Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Investigate the effect of gelatin film containing probiotics of *Bifidobacterium bifidum* and *Lactobacillus rhamnosus* on the survival of *Staphylococcus aureus* and physicochemical properties of rainbow trout fillet

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ARTICLE INFO

Article History:
Received:2023/6/12
Accepted: 2024/2/3

Keywords:

Film,
gelatin,
Probiotics,
rainbow trout

DOI: **10.22034/FSCT.21.148.62.**

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ABSTRACT

The aim of this study is to investigate the effect of gelatin film containing *Bifidobacterium bifidum* and *Lactobacillus rhamnosus* bacteria on the survival of *Staphylococcus aureus* bacteria inoculated into rainbow trout meat as well as its physicochemical characteristics for 12 days during storage at refrigerator. *Staphylococcus aureus* was inoculated into fish samples with a concentration of 10^6 log cfu/ml. Then the treatments (control group, rainbow trout samples packed with gelatin film, gelatin film containing each probiotic bacteria *Bifidobacterium bifidum* and *Lactobacillus rhamnus* with a concentration of 10^9 log cfu/ml separately and gelatin film containing both probiotics) were prepared. Samples were packed in polythene bags and stored in refrigerator. Probiotic bacteria viability and *Staphylococcus aureus* bacteria count, pH, TVB-N, PV and TBARS were evaluated. According to the results, the survival of probiotic bacteria showed a decreasing trend during the study, and it was found that the survival of *Bifidobacterium bifidum* bacteria in gelatin film treatment and gelatin film treatment containing both probiotics on the last day respectively 6.81 log cfu/g and 5.37 log cfu/g and the viability of *Lactobacillus rhamnosus* bacteria was also reduced by 7.43 log cfu/g and 6.31 log cfu/g respectively in the mentioned treatments. Compared to the control group, probiotic films controlled the microbial growth of fish fillets well, while the gelatin film treatment containing both probiotics had the lowest rate. Chemical tests also had an increasing trend and their changes in all treated fish fillets were significantly lower than the untreated group (P<0.05). It was found that gelatin films containing probiotics, especially gelatin films containing *Lactobacillus rhamnosus* and *Bifidobacterium bifidum*, which had worked more successfully, had an effective antimicrobial effect against *Staphylococcus aureus* bacteria, and this films can increase the shelf life of rainbow trout and this The category of products has a good effect.

[DOI: 10.22034/FSCT.21.148.62]

1. Introduction

Today, rainbow trout is known as one of the most consumed species of fish in Iran and most parts of the world. Its popularity is increasing day by day and it plays an important role in people's food basket in Iran, and this species is one of the main export fish of Iran [1, 2]. The quality of fresh fish is a major concern for the fish farming industry and their applicants [2, 3]. The most important reason for changing the aroma, taste and other organoleptic characteristics, as well as reducing the shelf life of fish meat, is the oxidation of fats and the growth of bacteria on its surface, which can be those bacteria that are naturally present in fish. . The microbial composition of fish is effective on their spoilage, they are affected because the spoilage ability and metabolic properties of these microorganisms are very different and microbial interactions are effective in them. Apart from this, there are other sources of spoilage agents in fish, which include aquatic habitats, fish skin, tools and processing tools, and others. All over the world, bacterial fish diseases cause a lot of losses in the aquaculture industry, and the widespread use of antibiotics to control this problem may lead to problems such as environmental pollution, high cost, and antibiotic resistance, which is a concern of today's societies [4].

Staphylococcus aureus is a gram-positive bacterium and is classified as the third pathogen responsible for food poisoning in humans, and due to its heat-stable enterotoxins, it is an important factor in the spread of food poisoning, in addition, it can cause disease in animals. Things like mastitis. The pathogenicity of *Staphylococcus aureus* is caused by several factors, which include *Staphylococcus aureus* enterotoxin (SE), hemolysins, fibronectin-bound proteins, etc. These factors play an important role in the pathogenesis of *Staphylococcus aureus*. Symptoms of food poisoning with *Staphylococcus aureus* include vomiting, abdominal pain, stomach cramps, diarrhea, which are very common and appear shortly after consuming contaminated food. Sometimes the symptoms may be so severe that a person is hospitalized, especially in the elderly, pregnant women, children and people with weak immune systems [5].

Various methods are used to prevent spoilage in fish and loss of quality in them, but nowadays the use of edible films as alternative compounds for packaging various food products has become popular. In a study examining edible films with suitable properties for active packaging of meat, fish and seafood, they are highly perishable products. Edible films offer an interesting approach to preserving and packaging these foods. Edible films of biopolymers produced from food industry waste materials or low consumption sources of protein, lipids or polysaccharides are composed of biodegradable and edible and can act as carriers [6].

Thin layers with a thickness of less than 0.01 inch, which are materials that are used directly for the external part of products that are intended to improve their properties or protect

[DOI: 10.22034/FSCT.21.148.62]

them, are called edible film and coating. They are compounds that can be used for food products in terms of standard rules and are safe for humans, which are consumed together with food and have a great ability to prevent the entry of moisture and oxygen, preserve the aroma of the product, and for this reason, they are also used in food and pharmaceutical products. They are used and lead to an increase in the shelf life of the product. Edible films have antioxidant properties and biological activity, and it is possible to increase these properties by adding different compounds to them. The components of edible coatings include proteins, polysaccharides, lipids or gums, among these biopolymers, gelatin has the ability to form a good film, but has weak antibacterial and antioxidant activity [7].

Gelatin, which is obtained from the partial hydrolysis of collagen, is suitable for food use due to its low cost, non-toxicity, and biodegradability, and due to the improvement of food quality and shelf life of food protected with these compounds, and due to its suitable mechanical properties and high resistance to Compared to gases such as oxygen and various aromas in medium and low relative humidity, they have attracted more attention and are better known [8]. One of the compounds used today to produce edible films is the use of gelatin film with probiotic bacteria. According to the findings of a study by Mozafar et al. (2020), it was found that the addition of probiotics to the carboxymethyl cellulose-sodium caseinate film controlled the growth of *Listeria monocytogenes* bacteria in a type of fish [9]. In a study titled the performance of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* in edible coatings and films, based on this study, the initial concentration of both *Lactobacillus acidophilus* and *Bifidobacterium bifidum* remained relatively constant for 6 days and at the end of the study period The number of *Lactobacillus acidophilus* bacteria was in a similar range for all batches, and the survival of Bifidobacterium decreased slightly during storage compared to the initial concentration. In many cases, lactic acid bacteria promote health when consumed, but they also fight pathogenic microorganisms by competing with pathogens for the production of nutrients, producing metabolites such as organic acids and bacteriocins, etc. They have a protective effect in the product [10]. According to what was said, the aim of the present study is to investigate the effect of gelatin film containing probiotics *B. bifidum* and *L. rhamnus* on the survival of *Staphylococcus aureus* bacteria and the physicochemical characteristics of rainbow salmon fillet.

2. Material and methods

2-1-Preparation of studied probiotic bacteria

Lactobacillus rhamnosus (PTCC 1637) and *Bifidobacterium bifidum* (PTCC 1644) were purchased from Iran Scientific and Industrial Research Organization and were activated according to the protocol of Shahbazi et al. (2015) and Ebrahimi et al. (2018) [10, 11]. In this way, bacteria were first added to the MRS broth sterile culture medium and kept in

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incubator at 37 degrees Celsius for 24 hours. Next, a 48-hour re-cultivation was given in order to achieve the maximum number of active probiotics [11, 12].

