilus* ATCC4356, *L. casei*, *L. plantarum* subsp. *plantarum*, *L. curvatus* BCS35, *L. brevis* CD0817, *L. paracasei* L26, *Bifidobacterium lactis* B94, *L. delbrueckii* subsp. *bulgaricus, L. reuteri, P. acidilactici*, and *Enterococcus faecium* BNM58 were used by the dipping method to coat the fish fillet. In this study, biological protection technique was used to maintain the quality of hake (*Merluccius merluccius*), spanish mackerel (*Scomberomorus commerson*), mackerel (*Megalaspis cordyla*), sea bass (*Lateolabrax Japonicus*), tilapia (*Oreochromis niloticus*), swordfish (*Xiphias gladius*), rainbow trout (*Oncorhynchus mykiss*), four-spot megrim (*Lepidorhombus boscii*) and ribbon (*Trichiurus lepturus*) fillets.

The quality of ready-to-eat swordfish fillets with or without *L. paracasei* IMPC 2.1 probiotic strain was evaluated during 3 months of refrigerated storage. Experimental and control treatments were evaluated during 7, 14, 30, 60, and 90 days of storage in terms of microbiological tests, fatty acid profile, and malondialdehyde content. Lactic acid bacteria, yeasts, and molds, Enterobacteriaceae, and Pseudomonas were determined Giribaldi et al. (2019). The quality of tilapia fish was kept in the refrigerator with *L. plantarum* 1.19 coating and Lactic acid bacteria, and the composition of gram-positive and gram-negative bacteria, and the sensory were investigated Rong et al. (2015).

Cell-free cultures of *L. plantarum* SKD4 and *P. stilesii* SKD11 bacteria were used to increase shelf life and preserve the freshness of ribbon fillet in the refrigerator (4 ^{o}C) or at room temperature (25 ^{o}C) Du-Min et al. (2021).

Sea bass was coated with 2 types of formulas including alginate fermented by *L. reuteri* at 24 and 48 hours and another alginate with glycerol. The effects of treatments were evaluated through microbial and sensory tests Angiolillo et al. (2018).

L.sakei ATCC 15521 was used to improve the shelf life of Horse Mackeral fish fillets at a temperature of 6±1 ºC against the pathogenic bacteria *Staphylococcus aureus* ATCC 25923 and was measured by microbial and biochemical analyses Nath et al. (2014).

Tilapia fillets were tested with *L. plantarum* JCM 1149 and *L. acidophilus* ATCC4356 bacteria. The preserved fillets were kept at 5 ºC and the changes in lactic acid bacteria, mesophilic, psychrophilic, and coliform bacteria were investigated for 30 days Castillo-Jiménez et al. (2017).

Rainbow trout fillets were treated using suspension of *L. reuteri*, *L. brevis*, *L. delbrueckii*, and their combination. Fillets immersed in 2% lactic acid and fillets without additives were considered as control treatments. The treatments were kept in the refrigerator for 5 days and frozen for six months Seifzadeh et al. (2020).

Spanish mackerel fish were coated with *L. casei* and kept at a refrigerator temperature (4 ºC). The treatments were evaluated on the first, third, seventh, fifteenth, and thirtieth days through chemical tests including TVB-N and pH, and microbial tests including the total number of bacteria Gholami et al. (2021).

The effect of using sodium alginate and whey protein food coatings containing *L. plantarum* subsp. Plantarum and *P. acidilactici* were performed on rainbow trout fillets at refrigerator temperature. The treatments were evaluated physically including color and texture on days 0, 7, 14, and 21 Alizadeh et al. (2019).

Cultures containing *L. curvatus* BCS35 and *E. faecium* BNM58, cell-free supernatant, and lyophilized bacteriocin on hake and four-spot megrim fish were kept in polystyrene boxes at 0-2 ºC using ice for 14 days. The samples were examined in terms of microbial and sensory tests Gómez-Sala et al. (2016).

Swordfish fillets were marinated in a 3% sodium salt solution containing 7 logCFU/g of *L. paracasei* IMPC 2.1 bacteria for 2, 7, and 15 days and were stored at 4 ºC for 120 days after packaging. The treatments were analyzed in terms of pH, water activity, Lactobacillus bacteria during storage and processing, Salmonella, and *Listeria monocytogenes*. Sampling was done on days 0, 7, 30, 60, 90, and 120 of storage Valerio et al. (2015).

