



## Effects of *Lactococcus lactis* (*L. lactis*) subsp. *lactis* Supernatant on the Shelf Life of Vacuum-packaged *Oncorhynchus mykiss* Fillets

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### Abstract

The present investigation was done to study the effects of *Lactococcus lactis* (*L. lactis*) subsp. *lactis* on the shelf life of the vacuum-packaged *Oncorhynchus mykiss*. Fish fillets were prepared and divided into 5 different treatment groups including control (distilled water), 2% and 4% supernatant, and  $10^6$  CFU/g *L. lactis* subspecies *lactis*. The pH, Thiobarbituric Acid Reactive Substances (TBARS), Total volatile Nitrogen (TVN), and Peroxide Value (PV) of the fillets were determined on days 0, 5, 10, and 15 while maintained at 4°C. Protein expression and destruction were analyzed using the SDS-PAGE. The organoleptic assessment was done using five expert sensory panelists. Contents of TBARS, TVN, pH, and PV were increased throughout the storage period ( $P < 0.05$ ). An increase in the concentration of supernatant caused a significant decrease in the content of TBARS, TVN, pH, and PV ( $P < 0.05$ ). The highest and lowest contents of TBARS, TVN, pH and PV on 15<sup>th</sup> day were belonged to the control ( $3.367 \pm 0.04$  mg MDA/kg) and pure bacteria ( $0.70 \pm 0.02$  mg MDA/kg), control ( $87.20 \pm 6.40$  mg/100g) and 4% supernatant ( $40.79 \pm 0.61$  mg/100g), pure bacteria ( $6.23 \pm 0.04$ ) and 4% supernatant ( $5.44 \pm 0.07$ ) and control ( $12.22 \pm 0.01$  meq/kg) and 4% supernatant ( $3.08 \pm 0.06$  meq/kg) groups, respectively. Protein destruction was lower in the fillet samples treated with pure bacteria and 4% supernatant. The highest scores of the odor, flavor, texture, and color were obtained for fillets treated with 4% supernatant, pure bacteria, pure bacteria, and 4% supernatant and pure bacteria, respectively. The results revealed that treating *O. mykiss* fillets with 4% supernatant and  $10^6$  CFU/g of pure *L. lactis* subsp. *lactis* can extend the shelf life of *O. mykiss* fillets.

**Keywords:** Fish, *L. lactis* subsp. *lactis*, *Oncorhynchus mykiss*, Shelf life, Vacuum package



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## Introduction

It is well-known that fish is an important source of high-quality animal proteins for human nutrition. Furthermore, fish is a rich source of protein, fatty acids, vitamins, and minerals (Larsen *et al.*, 2011). *O. mykiss* or Rainbow trout is one of the most extensively cultured and traded fish in Iran and many other countries of the world. Besides its nutritional value, the large and stable production quantity of rainbow trout makes it an important fish species for the seafood market. It is one of the major sources of protein, minerals, vitamins, and  $\omega$ -3 long-chain polyunsaturated fatty acids (LC-PUFAs) including eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3) which have been demonstrated to have valuable and special health effects to inhibit cardiovascular disease, lower cholesterol levels, and blood viscosity, and reinforce memory and thinking ability for humans (Larsen *et al.*, 2011). However, *O. mykiss* fillets are very prone to oxidation and rancidity. The oxidation reactions can cause alterations in the texture, color, and nutritional value of the final product (Chytiri *et al.*, 2004; Fuentes-Amaya *et al.*, 2015). Degradation of polyunsaturated fatty acids (PUFAs) caused by self-acting or enzymatic oxidation throughout different storage circumstances and processing operations can simply result in the development of undesirable oxidation products such as hydroperoxides, peroxides, aldehydes, conjugated dienes/trienes, ketones, and others (Chytiri *et al.*, 2004; Fuentes-Amaya *et al.*, 2015). Additionally, fish are very susceptible to microbial spoilage. The high water activity ( $a_w$ ) and the presence of corrosive tissues and proteins are the main factors that make it susceptible to chemical and microbial spoilage (Chytiri *et al.*, 2004; Fuentes-Amaya *et al.*, 2015). Therefore, microbiological, enzymatic, and chemical decomposition analyses are used to determine the shelf life of fish and marine products during processing and storage (Chytiri *et al.*, 2004; Fuentes-Amaya *et al.*, 2015).