2-2-Preparation of the inoculation amount of the studied bacteria

To prepare the amount of bacterial inoculation, MRS broth culture was used, from which a re-culture was prepared after 24 hours and kept in incubator for 48 hours. Bacterial sediment was separated using a refrigerated centrifuge (Sigma, Germany) at 4 degrees Celsius, with 4000 rpm for 15 minutes. After the complete settling of the bacterial sediment, the supernatant was drained and 2 ml of sterile physiological serum solution of 0.9% was added to the tubes. Then the tubes were vortexed to distribute the bacterial sediment in the physiological serum uniformly. The act of centrifuging and washing the bacteria with sterile physiological serum was repeated twice and finally, after draining the supernatant, another 2 ml of sterile physiological serum was added to the tubes and finally the tubes were vortexed. To prepare the bacterial inoculation dose, it was done as follows: the spectrophotometer (Cadex, Canada) was set to 600 nm wavelength and zeroed with a cuvette containing sterile physiological serum. From the prepared bacterial suspension, an appropriate amount was transferred into the cuvette and the light absorption of the bacteria was set to 1. Then the bacterial suspension was prepared with a concentration of 10 times the optical absorption of 1 [13]. From the prepared bacterial suspension, 1 ml was taken and by transferring it to a tube containing 9 ml of 0.1% sterile peptone water, successive dilutions up to -6 were prepared. Dilutions -5 and -6 were cultured in plates containing specific culture mediums in three replicates and after 48 hours of incubation using an anaerobic jar (Merck, Germany) and gas pack type A (Merck, Germany), the plots were counted. The number of bacteria was calculated as 1×10^9 cfu/ml [13].

2-3-preparation of gelatin film

Cold water fish skin gelatin (Merck, Germany) was purchased. Gelatin solutions (3.5 g) were mixed by dissolving each in 100 ml of distilled water with a magnetic stirrer at room temperature. Then, 30% (w/w) glycerol (Merck, Germany) and 0.02 g of Tween 80 were mixed as an emulsifier in the hydrocolloids for 15 minutes. The solutions were then sterilized at 121 °C for 15 min and allowed to cool to room temperature before adding probiotics. After preparing gelatin base solution, 109 cfu/ml of each of *L. rhamnus* and *B. bifidum* bacteria were added to the solution according to different conditions. It was stirred for 15 minutes with an ultrathorax device (IKA T18 digital) at 10,000 rpm. To prepare the films, 20 ml of the solutions were poured on sterile Petri dishes (inner diameter 8 cm) and dried at 30 °C for 12 hours to obtain a uniform thickness. Films filled with probiotics were stored in plastic bags and stored at 4 degrees Celsius [12, 14].

2-4- Investigating the survival of *L. ramensus* and *B. bifidum* in gelatin film

[DOI: 10.22034/FSCT.21.148.62]

Counting of probiotic bacteria was done according to Iranian national standard number 11325. In order to check the viability of the studied probiotic bacteria in the gelatin film, 1 gram of the prepared films was mixed with 9 ml of 0.1% peptone water inside the test tube and vortexed for ten minutes. After vortexing it, a series of dilutions was added by adding 1 ml to 9 ml of 0.1% sterile peptone water. After vortexing the tube, successive dilutions up to -7 were prepared in the same way, and then 100 microliters were taken from each dilution and used to count the number of *L. rhamnus* and *B. bifidum* bacteria for twelve days (zero, 3, 6, 9, 12) were cultured on plates containing MRS agar and MRS media with 0.05% cysteine respectively in three replicates with an L-shaped rod and cultured for 48-72 hours at a temperature of 37 degrees Celsius under sterile conditions. Aerobics was placed in incubator, and after that, the number of bacteria was counted in the specified environments. The counting results were reported in terms of cfu/g [10].

5-2-Preparation of rainbow salmon samples

Rainbow salmon was purchased from one of the reputable fish breeding centers and transported to the laboratory under completely sterile conditions and on ice. Immediately, its fillet was separated by scissors and a sterile scalpel, and as a sample, 10-gram pieces of fish meat were prepared and sterilized by immersion in 70% alcohol. After that, they were stored in a sterile ostomaker bag. Also, it was ensured that the samples were not contaminated with *Staphylococcus aureus*, for this purpose, 5 grams of fish fillets were dissolved in 45 ml of 0.1% peptone water and after preparing serial dilutions, the samples were cultured in Brad Parker Agar culture medium (Merck, Germany) and kept in incubator for 48 hours at a temperature of 30 degrees Celsius [15].

6-2-Preparation of *Staphylococcus aureus*

Staphylococcus aureus (ATCC 35218) was obtained from the Department of Health and Quality Control of Food, Faculty of Veterinary Medicine, University of Tehran. The general culture medium suitable for this bacterium is BHI agar (Merck, Germany), which cultured the bacterium two consecutive times in this medium, then placed in incubator at 37 degrees Celsius for 18 hours. Then, 4 to 5 colonies were removed by a sterile loop and transferred to tubes containing 5-10 ml of BHI broth medium and kept in incubator at 37 degrees Celsius for 18-24 hours. Then, the amount of light absorption was determined at 0.08-1 and the wavelength was 600 nm. The absorption between 0.08-1 is equivalent to 1.5×10^8 cfu/mL, and then the concentration of 10^6 cfu/ml was prepared [10]. In order to inoculate *Staphylococcus aureus* bacteria, the desired fish samples were placed inside the bacterial suspension containing 10^6 log cfu/ml for 5 minutes. In order to dry the surface of the samples, they were placed at room temperature for 30 minutes and then kept at the temperature of the refrigerator until the completion of other test steps [16].

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2-7- Counting *Staphylococcus aureus*

In order to count coagulase-positive *Staphylococcus aureus*, they were cultured on plates containing Bard-Parker agar medium and kept in incubator at 37±2 °C for 48±2 hours. For confirmation test, coagulase test was used in the tube [11].

8-2-Measuring the pH of the sample

The pH of the samples was measured based on the national standard of Iran No. 2852. 10 grams of rainbow salmon fillet sample was completely mixed and homogenized in 50 ml of distilled water and its pH was evaluated using a digital pH meter (Metrohm, Switzerland)[16].

9-2- Determination of peroxide number (PV)

Three grams of rainbow salmon fillet sample was placed in a bain-marie for 3 minutes at 60 degrees Celsius to melt the fat in it. Then 30 ml of acetic acid-chloroform at a ratio of 60 to 40 by volume and 0.5 ml of saturated potassium iodide were added to it and stirred for one minute in the dark. The solution was titrated with 0.01 normal thiosulfate until the solution became colorless, and using the following formula (formula number 1), the amount of peroxide was calculated in terms of milliequivalents of peroxide per 1000 grams of fat [16].

$$POV= V.N.1000/W$$

2-10-Measuring the amount of volatile nitrogen substances (TVB-N)

The volatile nitrogen content of the samples was measured by the macrocaldal method and the amount of volatile bases was expressed in terms of milligrams of nitrogen per 100 grams of fish sample [12].

$$\%\ mg\ TVB\text{-}N=100 \times 1.4 \times 0.1$$

2-11-Measurement of thiobarbituric acid index (TBARS)

This index was measured by adding 97.5 ml of distilled water and 2.5 ml of 4N hydrochloric acid to 10 grams of the homogenized sample. 5 ml of the liquid obtained from the distillation of this mixture was added to 5 ml of thiobarbituric acid reagent (Merck, Germany) and placed in a bain-marie at boiling temperature for 35 minutes. After cooling, the optical absorption of the pink solution was measured at 538 nm by a spectrophotometer. Thiobarbituric acid index (TBARS) was evaluated according to the following formula in terms of milligrams of malonaldehyde per kilogram of sample [12].

$$\text{TBARS value} = 7.8\ (\text{Measure of optical absorption at } 538\ nm)$$

12-2-Data analysis

SPSS version 26 software was used for statistical analysis of the data and the significance level was less than 0.05. The obtained results were analyzed by ONE WAY ANOVA method. The mean and standard deviation are the results of three replicates. Duncan's supplementary test was used to compare the means.