A bioactive film consisting of agar, containing green tea extract and probiotic strains (*L. paracasei* L26 and *B. lactis* B94) was used on hake fillets. These fillets were inoculated with 10^3-10^4 CFU/g *Shewanella putrefaciens* and *Photobacterium phosphoreum* to simulate the spoilage process, to an evaluation of the effect of the film during 15 days of storage. Hydrogen-disulfide-producing bacteria and total bacterial counts, total volatile nitrogenous bases, triethylamine, and pH were measured during the storage period López de Lacey et al. (2014).

3. RESULTS

According to Table 1, most of the studies conducted for the use of probiotics to increase the shelf life of fish fillets were conducted on rainbow trout and tilapia. The use of lactic acid bacteria to preserve the fillet increased the chemical, microbial, and sensory characteristics of the fillet. However, in some cases, a decrease in sensory characteristics was also reported. Based on the results, probiotic bacteria can be used with other compounds such as green tea and sodium alginate. Based on the obtained results, the application of other compounds along with these bacteria did not increase the effects of the investigated bacteria compared to the application alone to increase the quality and shelf life of fish fillets. Bacterial cell-free culture also demonstrated the ability to improve the microbial characteristics of fillets.

Studies to increase the quality of fillets using bacteria L. paracasei, L. plantarum, P. stilesii, L. reuteri, L. sakei, L. acidophilus, L. delbrueckii subsp. bulgaricus, L. reuteri, L. brevis CD0817, L. casei, P. acidilactici, L. curvatus BCS35, E. faecium BNM58, L. paracasei, B. lactis were performed.

Most of the studies were conducted using *L. plantarum* to maintain the fillet quality. At refrigerator temperature, in terms of microbial (4 months) and chemical (3 months) characteristics, the longest shelf life was related to swordfish and the application of *L. paracasei* IMPC 2.1 bacteria. Followed by a spanish mackerel fillet coated with *L. casei* for 30 days and a rainbow trout fillet coated with *L. plantarum* subsp. *Plantarum* was ranked second and third for 21 days. In the present study, the minimum shelf life of fish fillets in refrigerator conditions was 48 hours, which was related to tilapia fillets coated with *L. plantarum* 1.19.

Table 1

Table 1 The Results of the Use of Lactobacillus Bacteria on the Quality of Fish Fillet

Reference	Conclusion	Results	Storage conditions	Method of application	Fish fillet	Probiotic strain
Giribaldi et al. (2019)	The chemical characteristics of the fillet were preserved for 3 months.	Lipid profile and lipid oxidation during storage showed significant differences between products. It increased polyunsaturated fatty acids, monounsaturated fatty acids, and oleic acid and limited lipid oxidation.	Refrigerator	Bacteria	Swordfish	*L. paracasei* IMPC 2.1
Rong et al. (2015)	The storage time increased from 24 hours to 48 hours.	The total bacterial counts increased. Gramnegative bacteria predominated, and Pseudomonas and Aeromonas were relatively abundant. It changed the composition of Gram-positive bacteria. Micrococci was not detected and the proportion of Staphylococcus and sensory characteristics decreased.	Refrigerator	Bacteria	Tilapia	*L. plantarum* 1.19
Du-Min et al. (2021)	Shelf life increased to 72 hours	It led to an increase in shelf life, a delay in microbial growth, an improvement in food quality related to physicochemical parameters, maintaining quality, and a significant reduction in trimethylamine content.	25 ^{o}C	Cell-free suspension	Ribbon	*L. plantarum* SKD4 and *P. stilesii* SKD11
Angiolillo et al. (2018)	By increasing the fermentation time to 48 hours, was achieved better microbial and sensory quality.	The total bacterial counts decreased, and the proliferation of Pseudomonas microorganisms, hydrogen disulfideproducing bacteria, Enterobacteriaceae was delayed while maintaining the sensory characteristics.	4 ^{o}C	Bacteria with sodium alginate and glycerol	Sea bass	*L. reuteri*
Nath et al. (2014)	*L. sakei* was effective as a preservative for 15 days.	The number of Staphylococcus aureus, peroxide, and total volatile nitrogenous bases were higher in the control and aerobically packaged treatments compared to the coated treatments. These factors were more	6 ^{o}C	Bacteria	Mackerel	*L. sakei* ATCC 15521