The competence of numerous techniques of seafood preservation such as low temperature,

icing, and suitable packaging such as vacuum packaging, before the addition of synthetic or natural antioxidants, have been applied to monitor the progress of undesirable chemical, oxidative, enzymatic, and microbial changes in the product to increase its shelf-life (Gelman *et al.*, 2001; Giuffrida *et al.*, 2017). Vacuum packing is a technique to interrupt the microbial and chemical spoilage of fish. Vacuum packaging can be defined as the packaging of a product in a high-barrier package from which air is removed to avoid the growth of aerobic spoilage microorganisms, shrinkage, oxidation, and color deterioration (DeWitt & Oliveira, 2016; Özpolat *et al.*, 2014).

Novel research showed that the application of suitable packaging and using appropriate probiotic bacteria especially lactic acid bacteria (LAB) are efficient ways to prevent microbial and chemical spoilage of seafood products. LAB have long been used for changing the aromatic and textural properties of foods and for extending the shelf-life of various products such as milk, meat, poultry, fish, fruits, vegetables, and cereals. In most cases, the production of lactic and acetic acids and the resulting pH decrease are considered responsible for the inhibition of microbial and chemical spoilage (Chowdhury *et al.*, 2016; Nath *et al.*, 2014).

LAB are gram-positive microorganisms safely used in the food industry. Certain LAB strains possess probiotic properties for human and animal health (Salański *et al.* 2022; Fijan, 2014)

Krishnamoorthi *et al.* (2022) isolated bacteriocins producing *Lactococcus lactis* strain CH<sub>3</sub> and reported that bacteriocin has exhibited high antimicrobial, antibiofilm, and DPPH radical scavenging effects.

LAB can produce various antimicrobials including lactic acid, acetic acid, carbon dioxide, hydrogen peroxide, and bacteriocins that can inhibit spoilage and pathogenic organisms leading to extending shelf-life and enhancing the safety of food (Amor *et al.*, 2006) Despite the high importance of prevention from the microbial and chemical spoilage of fish

fillets, there were no previously published data about the application of LAB as an improving factor on the shelf life of fish fillets. Therefore, the present research was done to study the effects of *Lactobacillus lactis* (*L. lactis*) subsp. *lactis* on the shelf life of vacuum-packaged *O. mykiss* fillets.

## Material and Methods

### Fish Fillet samples

Forty kilograms of *O. mykiss* with an average weight of  $600 \pm 5$  g was randomly obtained from a Rainbow trout farm in Oshnavieh (Northwest of Iran) and immediately transferred to the National Artemia Research Center, Urmia, Iran in the ice box. Sample preparation was done according to the standard protocol of the Ministry of Health and Medical Education, Iran (Standard No. 1803929). All fishes were decapitated and eviscerated. Fish samples were then washed using sterile water. Washed samples were then filleted (100 g fillets) in a sterile hygienic condition.

### Bacterial culture and supernatant extraction

*L. lactis* subsp. *lactis* (PTCC 11454) was prepared from the Persian Type Culture Collection, Research Organization for Science and Technology (IROST), Iran. *Lactobacillus lactis* (*L. lactis*) subsp. *lactis* was cultivated 24 h in Nutrient Broth (NB, Merck, Germany) and stored at  $-80^{\circ}\text{C}$  in 50% glycerol until further experiment. The bacterium was then cultured in De Man Rogosa and Sharpe broth (MRS, Merck, Germany) medium according to Abbaspour *et al.* 2019. The bacterium was cultured again on MRS broth medium to obtain a concentration of  $10^6$  CFU/ml. The supernatant was obtained by refrigerator centrifuge (6000 rpm for 15 min) according to the method described by Scillinger *et al.* (1989) (Schillinger & Lücke, 1989). The supernatant was then filtered by a cellulose acetate filter (0.2  $\mu\text{l}$  mesh size). The pH of the filtered supernatant was then adjusted to 6.5 using sodium hydroxide to hide the pH antibacterial effects (1N, Merck, Germany). The obtained