3- Results and discussion

3-1-Survival of probiotic bacteria in gelatin films

[DOI: 10.22034/FSCT.21.148.62]

The survival of probiotic bacteria *B. bifidum* and *L. rhamnus* in gelatin film during 12 days of storage at 4 degrees Celsius is shown in Figure 1. As can be seen, there are no significant differences between the treatments on day zero ($p<0.05$). With the passage of time, the survival of probiotic bacteria in treatments has decreased. In the treatments of the film together with each of the probiotic bacteria alone (gelatin film + *L. rhamnus* and gelatin film + *B. bifidum*), the survival of *L. rhamnus* and *B. bifidum* was 8.98 log cfu/g and 9.01 log cfu/g, respectively. On the zero day of the study, log cfu/g reached 7.43 and 6.81, respectively, at the end of the study (day 12), and it was found that the highest survival rate of probiotic bacteria is related to *L. rhamnus*. In mixed treatments (gelatin film + *L. rhamnus* and *B. bifidum*), the survival of *L. rhamnus* bacteria increased from 8.99 log cfu/g on day 0 to 6.31 log cfu/g on day 12, and the survival of *B. bifidum* bacteria also increased from 9.02 log cfu/g reached 5.37 log cfu/g on day 12, which indicates the higher survival of *L. rhamnus* bacteria.

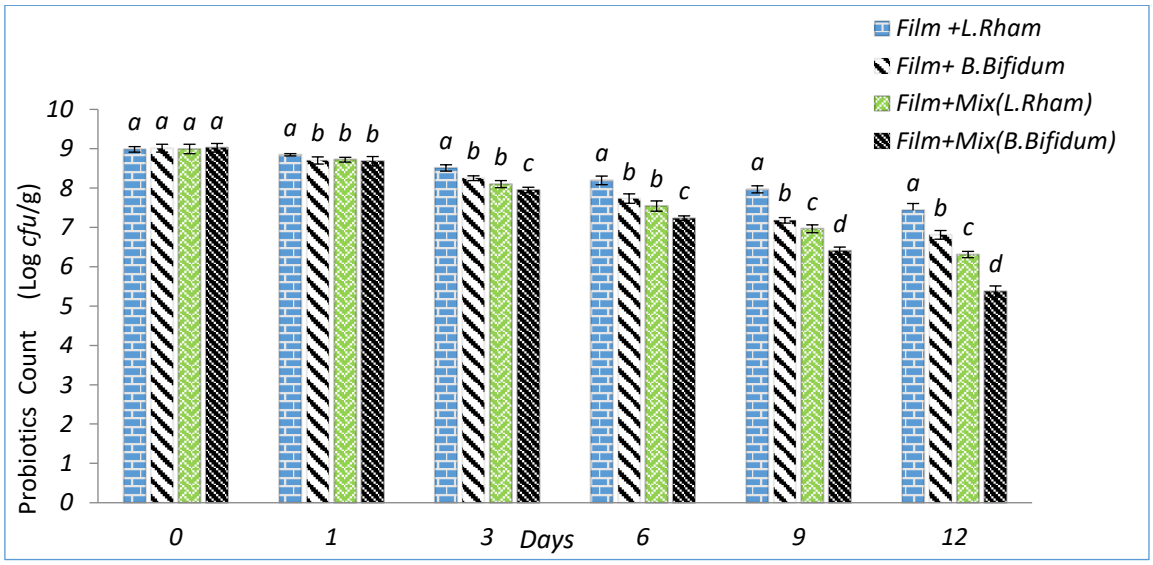

Figure 1. Survival of probiotic bacteria *B. bifidum* and *L. rhamnus* in gelatin film during storage at refrigerator temperature for 12 days (mean ± SD). Small and similar English letters in the diagram show no significant difference between the groups ($P> 0.05$).

2-3- The results of counting *Staphylococcus aureus* bacteria inoculated into rainbow salmon fillet

The results of counting *Staphylococcus aureus* bacteria in treatments of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* and *B. bifidum* during storage at refrigerator temperature for 12 days, along with the control treatment, are presented in Figure 2. As can be seen, there are no significant differences between the treatments on day zero ($p<0.05$). The growth of *Staphylococcus aureus* has been increasing in all treatments during the study. The count of *Staphylococcus aureus* in the control treatment was 5.98 log cfu/g at the beginning of the study and increased to 8.93 log cfu/g on the last day of the study. The count of *Staphylococcus aureus* in the treatments

[DOI: 10.22034/FSCT.21.148.62]

wrapped with gelatin film reached from 6.03 log cfu/g to 6.13 log cfu/g on day 12. It increased to 6.09 log cfu/g and 6.69 log cfu/g in the treatment of gelatin film with *L. rhamnus* bacteria and in the treatment of gelatin film with *B. bifidum* bacteria on day 12. From day zero to the end of the study, the count of *Staphylococcus aureus* in the treatment of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* and *B. bifidum* bacteria decreased significantly (P<0.05) compared to the control. This treatment increased the count of *Staphylococcus aureus* on day zero from 6.02 log cfu/g to log cfu/g reached 5.24 on the 12th day, which was the lowest among all groups on the last day and indicates the better performance of this treatment compared to other groups.

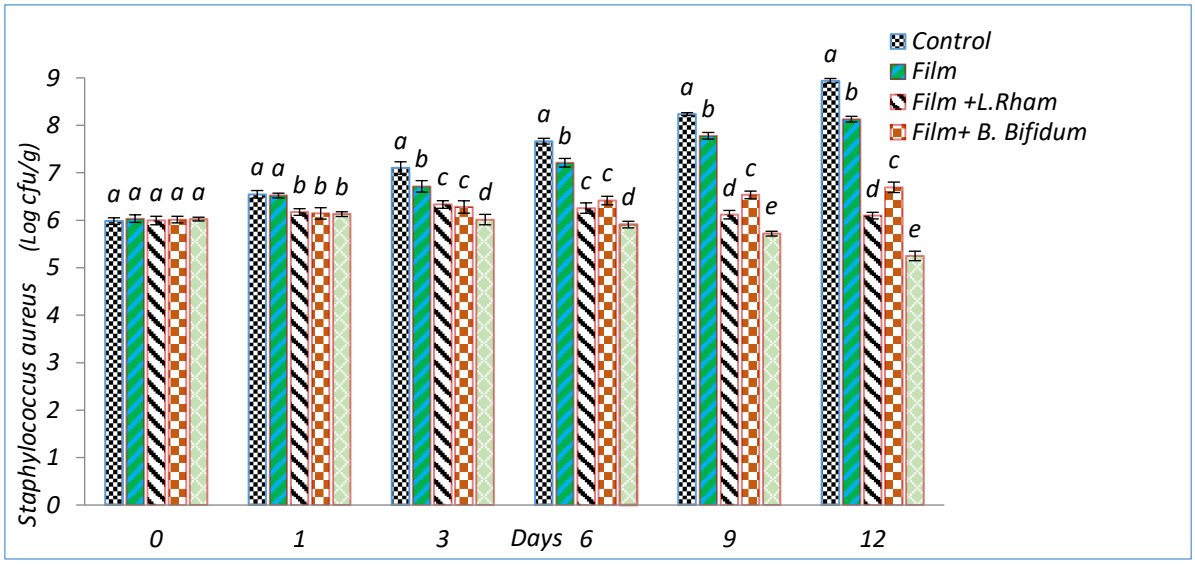

Figure 2. Survival of *Staphylococcus aureus* inoculated in treatments of rainbow rainbow trout fillets packaged with gelatin films containing *L. rhamnus* and *B. bifidum* during storage at refrigerator temperature for 12 days (mean ± SD). Small and similar English letters in the diagram show no significant difference between the groups (P> 0.05).