		in the treatment packaged by aerobic method compared to under vacuum. However, the free fatty acid and pH were reduced in the coated treatments packed by aerobic method compared to under vacuum. These factors were reduced in the non- coated treatment compared to the coated treatments.				
Castillo-Jiménez et al. (2017)	*L. plantarum* treatment compared to *L. Acidophilus* ATCC4356 maintained the microbiological and chemical quality of the fillet for 10 days. It had more ability to maintain the quality of the fillet.	Lactic acid bacteria for experimental treatments were 5.94 log CFU/g and coliform and psychrophilic bacteria were less than 2.7 log CFU/g. While in the control treatment, 1.2 log CFU/g of lactic acid bacteria were observed, and psychrophilic and coliform bacteria exceeded the permissible limit for human consumption after 10 days. Volatile nitrogen compounds were reduced in coated treatments compared to the control.	Refrigerator	Bacteria	Tilapia	*L. plantarum* JCM 1149 and *L. acidophilus* ATCC4356
Seifzadeh et al. (2019)	*L. brevis* was proposed as a preservative for the preservation of farmed rainbow trout fillets.	*S. aureus* and *Escherichia coli* were not observed in the experimental and control treatments. *L. delbrueckii* was unable to survive under freezing conditions. The number of Lactobacillus in the combined treatments decreased during the storage period under freezing conditions. A significant increase in the ratio of unsaturated to saturated fatty acids was not observed in L. brevis compared to *L. reuteri*. No significant difference was observed in the number of *L. brevis* during storage at freezing temperature.	Refrigerator and freezing	Bacteria	Rainbow trout	*L. delbrueckii* subsp. *bulgaricus* (PTCC 1737) ، *L. reuteri* (PTCC 1655) ، *L. brevis* CD0817 and combination of *Lactobacillus*
Gholami et al. (2021)	The quality of treated fillets was maintained for 30 days	On the 1, 3, 7, 15, and 30 days, the chemical tests of TVB-N and pH and the total bacterial counts were lower compared to the control sample.	Refrigerator	Bacteria	Spanish mackerel	*L. Casei*
Alizadeh et al. (2019)	The use of higher percentages of sodium alginatecontaining probiotic bacteria can be recommended for keeping fillets for 21 days.	On the 21st day, the redness index of the fillet increased and the yellowness index decreased. Regarding texture indicators such as force, depth of penetration, and strength, the use of coating containing lactic bacteria in higher percentages of sodium alginate and whey protein resulted in better preservation of fillet texture quality compared to other treatments	Refrigerator	Bacteria with sodium alginate and whey protein	Rainbow trout	*L. plantarum subsp. Plantarum* and *P. acidilactici*
Gómez-Freitas et al. (2016)	strain *L. curvatus* BCS35 can be considered a preservative. It preserved fish quality for 14 days.	In hick and megrim, the number of bacteria was significantly lower than in the control groups, in addition, the presence of Listeria species was inhibited in megrim treatments. In the evaluation of the sensory	On ice (0-2 °C)	Bacteria and cellfree suspension	Hake and four-spot megrim	*L. curvatus* BCS35 and *E. faecium* BNM58

		characteristics, the biopreserved batches had more value than the control batches.				
Valerio et al. (2015)	This strain was able to maintain the quality of swordfish fillet for 4 months.	The number of Lactobacillus bacteria increased from 8.57 in the 2-day treatment to 8.65 in the 7-day treatment and decreased to 8.40 in the 15-day treatment. The number of Lactobacillus bacteria increased from 7.27 on day 0 to 8.10 on day 90 and then decreased to 7.61 on day 120. But it was negative on 07 days and increased from 5.94 on day 30 to 6. 36 on day 120.	Refrigerator	Bacteria	Swordfish	*L. paracasei* IMPC 2.1
López de Lacey et al. (2014)	The shelf life of hake increased by at least a week (15 days)	It decreased trimethylamine and pH. The total bacterial counts, H2S-producing microorganisms, and TVB-N in the coated fillet remained below the permissible limit.	Refrigerator	Bacteria with green tea film and agar	Hake	*L. paracasei* L26 plus *B. lactis* B94