supernatant concentrate was considered 100%. Distilled water was used to prepare 2% and 4% concentrations of bacterial supernatant (Erkan *et al.*, 2007; Sarika *et al.*, 2012; Shamloofar *et al.*, 2015).

### Fish fillets inoculation

Fillets of the *O. mykiss* were immersed in 2% and 4% supernatant and live cells ( $10^6$  CFU/ml) of *L. lactis* subsp. *lactis*. Initially, 100 g of fish fillets were immersed in 2% and 4% supernatant and live cells and maintained for 15 min. Therefore, 4 different treatment groups were studied including fish fillet samples treated with 2% and 4% supernatant, pure bacteria, and also those of the control group. Plastic bags were then used for vacuum packaging according to the method described by Tufail *et al.*, 2011. All samples were packed in nylon bags and vacuumed using Multivac-Germany apparatus at room temperature and stored at  $4^{\circ}\text{C}$ . Samplings were carried out on days 1, 5, 10, and 15 after packaging. All samples were analyzed for chemical characteristics (Thiobarbituric Acid Reactive Substances (TBARS), pH, Peroxide Value (PV), and Total Volatile Nitrogen (TVN)) and sensory properties.

### TBARS analysis

Lipid oxidation was monitored by the evaluation of thiobarbituric acid reactive substances (TBARS) according to the procedure described by (Salih *et al.*, 1987). Five g of fish fillet was minced and then mixed with 25 mL of trichloroacetic acid (20%) (Sigma Aldrich, USA) and 20 mL of distilled water and then centrifuging for 10 min with the revolving speed of 8000 rpm, and the filtrate was diluted with ultrapure water to 50 mL. The mixture of 10 mL of diluent and 10 mL of TBA solution was heated in a boiling water bath ( $95\text{--}100^{\circ}\text{C}$ ) for 15 min to develop a pink color and then cooled with running tap water for 5 min. The absorbance of the cooled supernatant was measured at 532 nm by a spectrophotometer (UV-1800, Instruments of Mfg. Co. Ltd.,

Suzhou, China). The amount of TBARS was expressed as mg of MDA/kg sample.

### pH analysis

The pH value was determined for the homogeneous mixtures of fish fillets with distilled water (1:10, w/v), using a digital pH meter (713 pH meter, Metrohm Herisau, Switzerland) as described by Benjakul *et al.*, 2006.

### PV analysis

The peroxide value of fish fillets was determined by the method described by Shon and Chin, 2008. Briefly, 5 g of fish fillet was heated in a water bath for 3 min at 60 °C followed by thorough mixing through agitation to dissolve the fat and homogenize the sample after the addition of 30 mL of acetic acid-chloroform solution (3:2 v/v). After filtration 0.5 mL saturated potassium iodide solution was added to the filtrate before transferring it into a burette. The sample was titrated against a standard solution of sodium thiosulfate (25 g/L) using starch solution as an indicator. The peroxide value was calculated and expressed in milliequivalent peroxides per kg (meq/kg) of the fish sample using the following formula:

$$PV = (S \times N / W) \times 100$$

Whereas S= Volume of titration in mL, N= Normality of the sodium thiosulfate solution, W= Sample weight in kg.

### TVN analysis

The total volatile basic nitrogen (TVN) of fish fillet was measured according to the method described by (Malle and Poumeyrol, 1989). Briefly, 10 g fish fillet, 1 g magnesium oxide (Merck, Darmstadt, Germany), and 60 mL distilled water were placed in a distilling flask. Samples were boiled and distilled into 40 mL of boric acid (Merck) containing methyl red as an indicator. After the distillation, the contents of the conical flask were titrated with H<sub>2</sub>SO<sub>4</sub> (Merck) and TVN was expressed as mg of N per 100 g muscle.