The survival of probiotic bacteria *B. bifidum* and *L. rhamnus* in gelatin film and the results of evaluating the effect of gelatin film containing *L. rhamnus* and *B. bifidum* during 12 days of study at refrigerator temperature on the survival of *Staphylococcus aureus* bacteria in rainbow salmon fillet samples in chart 1 and 2 have been reported. According to the results of this study, gelatin film alone did not have a favorable antimicrobial effect compared to gelatin films containing probiotic bacteria, and only probiotic films had antimicrobial properties, and these results are in agreement with the studies of Saba et al., 2016; Han et al., 2017; Rezaei and Shahbazi, 2018 are consistent [18, 19, 20]. In the study and research conducted by Rezaei and Taghizadeh Andvari (2011) regarding the effect of 4% gelatin coating on the quality of rainbow salmon fillet during a period of 20 days at refrigerator temperature, it was found that gelatin coating alone has no effect. It does not control the growth of bacteria, and in fact, it does not have biological and antibacterial activity, and these results are consistent with the findings of the present study [21]. In a research by Fazal Ara et al. (2017), it was found that gelatin coating alone lacks the necessary ability to improve the

[DOI: 10.22034/FSCT.21.148.62]

shelf life of ostrich fillets, but as a coating and carrier for compounds with antimicrobial effect, it can be effective in increasing the shelf life, and these results are consistent with The findings of the present study are consistent [22]. According to the results of this study, the gelatin film increased the viability of probiotic bacteria. In this regard, in a study aimed at the survival of different probiotics *Lactobacillus plantarum* and Lactobacillus casei during storage periods in gelatin films and low methoxyl pectin, the results showed that the number of viable probiotic cells decreased in the drying phase of film forming solutions. Storage temperature was an effective factor in the survival of probiotics [16]. Also, in a study, they investigated the survival time of *L. rhamnus* bacteria on probiotic films made from corn starch, gelatin, sodium caseinate, and soy protein concentrate and reported that the number of bacteria increased in protein-based films, which is consistent with the results. The present study is consistent. While in a study, the effect of *Lactobacillus acidophilus* and *B. bifidum* in edible coatings and films was investigated. According to the results, the amount of studied bacteria was constant at the beginning and decreased slightly during the storage period. It was found that the use of gelatin film containing *Lactobacillus acidophilus* and *B. bifidum* and the application of high hydrostatic pressure (200 MPa/10 minutes/temperature of 20 degrees Celsius) at the same time significantly increased the shelf life of the studied fish fillet [23]. As explained, gelatin films containing probiotic bacteria *L. rhamnus* and *B. bifidum* caused a significant decrease in the number of *Staphylococcus aureus* bacteria in rainbow salmon fillets stored at refrigerator temperature ($P < 0.05$), which is consistent with some studies in These fields match. In a study by Altieri et al. (2005), adding *B. bifidum* to a type of fish has inhibited the growth of spoilage bacteria including *Schwanella putrifiense*, Pseudomonas species and *Photobacterium phosphorium* [24]. In a study, film containing *Lactobacillus paracasei* and *Bifidobacterium lactis* caused a significant reduction in microbial spoilage indicators in a type of fish kept at refrigerator temperature for 15 days [14].

In a research, Fazal Ara et al. (2017) investigated the effect of gelatin coating with a specific concentration containing a type of herbal antimicrobial compound on the microbial characteristics of ostrich fillet at refrigerator temperature for 15 days. According to the findings, this coating has had a significant effect on reducing the increasing trend of the number of spoilage-producing bacteria, which is consistent with the findings of the present study [22]. In a study, Salehi (2013) proved the effect of lactobacilli isolated from local foods in controlling the growth of *Staphylococcus aureus* bacteria [25]. The results of this study showed that *L. rhamnus* and *B. bifidum* have the highest survival rate of probiotic bacteria. The lower survival rate of *Bifidobacterium bafidum* can be attributed to its greater sensitivity to the reduction of nutrients, water activity, oxygen level and storage temperature, which is consistent with the results of a study by Homayouni et al. (2008). In the mentioned study, the survival rate of lactobacilli is

[DOI: 10.22034/FSCT.21.148.62]

significantly higher than bifidobacteria during long-term storage of synbiotic ice cream [27].

3-4- pH evaluation results

The results of evaluating the effect of gelatin film containing *L. rhamnus* and *B. bifidum* bacteria during 12 days of study at refrigerator temperature on the pH of rainbow salmon samples are presented in Table 1. As can be seen, the trend was increasing in all groups and no significant difference was observed between the treatments on day zero (P<0.05). Based on the obtained results, the pH of the control group increased from 17.6 on day 0 to 29.7 on the last day of the study. While on the 12th day, there was a significant difference between the control group and other treatments (P<0.05). On day 12, rainbow salmon fillets packed with gelatin film equal to 7.01 in the samples of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* equal to 6.49 in the samples of colored salmon fillets. The rainbow trout packed with gelatin film containing *B. bifidum* was equal to 6.50 and in the rainbow salmon fillet samples packed with gelatin film containing *L. rhamnus* and *B. bifidum* was equal to 6.30. A significant difference (P<0.05) was observed in the pH value of rainbow salmon samples packed with gelatin coating containing *L. rhamnus* and *B. bifidum* compared to control treatment samples on all days of the test except day zero. During the conducted study, the lowest pH difference with the control treatment was related to the treatment of rainbow salmon fillets packed with gelatin film alone, and the biggest difference in pH level was related to the samples treated with gelatin containing *L. rhamnus* and the gelatin treatment containing *B. bifidum*. . And the highest difference (the lowest pH value) was related to rainbow salmon samples packed in gelatin containing *L. rhamnus* and *B. bifidum*.

Table 1. Average pH changes in different treatments of rainbow trout kept at refrigerator temperature (mean ± SD). Small and similar English letters in the diagram show no significant difference between the groups (P> 0.05).

Treatment	Day					
	0	**1**	**3**	**6**	**9**	**12**
Control	$0.03^{a} \pm 6.17$	$0.03^{a} \pm 6.51$	$0.01^{a} \pm 6.81$	$0.01^{a} \pm 6.89$	$0.03^{a} \pm 7.15$	**$0.04^{a} \pm$**
Film	$0.03^{a} \pm 6.17$	$0.04^{a} \pm 6.48$	$0.01^{b} \pm 6.63$	$0.02^{b} \pm 6.71$	$0.02^{b} \pm 6.88$	**$0.02^{b} \pm$**
B.b	$0.03^{a} \pm 6.17$	$0.02^{b} \pm 6.35$	$0.02^{c} \pm 6.39$	$0.02^{c} \pm 6.35$	$0.01^{c} \pm 6.47$	**$0.01^{c} \pm$**
L.r	$0.03^{a} \pm 6.17$	$0.01^{c} \pm 6.30$	$0.01^{d} \pm 6.34$	$0.01^{d} \pm 6.31$	$0.02^{d} \pm 6.40$	**$0.01^{c} \pm$**
B.b+Lr	$0.03^{a} \pm 6.17$	$0.02^{d} \pm 6.18$	$0.01^{e} \pm 6.22$	$0.02^{e} \pm 6.25$	$0.01^{e} \pm 6.26$	**$0.03^{d} \pm$**