4. DISCUSSION

Despite the high nutritional value, seafood acts as a major source of foodborne pathogens including Salmonella spp., *E. coli*, *L. monocytogenes*, Campylobacter sp, and so on. The importance of food safety is increasing continuously. In this direction, various methods have been considered, such as the widespread use of chemical preservatives, to prevent the spread of pathogenic microorganisms, extend the shelf-life of food, and prevent economic losses. The relationship between the association of these preservatives with cancer and other health-related risks has necessitated researchers to explore safer alternatives including bio-preservation or their protective cultures Mei et al. (2019), Rout George et al. (2018).

As the results show (Table 1), the use of probiotic lactobacillus bacteria to improve the quality and increase the shelf life of fish fillets is a new biotechnological research. Some Lactobacillus bacteria have high salt tolerance. They can tolerate low pH and high concentrations of bile salts and gastric juice. Also, despite the ability to stick to the cells of the human body, they are not able to invade and penetrate the cells. Therefore, they can be considered potential probiotics for human health and can be used to process and preserve food such as aquatics Seifzadeh et al. (2019). As presented in Table 1, the total number of aerobic bacteria in the samples treated with lactic acid bacteria increased compared to the control. The increase could be due to the presence of a coating containing bacteria on the fish fillet. In addition, bacteria face changing environmental conditions during the stages of food production and storage, and cold is one of the key elements in the production of new food to maintain food safety. Spoilage bacteria and food pathogens can adapt to the cold. In this condition, an increase in the composition of cyclic and unsaturated fatty acids as a reaction to the rapid decrease in temperature (cold shock) happens in numerous bacteria, and therefore they can survive in cold temperatures, as the results show, these bacteria remain in the refrigerator and have continued to grow Seifzadeh et al. (2019).

According to Table 1, the number of Staphylococcus bacteria in fillets treated with lactic acid bacteria decreased during storage. According to the report of Seifzadeh et al. (2019), *L. brevis*, one of the Lactobacilli of plant flora, has shown probiotic ability. The main metabolites of *L. brevis* include lactic acid and ethanol. *L.*

brevis produces carbon dioxide gas during lactic acid fermentation. Therefore, it can prevent the growth of some bacteria, including Staphylococcus. However this bacterium causes the bitter taste and smell of fish, so it is not recommended for keeping fillets. Rong et al. (2015) also observed decreased Staphylococcus bacteria due to using *L. plantarum* on tilapia fish fillets. However, *L. plantarum* 1.19 did not have an obvious antagonistic effect against Gram-negative bacteria such as Pseudomonas and Aeromonas, which were dominant in the spoilage process of tilapia fillets, so the shelf life of samples treated with LAB was not prolonged. Also, these researchers stated the reduction of sensory characteristics. According to studies conducted by various researchers, although this bacterium improved some microbial characteristics it could not increase the shelf life and maintain the sensory characteristics due to processing is inevitable. Therefore, using species such as *L. plantarum* is not suggested to maintain the quality of fish fillets.

The use of *L. reuteri* as a food supplement is accepted, and in general, the daily consumption of live cells of this Lactobacillus is allowed even in immunocompromised people. Rutherin includes hydrated, non-hydrated, and dimeric forms of 3-hydroxypropional aldehyde. Because reuterin is resistant to proteases and lipases, it has a great ability as a food preservative. It also has strong antimicrobial properties against Gram-positive and Gram-negative bacteria, yeasts, molds, and protozoa, which made it to be noticed in the industry. Of course, reuterin in combination with polyphenols causes a bitter taste, so its combination with plant extracts is not recommended for fillet preservation Seifzadeh et al. (2019). Seifzadeh et al. (2019) stated that probiotic lactobacilli prevent the initial attachment and establishment of pathogenic bacteria in the host's body due to their antimicrobial properties and especially aggregation ability, which was confirmed by Angiolillo et al. (2018). During anaerobic fermentation of glycerol, *L. reuteri* produces reuterin as an intermediate metabolite, which has antimicrobial properties. Therefore, in the study of recent researchers, the properties of *L. reuteri* bacteria in preventing the growth of spoilage bacteria are also affected by the production of reuterin Angiolillo et al. (2018). Seifzadeh et al. (2019) and Angiolillo et al. (2018) observed the reduction of microorganisms causing spoilage along with the preservation of sensory characteristics in fillets of sea bass and farmed salmon coated using *L. reuteri*.