### Sensory evaluation

Organoleptic analysis was performed according to the method described by Ndaw *et al.* (2008). The cooking procedure was done using liquid vegetable oil (Oila, Iran) and a constant amount of edible salt (Sepidan, Iran). The fresh samples of fish fillets were cooked at 270 °C for 20 min. The odor, texture, flavor, and color of fish fillet samples were evaluated by five expert panelists famed for the organoleptic properties of fish fillet samples. Three to ten-point scales were achieved by panelists according to Codex guidelines for the sensory evaluation of fish and shellfish in laboratories (Codex, 1999).

Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis

SDS-PAGE test was performed according to the method of Lamli (1970) Tissue denaturation of fish fillet samples was tracked using the SDS-PAGE. SDS-PAGE analysis was done using 5% polyacrylamide stacking gel (Thermofisher Scientific, Germany) and 15% resolving gel according to the method described by (Boulares *et al.*, 2013).

### Statistical analysis

Results of chemical and sensory analysis were reported as Mean ± Standard deviation (SD). Data were analyzed with SPSS 21.0 statistical software (SPSS Inc., Chicago, IL, USA) using the analysis of variance test (ANOVA). The Least Significant Differences (LSD) procedure was used to test for differences between means at the 0.05 significance level.

### Results

Table 1 represents the results of the TBARS of the fish fillet samples treated with 2% and 4% supernatant, pure bacteria, and control group during storage conditions. The contents of TBARS of all samples were increased after 15 days of cold storage ( $P < 0.05$ ). The highest and lowest contents of the TBARS in fillet samples on the 15<sup>th</sup> day were related to the control group ( $3.367 \pm 0.04$  mg/kg) and pure bacteria group ( $0.70 \pm 0.02$  mg/kg). An increase

in the concentration of supernatant caused a significant decrease in the content of TBARS ( $P < 0.05$ ).

**Table 2** represents the content of TVN of the fish fillet samples treated with 2% and 4% supernatant, pure bacteria, and control group during the maintenance period. The contents of TVN were increased during cold storage ( $P < 0.05$ ). The highest and lowest contents of the TVN on day 15 were related to fillet samples of the control group ( $87.20 \pm 6.40$  mgN/100g) and 4% supernatant group ( $40.79 \pm 0.61$  mgN/100g). An increase in the concentration of supernatant caused a significant decrease in the content of TVN ( $P < 0.05$ ).

**Table 3** represents the pH of the fish fillet samples treated with 2% and 4% supernatant, pure bacteria, and control group during the maintenance period. The pH values were increased during cold storage ( $P < 0.05$ ). The highest and lowest pH values at day 15 were related to fillet samples of pure bacteria ( $6.23 \pm 0.04$ ) and 4% supernatant ( $5.44 \pm 0.07$ ). An increase in the concentration of supernatant caused a significant decrease in the pH value ( $P < 0.05$ ).

**Table 4** represents the PV of the fish fillet samples treated with 2% and 4% supernatant, pure bacteria, and control group during the maintenance period. We found that the content of PV was increased in the maintenance period ( $P < 0.05$ ). The highest and lowest contents of the PV on day 15 were related to fillet samples of the control group ( $12.22 \pm 0.01$  meq/kg) and 4% supernatant group ( $3.08 \pm 0.06$  meq/kg). An increase in the concentration of supernatant caused a significant decrease in the content of PV ( $P < 0.05$ ).

**Table 5** represents the results of the organoleptic tests for different groups of fish fillet samples at the end of the maintenance period. Sensory evaluators gave the highest scores of the odor, flavor, texture, and color to fish fillet samples treated with 4% supernatant ( $8.00 \pm 0.00$ ), pure bacteria ( $7.00 \pm 0.40$ ), pure bacteria, and 4% supernatant ( $7.75 \pm 0.28$  and  $7.75 \pm 0.28$ , respectively) and pure bacteria ( $8.50 \pm 0.28$ ), respectively.