The general trend in all groups has been increasing. The increase in pH is generally due to the production of alkaline compounds such as ammonia and amines and through the breakdown of proteins, but in the control group due to more bacterial contamination, more nitrogenous compounds were also produced, which is consistent with the results of the studies of Luto et al. (2014) and Fan et al. colleagues (2009) is consistent [28, 29]. On day 12, pH of rainbow salmon fillets packed with gelatin film equals 7.01, in samples of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* equal to 6.49, gelatin film containing *B. bifidum* It was equal to 6.50 and the gelatin film containing *L. rhamnus* and *B. bifidum* was equal to 6.30, which was the lowest pH value related to this treatment. The decrease in pH, especially in the gelatin group containing *L. rhamnus* and *B. bifidum*, is due to the presence of lactic acid in it, and it has been transferred to the sample, which is consistent with the study of Lopez et al. in 2012 and Fan

[DOI: 10.22034/FSCT.21.148.62]

et al. (2009) [14, 28]. Probiotic microorganisms reduce the pH of the environment by fermenting lactose and producing lactic acid and butyric acid and are effective in controlling the growth of pathogenic agents that are not resistant to these conditions. In a study by Talwalker and colleagues in 2004, they stated that Bifidobacterium is capable of fermenting lactose and producing acetic acid, which itself contributes to the drop in pH [30]. In 1997, Dioshe et al. observed a gradual decrease in pH in yogurt containing probiotics, which they attributed to fermentation, and it is consistent with the results of this study [31]. Also, in a research conducted by Fazal Ara et al. (2017), the effect of gelatin coating (4%) containing Shirazi thyme (1.5%) on the chemical properties of ostrich fillet at 4 degrees Celsius and in a period of 15 days was investigated. it placed. According to the findings, Shirazi gelatin-thyme treatment showed a lower pH level during the storage period than the other three groups, which is consistent with the findings of the present study [22].

5-3- The results of the evaluation of the amount of peroxide index (PV)

The results of measuring the amount of peroxide in the treatments of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* and *B. bifidum* during storage at refrigerator temperature for 12 days, along with the control treatment, are presented in Table 2. Based on the obtained results, a significant difference ($P<0.05$) was observed in the peroxide value of the control treatment compared to all other treatments from the third day to the twelfth day. But the general trend in all treatments was increasing. The peroxide value of the control treatment was 0.96 meq/kg on the zero days and reached 4.25 meq/kg on the last day. The results obtained in the comparison between the control treatment and the gelatin-wrapped treatment with the gelatin-wrapped treatments containing *L. rhamnus* have a significant difference ($P<0.05$). In the treatment of rainbow salmon packaged with gelatin film on the last day to 3.92 meq/kg, in the treatment of rainbow salmon fillet packed with gelatin film containing *L. rhamnus* on the last day to 06/meq/kg 3 and in the treatment of rainbow salmon fillets packed with gelatin film containing *B. bifidum* to 3.23 meq/kg and in the treatment of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* and *B. bifidum* equal to meq 2.58/kg (the lowest amount) increased. The peroxide number in the treatments wrapped with gelatin film containing *L. rhamnus* from day 0 to the end of the study was significantly ($P<0.05$) lower than the peroxide number in the control treatment.

Table 2. Average PV changes in different treatments of rainbow trout kept at refrigerator temperature (mean ± SD). Small and similar English letters in the diagram show no significant difference between the groups ($P> 0.05$).

Treatment	Day					
	0	**1**	**3**	**6**	**9**	**12**
Control	$0.03^{a} \pm 0.96$	$0.05^{a} \pm 1.66$	$0.05^{a} \pm 2.84$	$0.01^{a} \pm 3.03$	$0.01^{a} \pm 3.96$	$\mathbf{0.00^{a} \pm 4.25}$
Film	$0.03^{a} \pm 0.96$	$0.06^{a} \pm 1.63$	$0.01^{b} \pm 2.55$	$0.03^{b} \pm 2.66$	$0.02^{b} \pm 3.18$	$\mathbf{0.03^{b} \pm 3.92}$
B.b	$0.03^{a} \pm 0.96$	$0.06^{b} \pm 1.34$	$0.02^{c} \pm 1.69$	$0.03^{c} \pm 2.21$	$0.03^{c} \pm 2.77$	$\mathbf{0.01^{c} \pm 3.23}$

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L.r	$0.03^{a} \pm 0.96$	$0.04^{b} \pm 1.31$	$0.04^{c} \pm 1.67$	$0.02^{d} \pm 2.02$	$0.02^{d} \pm 2.54$	$\mathbf{0.02^{d} \pm 3.06}$
B.b+L.r	$0.03^{a} \pm 0.96$	$0.05^{b} \pm 1.29$	$0.01^{c} \pm 1.63$	$0.01^{e} \pm 1.90$	$0.01^{e} \pm 2.08$	$\mathbf{0.02^{e} \pm 2.58}$

Fat oxidation is a major problem in fresh fish and other marine products. Peroxide number (PV) is the product of primary oxidation of fats, and the higher the degree of unsaturation of fats, the more ready than substance is for oxidation [32]. It has been suggested that the maximum acceptable amount of peroxide for the optimal quality of fish is 7-8 meq/kg [33], which in this study, the control group and the samples packed with gelatin film on the last day were close to these limits. The general trend in all treatments was increasing; the highest amount was observed on the 12th day of the control group and reached from 0.96 meq/kg to 4.25 meq/kg, which is due to the activity of cold-loving bacteria, especially Pseudomonas species. In fact, bacteria Cold-loving foods can produce lipase and phospholipase enzymes while storing food in the refrigerator and subsequently increase the amount of short-chain fatty acids. This type of fatty acids is sensitive to oxidation and finally hydroperoxide is produced in the food [34]. Treatment of rainbow salmon fillets packed with gelatin film on the last day to 3.92 meq/kg, treatment of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* and *B. bifidum* respectively on the last day reached 3.06 meq/kg and 3.23 meq/kg. In the treatment of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* and *B. bifidum* increased by 2.58 meq/kg (the lowest amount). All samples of rainbow salmon fillets with film, especially containing probiotic bacteria, had less peroxide than the control, which indicates that this film and its contents were effective in reducing the fat oxidation of rainbow salmon fillets. Saki et al., (2017) in a study investigated the effect of chitosan-gelatin edible film on the characteristics of a type of fish kept in a refrigerator. According to this research, the samples with film and chitosan-gelatin coating compared to the control sample, in the tests of the index of fat oxidation (free fatty acid), had a lower level of oxidation, which is consistent with the findings of the present study [35]. The lower amount of PV and TVB-N in pure film group is consistent with other studies on salmon fillet (Dehghani et al., 2018) [36]. Oxidation raw materials (hydroperoxides) are unstable and prone to decomposition. Secondary oxidation products include ketones, aldehydes, hydrocarbons, alcohols, epoxy compounds and organic acids. A partial compound of fatty acids with three or more double bonds is malonaldehyde, which is formed by the breakdown of unsaturated fatty acids during fat oxidation [37].

6-3- The results of TVB-N evaluation

The results of the evaluation of volatile nitrogen substances in treatments of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* and *B. bifidum* during storage at refrigerator temperature for 12 days, along with the control treatment, are reported in Table 3. The general trend of volatile nitrogen substances in the samples was increasing

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during the study. The initial amount of volatile nitrogen in rainbow salmon fillet samples was 9.19 mg/100g on day zero, which reached 48.22 mg/100g on day 12. On the 12th day, this number for the treatment of rainbow salmon fillets packed with gelatin film equals 41/16 mg/100g, for the treatment of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* 17/100g mg/100g 23, for the treatment of rainbow salmon fillets packed with gelatin film containing *B. bifidum* mg/100g 23/26, for the treatment of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* and *B. bifidum* mg/100g 05 was 19.00, all treatments of rainbow salmon fillets wrapped with antimicrobial film showed a significant difference in the amount of volatile nitrogen substances compared to the control treatment ($P<0.05$). The lowest amount of volatile nitrogen substances in the entire study was related to the treatment wrapped with gelatin film containing *L. rhamnus* and *B. bifidum*.