Following the use of lactic acid bacteria to maintain the quality of fish fillets, several studies have used cell-free suspension of lactic acid bacteria to investigate the antimicrobial activity against several human pathogenic bacteria. As can be seen in Table 1, different strains of *L. plantarum* including SkD4, 1.19, and JCM have been used to maintain the quality of ribbon and tilapia fillets, but they have exhibited different times to maintain the quality of the fillet. SkD4 strains increased the shelf life of ribbon fillets to 72 h and 1.19 and JCM increased the shelf life of tilapia to 48 h and more than 10 d, respectively. Considering that these strains belong to the same bacteria, it can be said that different strains show different effects on the shelf life of fillets. Also, considering that the SkD4 strain was used as a cell-free suspension for fillet processing and the study was carried out on different species of aquatic animals, ribbon fish has more fat compared to tilapia, considering that *L. plantarum* It is a species that can produce bacteriocin Shih-Chun et al. (2014), but based on the results of strain SkD4 compared to strain 1.19, it has a greater ability to maintain fillet quality. This point shows that the cell-free culture of this bacterium is more capable of maintaining the quality characteristics of the fillet during storage compared to the use of bacteria alone. In addition, ribbon fillets were stored at room temperature, and considering the storage conditions, it can be argued that the SkD4 strain has a greater ability to maintain the quality of aquatic fillets compared to the

1.19 and JCM strains. This report also confirmed that the cell-free culture of lactic acid bacteria contains several types of antimicrobial compounds and has antimicrobial properties. The ability of SkD4 strain may be influenced by *P. stilesii* SKD11. It is one of the bacteriocin producing bacteria and can enhance the antimicrobial effects of this strain Rong et al. (2015), Du-Min et al. (2021), Castillo-Jiménez et al. (2017).

L. plantarum subsp. *plantarum* was also used to maintain the quality characteristics of rainbow trout. It could improve the quality of fish fillets for a longer period (21 d) compared to recent strains Alizadeh et al. (2019). Since trout is considered a fatty species and this strain was used along with sodium alginate coating, which according to the report of Seifzadeh and Motallebi (2013) sodium alginate has antioxidant and antimicrobial power, the shelf life increases fillets treated with *L. plantarum* may be affected by the properties of sodium alginate. Also, *L. plantarum* subsp. *plantarum* was used in combination with *P. acidilactici* to coat rainbow trout fillet, as a result of which its shelf life can be increased more compared to SkD4, 1.19, and JCM strains of this bacterium. But the preservation of the quality of the fillet provided by this bacterium was lower compared to other species such as *L. paracasei* IMPC 2.1 and *L. casei*, which shows that although sodium alginate had an effect in increasing the ability of this bacterium, it was not able to compete with the mentioned species in terms of ability.

In addition to the microbial characteristics, another mechanism of action of lactic acid bacteria to maintain the quality of fillets is related to the effect of these bacteria to prevent the progress of lipid oxidation and delay its onset Seifzadeh (2021). Based on the studies conducted by other researchers, it was proved that Lactobacillus bacteria species have antioxidant properties, and as seen in the results (Table 1), in the fillets treated with *L. casei*, *L. plantarum* SKD4 and *L. paracasei* IMPC 2.1, chemical characteristics including trimethylamine, peroxide value, and fat oxidation decreased during storage Du-Min et al. (2021), Gholami et al. (2021), Valerio et al. (2015).