**Fig. 1** represents the SDS-PAGE electrophoretic pattern of soluble proteins of different treatments of fish fillet samples on the first day of the maintenance period. There were no significant differences in the diversity of soluble proteins and their expression. A slight difference of about 80 KD was seen between the expression of proteins of fish fillet samples of the control and other groups. Furthermore, a slight difference was seen about 30 KD between the expression of proteins of fish fillet samples of pure bacteria and other groups. **Fig. 2** represents the SDS-PAGE electrophoretic pattern of soluble proteins of different treatments of fish fillet samples at day 5 of the maintenance period. Severe differences were seen in the diversity of soluble proteins and their expression in 57 KD and higher than 93 KD bands. The severity of expression soluble proteins or their survival amongst other proteins was higher between studied samples. **Fig. 3** represents the SDS-PAGE electrophoretic pattern of soluble proteins of different treatments of fish fillet samples on day 10 of the maintenance period. Severe differences were also seen in the diversity of soluble proteins and their expression in 57 KD and higher than 93 KD bands. The severity of expression soluble proteins or their survival amongst other proteins was higher between control and supernatant treatments (2% and 4%). **Figure 4** represents the SDS-PAGE electrophoretic pattern of soluble proteins from different treatments of fish fillet samples on day 15 of the maintenance period. Severe differences were also seen in the diversity of soluble proteins and their expression especially in the expression of soluble proteins in the ranges between 57 to 93 KD which represented higher proteolysis.

Table 1- The TBARS of the treated fish fillet samples

Storage period (day)	Pure bacteria (10 <sup>6</sup> CFU/g)	TBARS (mg/kg)		
		Supernatant		Control
		2%	4%	
1	0.03±0.008 <sup>A*d</sup>	0.015±0.00 <sup>Bc</sup>	0.013±0.00 <sup>Bc</sup>	0.014±0.00 <sup>Bd</sup>
5	0.052±0.00 <sup>Bc**</sup>	0.330±0.04 <sup>Cc</sup>	0.188±0.05 <sup>Db</sup>	0.914±0.03 <sup>Ac</sup>
10	0.650±0.03 <sup>Cb</sup>	1.520±0.11 <sup>Ab</sup>	1.100±0.09 <sup>Ba</sup>	1.668±0.04 <sup>Ab</sup>
15	0.70±0.02 <sup>Da</sup>	2.770±0.08 <sup>Ba</sup>	0.960±0.08 <sup>Ba</sup>	3.367±0.04 <sup>Aa</sup>

\*Dissimilar capital letters in each row show a significant statistical difference ( $P < 0.05$ ).

\*\*Dissimilar small letters in each column show significant statistical differences ( $P < 0.05$ ).

Table 2- The TVN of the treated fish fillet samples

Storage period (day)	Pure bacteria (10 <sup>6</sup> CFU/g)	TVN (mgN/100g)		
		Supernatant		Control
		2%	4%	
1	10.30±0.11 <sup>A*d</sup>	10.00±0.35 <sup>Ad</sup>	8.8±0.31 <sup>Cd</sup>	9.50±0.50 <sup>Bd</sup>
5	23.13±0.43 <sup>Bc**</sup>	24.05±0.50 <sup>Bc</sup>	18.86±1.39 <sup>Cc</sup>	42.70±1.05 <sup>Ac</sup>
10	33.50±0.87 <sup>Db</sup>	43.80±0.91 <sup>Cb</sup>	47.25±2.21 <sup>Ba</sup>	55.9±2.10 <sup>Ab</sup>
15	53.25±0.56 <sup>Ca</sup>	78.6±0.60 <sup>Ba</sup>	40.79±0.61 <sup>Bb</sup>	87.20±6.40 <sup>Aa</sup>

\*Dissimilar capital letters in each row show significant statistical differences ( $P < 0.05$ ).