Table 3. Average TVB-N changes in different treatments of rainbow trout kept at refrigerator temperature (mean ± SD). Small and similar English letters in the diagram show no significant difference between the groups ($P> 0.05$).

Treatment	Day					
	0	**1**	**3**	**6**	**9**	**12**
Control	$0.02^{a} \pm 9.19$	$0.06^{a} \pm 12.58$	$0.05^{a} \pm 25.18$	$0.03^{a} \pm 28.64$	$0.02^{a} \pm 42.01$	**$0.03^{a} \pm 48.22$**
Film	$0.02^{a} \pm 9.19$	$0.05^{a} \pm 12.55$	$0.01^{b} \pm 21.14$	$0.04^{b} \pm 27.03$	$0.01^{b} \pm 33.34$	**$0.04^{b} \pm 41.16$**
B.b	$0.02^{a} \pm 9.19$	$0.04^{b} \pm 11.10$	$0.03^{c} \pm 13.22$	$0.02^{c} \pm 16.17$	$0.06^{c} \pm 20.18$	**$0.02^{c} \pm 26.23$**
L.r	$0.02^{a} \pm 9.19$	$0.05^{b} \pm 11.08$	$0.02^{c} \pm 13.19$	$0.02^{d} \pm 15.14$	$0.04^{d} \pm 19.45$	**$0.03^{d} \pm 23.17$**
B.b+L.r	$0.02^{a} \pm 9.19$	$0.07^{c} \pm 10.99$	$0.02^{d} \pm 11.79$	$0.04^{e} \pm 12.01$	$0.05^{e} \pm 15.32$	**$0.01^{e} \pm 19.05$**

Total Volatile Nitrogen (TVB-N), which mainly consists of ammonia and amines, is used as an indicator of spoilage in fish meat. By increasing the activity of bacteria and enzymes, this index also increases and causes an unpleasant taste in fish. The maximum acceptable amount of volatile nitrogen substances is suggested to be 25 mg per 100 grams of fish meat sample. In this study, according to the results of the evaluation of volatile nitrogen substances in treatments of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* and *B. bifidum* during storage at refrigerator temperature for 12 days, along with the control treatment in Table 3, it is determined It was exceeded in the control group from the third day, from the gelatin film group alone from the sixth day, in the gelatin film group containing *B. bifidum* probiotic on the last day (day 12) and in the other groups, i.e. the gelatin film group containing *L. rhamnus* and gelatin film group containing *B. bifidum* and *L. rhamnus* probiotics, until the end of the study period, the amount of volatile nitrogenous bases was reported within the permissible limits. The general trend of volatile nitrogen content in the samples was increasing during the study, and as mentioned, the increase in the amount of total volatile bases during the storage period of fish fillets at refrigerator temperature can be caused by the partial dehydrogenation of fish tissue, and the production of volatile metabolites in the presence of oxygen and lipid oxidation [38]. The initial amount of volatile nitrogen

[DOI: 10.22034/FSCT.21.148.62]

substances in fish fillet samples reached from 9.19 mg/100g to 48.22 mg/100g, which was the highest amount and the reason for this is the higher microbial load. On the 12th day, this number for the treatment of rainbow salmon fillets packed with gelatin film equals 41/16 mg/100g, for the treatment of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* 17/100g mg/100g 23, for the treatment of rainbow salmon fillets packed with gelatin film containing *B. bifidum* mg/100g 23/26, for the treatment of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* and *B. bifidum* mg/100g 05 19/ which was the lowest amount. In a research conducted by Fazal Ara et al. (2017), the effect of gelatin coating (4%) containing Shirazi thyme (1.5%) on the chemical properties of ostrich fillet at a temperature of 4 degrees Celsius and in a period of 15 days was investigated. it placed. According to the findings, in terms of chemical factors, the gelatin treatment containing Shirazi thyme showed a lower amount of total volatile nitrogen during the storage period than the other three groups, which is consistent with the findings of the present study [22].

7-3- The results of evaluating the amount of thiobarbituric acid index (TBARS)

The results of TBARS evaluation in treatments of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* and *B. bifidum* during storage at refrigerator temperature for 12 days, along with the control treatment, are reported in Table 4. The general trend in the samples was increasing during the study. The amount of TBARS in rainbow salmon fillet samples was 0.41 on day zero, which reached 3.27 on day 12. On day 12, this number for the treatment of rainbow salmon fillets packed with gelatin film equals 2.99, for the treatment of rainbow salmon fillets packed with gelatin film containing *L. rhamnus*, for the treatment of fish fillets 1.80 Rainbow salmon packaged with gelatin film containing *B. bifidum* 2.03, for the treatment of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* and *B. bifidum* was 1.41, which is the lowest amount of TBARS in the entire study related to the treatment of rainbow salmon fillets packed with gelatin film containing *L. rhamnus* and It was *B. bifidum*.

Table 4. Average TBARS changes in different treatments of rainbow trout kept at refrigerator temperature (mean ± SD). Small and similar English letters in the diagram show no significant difference between the groups (P> 0.05).

Treatment	Day					
	0	**1**	**3**	**6**	**9**	**12**
Control	$0.02^{a} \pm 0.41$	$0.02^{a} \pm 1.01$	$0.01^{a} \pm 1.70$	$0.02^{a} \pm 2.18$	$0.03^{a} \pm 2.98$	**$0.02^{a} \pm 3.27$**
Film	$0.02^{a} \pm 0.41$	$0.03^{a} \pm 0.98$	$0.02^{b} \pm 1.49$	$0.03^{b} \pm 2.02$	$0.01^{b} \pm 2.81$	**$0.03^{b} \pm 2.99$**
B.b	$0.02^{a} \pm 0.41$	$0.03^{b} \pm 0.74$	$0.01^{c} \pm 1.27$	$0.01^{c} \pm 1.45$	$0.03^{c} \pm 1.73$	**$0.01^{c} \pm 2.03$**
L.r	$0.02^{a} \pm 0.41$	$0.03^{b} \pm 0.72$	$0.01^{c} \pm 1.25$	$0.02^{d} \pm 1.32$	$0.02^{d} \pm 1.54$	**$0.02^{d} \pm 1.80$**
B.b+L.r	$0.02^{a} \pm 0.41$	$0.01^{b} \pm 0.73$	$0.02^{d} \pm 1.03$	$0.01^{e} \pm 1.19$	$0.02^{e} \pm 1.29$	**$0.03^{e} \pm 1.41$**

It has been suggested that the maximum acceptable amount of thiobarbituric acid for the optimal quality of fish is 5 mg of malonaldehyde-equivalane per kilogram of

[DOI: 10.22034/FSCT.21.148.62]

sample, while up to 8 mg of malonaldehyde-equivalane per kilogram of sample can be used [39], which in this study, the control group And the samples packed with gelatin film were close to this on the last day. The general trend during the study was incremental. The amount of TBARS in fish fillet samples was 0.41 on day 0, which reached 3.27 on day 12, which was the highest amount, and for the treatment wrapped with gelatin film containing *L. rhamnus* and *B. bifidum*, it was 1.41, which is the lowest amount. It has been suggested that the maximum acceptable amount of thiobarbituric acid for the optimal quality of fish is 5 mg of malonaldehyde akyvalane per kilogram of sample, while up to 8 mg of malonaldehyde akyvalane per kilogram can be used [39]. So far, many studies have not been conducted on the effect of probiotic films on the chemical spoilage of fresh food. In a study by Kaya and Akso, 2005, the effect of direct addition of probiotic bacteria on the chemical spoilage of processed meats [40] and a type of fermented meat product was investigated, and according to the findings, they reported that probiotic bacteria can increase the amount of PV and TVB-N. compared to the control group [41].