L. paracasei strains such as L26 and IMPC 2.1 have been used for hake and swordfish species, respectively, which increased shelf life for at least 1 week for hake and 3 months for swordfish Giribaldi et al. (2019), López de Lacey et al. (2014). Although strain L26 was used together with green tea film, and like strain L26, green tea film has antioxidant properties in addition to antibacterial properties and alone can increase shelf life López de Lacey et al. (2014). However as stated in the results, the association of L26 strain with green tea film was able to maintain the chemical characteristics of the fillet in a short period, which is probably due to the antibacterial effects of green tea polyphenols on bacteria. This point shows that the association of probiotic bacteria with plant extracts does not always lead to an increase in their antibacterial and antioxidant effects. However, the IMPC 2.1 strain alone increased the shelf life of the fillet for a longer time compared to the L26 strain. Considering that hake is one of the low-fat species, but swordfish is one of the high-fat types of aquatic species, it can be seen that this strain has sufficient efficiency to maintain the chemical characteristics and long-term storage of fish fillets. There is no report available for bacteriocin production by *L. paracasei* L26 and *L. paracasei* IMPC 2.1 Giribaldi et al. (2019), López de Lacey et al. (2014). Of course, the ability of these bacteria to limit the growth of unwanted organisms is through competition for nutrients and/or the production of a wide range of antimicrobial metabolites such as organic acids, diacetyl, acetone, hydrogen peroxide, carbon dioxide, rotricycline, antifungal peptides, and related unspecified compounds. The primary antimicrobial effect of lactic acid bacteria is due to the production of lactic acid, which tends to lower the pH of food and, in most cases, inhibits microbial growth. Lactic acid bacteria and their metabolites are widely used in the preservation of agricultural products and meat, and some strains have been reported as antagonists of pathogens and food spoilage microorganisms. Probiotic bacteria can compete with pathogenic bacteria in different ways, such as destroying toxin receptors and strengthening the immune system Seifzadeh et al. (2019).

As shown in the results, *L. curvatus* BCS35 can increase the shelf life of hake and four-spot megrim fillets to 14 days Gómez-Sala et al. (2016). Since *L. curvatus* BCS35 produces two types of bacteriocins, it was able to display this capability along with other antimicrobial mechanisms of Lactobacillus bacteria. Also, this bacterium was used together with *E. faecium* BNM58 for the biological protection of hake and four-spot megrim fish, and since *E. faecium* BNM58 can prevent the growth of bacteria that cause spoilage and disease of seafood, it is possible that the effects of *L. curvatus* BCS35 in protecting of hake and four-spot megrim fillets related to the characteristics of this bacterium. However, there was no report of bacteriocin production by *E. faecium* BNM58. According to Nath et al. (2014), the shelf life of mackerel coated with *L.sakei* ATCC 15521 increased to 15 days. Gholami et al. (2021) stated that *L. casei* was able to maintain the quality characteristics of Spanish mackerel for 30 d, which, in addition to the antimicrobial mechanisms used by Lactobacillus bacteria described, the effects of bacteriocin production by these bacteria cannot be ignored. Because the relationship of several species of lactic acid bacteria with fish spoilage has been proven, therefore, not all species of this group of bacteria can be used as biological preservatives. Also, increasing the number of these bacteria to more than the limit declared by the national and international organizations can be another factor to start spoilage, so the shelf life of fillets treated with these bacteria is limited at refrigerator temperature. However, as can be seen in the present study, the shelf life of fish fillets in refrigerator conditions was variable, which may be influenced by the microbial quality of the raw materials, and the conditions of catching and transporting to the processing plant López de Lacey et al. (2014), Sharma et al. (2022).

5. CONCLUSION

The processing of fish fillets with probiotics enables the preparation of functional foods, which represents a way to achieve a functional diet plan for patient consumers with modified diets, such as cholesterol-free or lactose-free diets. As mentioned, Lactobacillus bacteria were able to increase the shelf life of fish fillets, which was related to maintaining the microbial and chemical characteristics. Also, the use of lactic acid bacteria alone or together with other biological and natural treatments may help to increase the shelf life and safety of food. *L. paracasei* IMPC 2.1 bacterium was able to maintain the quality characteristics of swordfish fillet for up to 4 months at refrigerated temperature, and compared to other bacteria, it showed a greater ability to maintain the quality of the fillet. However, since the sensory factors are also one of the characteristics whose reduction leads to the non-acceptance of the product, it is necessary to examine the sensory characteristics along with the chemical and microbial factors, which were not considered in many studies. Because this bacterium is to be introduced as a biological preservative to the food industry for the enrichment of food products, it requires wider research, and in addition to the sensory characteristics, the effects of this bacterium should also be investigated on other aquatic species with high, low, and medium fat.

CONFLICT OF INTERESTS

None.

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