\*\*Dissimilar small letters in each column show significant statistical differences ( $P < 0.05$ ).

Table 3- The pH of the treated fish fillet samples

Storage period (day)	Pure bacteria (10 <sup>6</sup> CFU/g)	pH		
		Supernatant		Control
		2%	4%	
1	5.36±0.13 <sup>A*b</sup>	4.96±0.07 <sup>Bc</sup>	5.10±0.03 <sup>Bb</sup>	5.10±0.04 <sup>Ba</sup>
5	6.03±0.04 <sup>Aa**</sup>	5.13±0.03 <sup>Bc</sup>	5.18±0.06 <sup>Bb</sup>	5.26±0.06 <sup>Ba</sup>
10	6.42±0.03 <sup>Ba</sup>	6.72±0.03 <sup>Aa</sup>	5.83±0.03 <sup>Ca</sup>	5.49±0.24 <sup>Ca</sup>
15	6.23±0.04 <sup>Aa</sup>	5.65±0.12 <sup>Bb</sup>	5.44±0.07 <sup>Cb</sup>	5.61±0.16 <sup>Ca</sup>

\*Dissimilar capital letters in each row show significant statistical differences ( $P < 0.05$ ).

\*\*Dissimilar small letters in each column show significant statistical differences ( $P < 0.05$ ).

Table 4- The PV of the treated fish fillet samples

Storage period (day)	Pure bacteria (10 <sup>6</sup> CFU/g)	PV (meq/kg)		
		Supernatant		Control
		2%	4%	
1	1.09±0.03 <sup>A*c</sup>	1.17±0.05 <sup>Ac</sup>	0.00	0.00
5	1.13±0.02 <sup>Bc**</sup>	1.37±0.21 <sup>Bc</sup>	1.08±0.06 <sup>Bc</sup>	3.82±0.13 <sup>Ac</sup>
10	1.53±0.17 <sup>Cb</sup>	3.56±0.13 <sup>Bb</sup>	3.66±0.52 <sup>Ba</sup>	4.20±0.00 <sup>Ab</sup>
15	4.20±0.07 <sup>Da</sup>	7.40±0.11 <sup>Ba</sup>	3.08±0.06 <sup>Cb</sup>	12.22±0.01 <sup>Aa</sup>

\*Dissimilar capital letters in each row show significant statistical differences ( $P < 0.05$ ).

\*\*Dissimilar small letters in each column show significant statistical differences ( $P < 0.05$ ).

Table 5- The sensory evaluation of the treated fish fillet samples period

Treatments	Odor	Organoleptic characters		
		Flavor	Texture	Color
Pure bacteria (10 <sup>6</sup> CFU/g)	4.25±0.25 <sup>B*</sup>	7.00±0.40 <sup>A</sup>	7.75±0.28 <sup>A</sup>	8.50±0.28 <sup>A</sup>
Supernatant	2%	3.70±0.47 <sup>C</sup>	3.70±0.25 <sup>C</sup>	4.20±0.47 <sup>B</sup>
	4%	8.00±0.00 <sup>A</sup>	6.70±0.25 <sup>B</sup>	7.75±0.25 <sup>A</sup>
Control	3.00±0.00 <sup>C</sup>	3.00±0.00 <sup>C</sup>	3.00±0.00 <sup>C</sup>	3.00±0.00 <sup>C</sup>

\*Dissimilar capital letters in each column show significant statistical differences ( $P < 0.05$ ).