4 .Conclusion

According to the results of chemical tests, especially the amount of volatile nitrogen bases in this study, it was found that gelatin films containing *L. rhamnus* and *B. bifidum* were able to significantly increase the shelf life of rainbow salmon fillets. According to the test results of volatile nitrogen bases and the suggested limit of 25 mg/100g, in control samples, shelf life starts from the third day, samples packed with gelatin film from the sixth day, and in samples containing probiotic bacteria, spoilage starts at least from the ninth day. It has been found that the shelf life has increased by at least 6 days. Also, according to the findings, gelatin film alone has the least effect and films containing probiotics, especially gelatin films containing two probiotic bacteria *L. rhamnus* and *B. bifidum*, have the most antimicrobial effect and the most prevention of chemical changes in rainbow salmon fillets. Therefore, the gelatin film together with the studied probiotic bacteria can increase the shelf life of rainbow salmon fillet and can be used in the food industry for active packaging in food products, especially fish meat.

5- Acknowledgments: I am hereby grateful for the financial support of Amol University of Special Modern Technologies in conducting this research.

5.References

[1] Tabatabaei Moradi L, Sharifan A, Larijani K. Antimicrobial activity of lemon and peppermint essential oil in edible coating containing chitosan and pectin on rainbow trout (Oncorhynchus mykiss) fillets. J Med Microbiol Infect Dis. 2015;3(1):38–43.

[2] Raeisi M, Tajik H, Aliakbarlu J, Valipour S. Effect of carboxymethyl cellulose edible coating containing *Zataria multiflora* essential oil and grape seed extract on chemical attributes of rainbow trout meat. In: Veterinary research forum: an international quarterly journal. Faculty of Veterinary Medicine, Urmia University, Urmia, Iran; 2014. p. 89.

[DOI: 10.22034/FSCT.21.148.62]

[3] Tafi AA, Meshkini S, Tukmechi A. Effects of Chitosan on some immune response of rainbow trout (Oncorhynchus mykiss) and enhance resistance against a pathogenic *Aeromonas hydrophila* fallowing experimental infection. J Anim Res (Iranian J Biol. 2014;26(4):468–77.

[4] Parthasarathy R, Ravi D. Probiotic bacteria as growth promoter and biocontrol agent against *Aeromonas hydrophila* in Catla catla(Hamilton, 1822). Indian J Fish. 2011;58(3):87–93.

[5] Fetsch A, Contzen M, Hartelt K, Kleiser A, Maassen S, Rau J. *Staphylococcus aureus* food-poisoning outbreak associated with the consumption of ice-cream. Int J Food Microbiol. 2014;187:1–6.

[6] Umaraw P, Munekata PES, Verma AK, Barba FJ, Singh VP, Kumar P, et al. Edible films/coating with tailored properties for active packaging of meat, fish and derived products. Trends Food Sci Technol. 2020;98:10–24.

[7] Baldwin EA, Hagenmaier R, Bai J. Edible coatings and films to improve food quality. CRC press; 2011.

[8] Pereda M, Ponce AG, Marcovich NE, Ruseckaite RA, Martucci JF. Chitosan-gelatin composites and bi-layer films with potential antimicrobial activity. Food Hydrocoll. 2011;25(5):1372–81.

[9] Mozaffarzogh M, Misaghi A, Shahbazi Y, Kamkar A. Evaluation of probiotic carboxymethyl cellulose-sodium caseinate films and their application in extending shelf life quality of fresh trout fillets. Lwt. 2020;126:109305.

[10] De Lacey AML, López-Caballero ME, Gómez-Estaca J, Gómez-Guillén MC, Montero P. Functionality of *Lactobacillus acidophilus* and *Bifidobacterium bifidum* incorporated to edible coatings and films. Innov Food Sci Emerg Technol. 2012;16:277–82.

[11] Shahbazi Y, Ahmadi F, Karami N. Screening, determination and confirmation of tetracycline residues in chicken tissues using four-plate test, ELISA and HPLC-UV methods: comparison between correlation results. Food Agric Immunol. 2015;26(6):821–34.

[12] Ebrahimi B, Mohammadi R, Rouhi M, Mortazavian AM, Shojaee-Aliabadi S, Koushki MR. Survival of probiotic bacteria in carboxymethyl cellulose-based edible film and assessment of quality parameters. LWT. 2018;87:54–60.

[13] Banuree SA, Noori N, Gandomi H, Khanjari A, Karabagias IK, Faraki A, Ghadami F, Azizian A, Banuree SZ. Effect of Stevia rebaudiana aqueous extract and microencapsulation on the survivability of *Bifidobacterium bifidum* Bb12 and *Lactobacillus acidophilus* La5 in functional ice cream. International Journal of Food Science & Technology. 2022 Nov 1.

[14] Wikström F, Williams H, Trischler J, Rowe Z. The importance of packaging functions for food waste of different products in households. Sustainability. 2019;11(9):2641.

[15] Kazemeini H, Azizian A, Shahavi MH. Effect of chitosan nano-gel/emulsion containing *bunium persicum* essential oil and nisin as an edible biodegradable coating on *Escherichia coli* O_{157}: H*7* in rainbow trout fillet. Journal of Water and Environmental Nanotechnology. 2019 Oct 1;4(4):343-9.

[16] Khodaei D, Hamidi-Esfahani Z, Lacroix M. Gelatin and low methoxyl pectin films containing probiotics: Film characterization and cell viability. Food Biosci. 2020;36:100660.

[17] Cook PA, Wheater P. Using statistics to understand the environment. Routledge; 2005.

[18] Saba MK, Sogvar OB. Combination of carboxymethyl cellulose-based coatings with calcium and ascorbic acid impacts in browning and quality of fresh-cut apples. LWT-Food Sci Technol. 2016;66:165–71.

[19] Han Y, Wang L. Sodium alginate/carboxymethyl cellulose films containing pyrogallic acid: Physical and antibacterial properties. J Sci Food Agric. 2017;97(4):1295–301.

[20] Shahbazi Y. Characterization of nanocomposite films based on chitosan and carboxymethylcellulose containing *Ziziphora clinopodioides* essential oil and methanolic Ficus carica extract. J Food Process Preserv. 2018;42(2):e13444.

[21] Andevari GT, Rezaei M. Effect of gelatin coating incorporated with cinnamon oil on the quality of fresh rainbow trout in cold storage. Int J food Sci Technol. 2011;46(11):2305–11.

[22] Fazlara A, Pourmadi M, Molaei F. The effect of gelatin-Avishan Shirazi (*Zataria multiflora* Bioss) coating on microbial, chemical and sensorial characteristics of ostrich fillets in refrigerated condition. J food Sci Technol. 2017;6(67).

[23] Soukoulis C, Singh P, Macnaughtan W, Parmenter C, Fisk ID. Compositional and physicochemical factors governing the viability of *Lactobacillus rhamnosus* GG embedded in starch-protein based edible films. Food Hydrocoll. 2016;52:876–87.

[24] Al-Holy MA, Al-Nabulsi A, Osaili TM, Ayyash MM, Shaker RR. Inactivation of *Listeria innocua* in brined white cheese by a combination of nisin and heat. Food Control. 2012;23(1):48–53.

[25] Salehi M. Assessment antagonistic effect of Lactobacilli isolated from Iranian traditional food. J Innov Food Sci technol. 2013;5(1):79–84.

[DOI: 10.22034/FSCT.21.148.62]

[26] Sánchez-González L, Saavedra JIQ, Chiralt A. Physical properties and antilisterial activity of bioactive edible films containing *Lactobacillus plantarum*. Food Hydrocoll. 2013;33(1):92–8.
[27] Homayouni A, Azizi A, Ehsani MR, Yarmand MS, Razavi SH. Effect of microencapsulation and resistant starch on the probiotic survival and sensory properties of synbiotic ice cream. Food Chem. 2008;111(1):50–5.
[28] Fan W, Sun J, Chen Y, Qiu J, Zhang Y, Chi Y. Effects of chitosan coating on quality and shelf life of silver carp during frozen storage. Food Chem. 2009;115(1):66–70.
[29] Latou E, Mexis SF, Badeka A V, Kontakos S, Kontominas MG. Combined effect of chitosan and modified atmosphere packaging for shelf life extension of chicken breast fillets. LWT-Food Sci Technol. 2014;55(1):263–8.
[30] Talwalkar A, Miller CW, Kailasapathy K, Nguyen MH. Effect of packaging materials and dissolved oxygen on the survival of probiotic bacteria in yoghurt. Int J food Sci Technol. 2004;39(6):605–11.
[31] Dave RI, Shah NP. Ingredient supplementation effects on viability of probiotic bacteria in yogurt. J Dairy Sci. 1998;81(11):2804–16.
[32] Jay JM, Loessner MJ, Golden DA. Modern food microbiology. Springer Science & Business Media; 2008.
[33] Torian F, Rihani M, Shirvani Z. Investigation of fat oxidation parameters of precooked rainbow trout (Oncorhynchus mykiss) fillets treated with cumin (*Cuminum cyminum*) essential oil at -18°C. Journal of Veterinary Research. 2017;73(4):435–46.
[34] Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food Chem. 2010;120(1):193–8.
[35] Saki J, Khodanazary A, Hosseini SM. The effect of chitosan-gelatin composition and bi-layer coating and film on physicochemical, microbial and sensory properties of Johnius belangerii stored at refrigerator. J Res Innov Food Sci Technol. 2017;6(1):71–86.
[36] Dehghani P, Hosseini SMH, Golmakani M-T, Majdinasab M, Esteghlal S. Shelf-life extension of refrigerated rainbow trout fillets using total Farsi gum-based coatings containing clove and thyme essential oils emulsions. Food Hydrocoll. 2018;77:677–88.
[37] Jeon Y-J, Kamil JYVA, Shahidi F. Chitosan as an edible invisible film for quality preservation of herring and Atlantic cod. J Agric Food Chem. 2002;50(18):5167–78.
[38] Nowzari F, Shábanpour B, Ojagh SM. Comparison of chitosan–gelatin composite and bilayer coating and film effect on the quality of refrigerated rainbow trout. Food Chem. 2013;141(3):1667–72.
[39] No HK, Meyers SP, Prinyawiwatkul W, Xu Z. Applications of chitosan for improvement of quality and shelf life of foods: a review. J Food Sci. 2007;72(5):R87–100.
[40] Libera J, Karwowska M, Stasiak DM, Dolatowski ZJ. Microbiological and physicochemical properties of dry-cured neck inoculated with probiotic of Bifidobacterium animalis ssp. lactis BB-12. Int J Food Sci Technol. 2015;50(7):1560–6.
[41] Kaya M, Aksu MI. Effect of modified atmosphere and vacuum packaging on some quality characteristics of sliced ‘sucuk’produced using probiotics culture. J Sci Food Agric. 2005;85(13):2281–8.

[DOI: 10.22034/FSCT.21.148.62]

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مقاله علمی_پژوهشی

بررسی اثر فیلم ژلاتینی حاوی پروبیوتیک‌های *بیفیدوباکتریوم بیفیدوم* و *لاکتوباسیلوس رامنسوس* بر بقاء باکتری *استافیلوکوکوس اورئوس* و خصوصیات فیزیکوشیمیایی فیله ماهی قزل آلای رنگین کمان

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اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت: ۱۴۰۲/۳/۲۲

تاریخ پذیرش: ۱۴۰۲/۱۱/۱۴

کلمات کلیدی:

فیلم، ژلاتین،

پروبیوتیک،

قزل آلای رنگین کمان.

DOI: **10.22034/FSCT.21.148.62.**

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چکیده

هدف از این مطالعه، بررسی اثر فیلم ژلاتینی حاوی باکتری‌های *بیفیدوباکتریوم بیفیدوم* و *لاکتوباسیلوس رامنسوس* بر بقا باکتری *استافیلوکوکوس اورئوس* تلقیح شده به گوشت ماهی قزل آلای رنگین کمان و همچنین خصوصیات فیزیکوشیمیایی آن به مدت ۱۲ روز در طی نگهداری در دمای یخچال بوده است. *استافیلوکوکوس اورئوس* با غلظت 10^6 log cfu/ml به نمونه های ماهی تلقیح، سپس تیمارها(گروه کنترل، نمونه‌های ماهی قزل آلای رنگین کمان بسته‌بندی شده با فیلم ژلاتین، فیلم ژلاتین حاوی هر باکتری پروبیوتیک با غلظت 10^9 log cfu/ml به طور جدا و فیلم ژلاتین حاوی هردو باکتری تهیه شدند. نمونه‌ها در کیسه‌های پلی‌اتیلنی بسته‌بندی ودر دمای یخچال نگهداری شدند. زنده مانی باکتری‌های پروبیوتیک و شمارش باکتری *استافیلوکوکوس اورئوس*، pH ، TVB-N، PV و TBARS مورد ارزیابی قرار گرفت. بر طبق نتایج، زنده‌مانی باکتری‌های پروبیوتیک در طول مطالعه روندی کاهشی را نشان داد و مشخص شد که زنده‌مانی باکتری *بیفیدوباکتریوم بیفیدوم* در تیمار فیلم ژلاتین و تیمار فیلم ژلاتین حاوی هر دو باکتری پروبیوتیک در روز آخر به ترتیب log ۶/۸۱ cfu/g و log ۵/۳۷ cfu/g و زنده‌مانی باکتری *لاکتوباسیلوس رامنسوس* نیز در تیمارهای ذکر شده به ترتیب log ۷/۴۳ cfu/g و log ۶/۳۱ cfu/g کاهش یافت. فیلم‌های پروبیوتیک نسبت به گروه شاهد رشد میکروبی فیله‌های ماهی را به خوبی کنترل کردند در حالی‌که تیمار فیلم ژلاتین حاوی هر *دو پروبیوتیک* کمترین میزان را دارا بود. آزمون‌های شیمیایی نیز دارای روندی افزایشی بوده و تغییرات آن‌ها در تمامی فیله‌های ماهی تیمارشده به صورت معنی‌داری نسبت به گروه تیمار نشده کمتر بود ($P<0/05$). مشخص گردید فیلم‌های ژلاتین حاوی پروبیوتیک به خصوص فیلم‌های ژلاتین حاوی هر دو پروبیوتیک که موفق‌تر عمل کرده بودند، اثر ضدمیکروبی مؤثری علیه باکتری *استافیلوکوکوس اورئوس* داشته و این پوشش می‌تواند در افزایش عمر ماندگاری ماهی قزل‌آلای رنگین کمان و این دسته از محصولات، موثر باشد.

[DOI: 10.22034/FSCT.21.148.62]