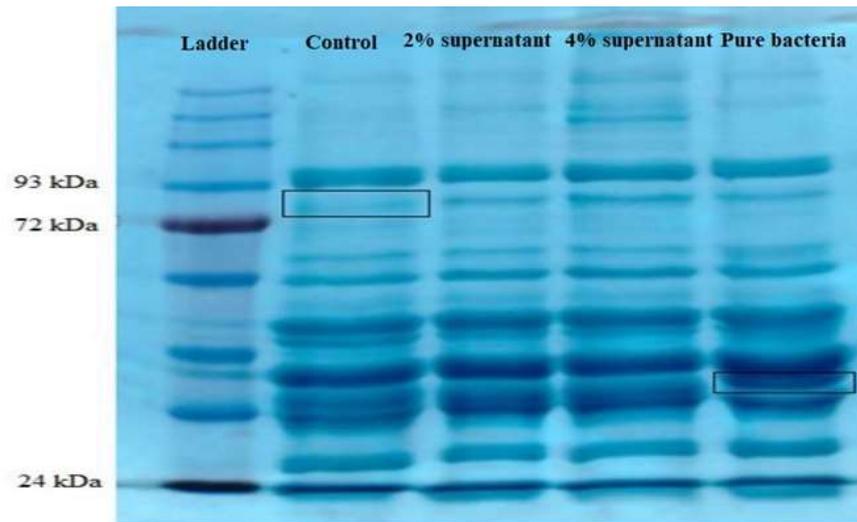


Fig. 1. SDS-PAGE electrophoretic pattern of soluble proteins of different treatments of fish fillet samples in the first day of storage period

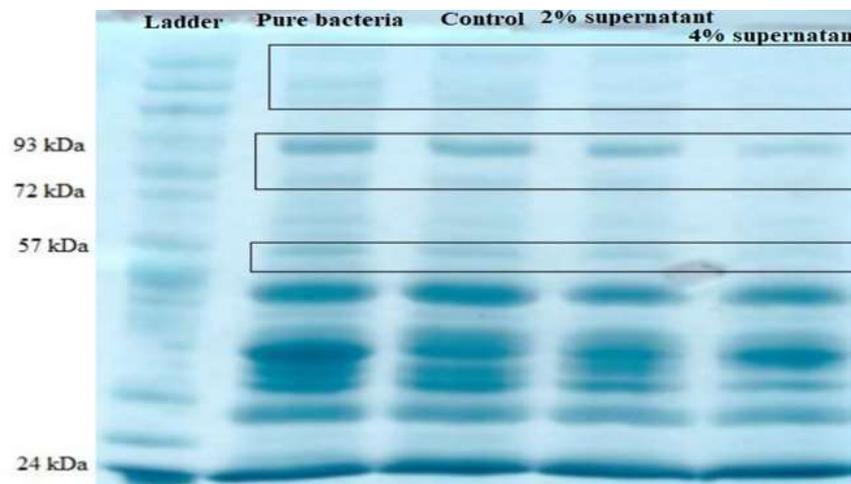


Fig. 2. SDS-PAGE electrophoretic pattern of soluble proteins of different treatments of fish fillet samples at day 5 of the storage period

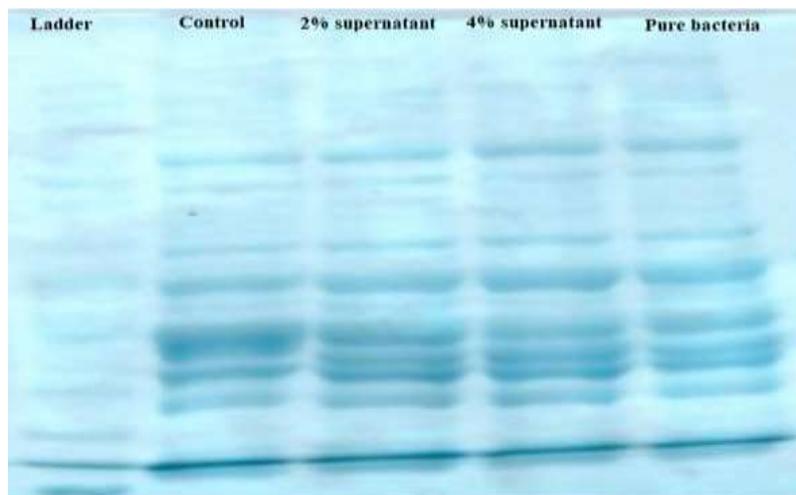


Fig. 3. SDS-PAGE electrophoretic pattern of soluble proteins of different treatments of fish fillet samples at day 10 of storage period

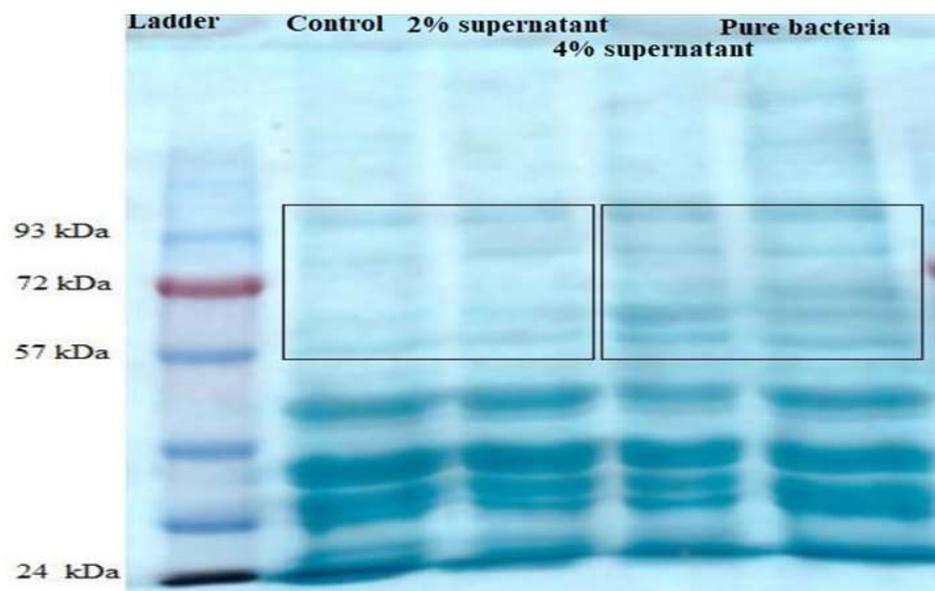


Fig. 4. SDS-PAGE electrophoretic pattern of soluble proteins of different treatments of fish fillet samples at day 15 of the storage period

## Discussion

Application of LAB in combination with modified atmosphere packaging and even slight physicochemical treatments and low concentrations of natural traditional preservers may be considered as effective method to increase the shelf life and food safety of fish and marine products through the inhibition of chemical and microbial spoilage without changing the nutritional quality of food products (Pavličević *et al.*, 2013). The procedure of extension of shelf life by application of natural or controlled microorganisms and their antimicrobial components is recommended by novel scientific reports (Pavličević *et al.*, 2013).

Abbaspour *et al.* (2018) reported that the number of spoilage bacteria in 4% acid live cells in the storage time of *O. mykiss* fillets was less than human consumption limits (7 logcfu/ml) compared with control and 2% supernatant of *L. lactis* subspecies *lactis* which improved the sensory characteristics of the fillets.

The results revealed that the application of supernatant and pure culture of *L. lactis* subsp. *lactis* caused a significant decrease in the contents of TBARS, TVN, pH, and PV values

compared with the control group. Furthermore, the reduction effects were dose-dependent so an increase in the concentration of bacterial supernatant caused a significant decrease in the contents of TBARS, TVN, pH, and PV. Additionally, fillet samples treated with bacterial supernatants and also pure bacteria had lower protein destruction in the SDS-PAGE analysis. Moreover, fillet samples treated with bacterial supernatants and pure bacteria caused an increase in their sensory scores.

The increase in TVN value is attributed to autolytic enzymes and deamination, which lead to the formation of numerous volatile compounds such as dimethylamine, trimethylamine, ammonia, Trimethylamine Oxide (TMAO), hypoxanthine, and non-volatiles histamine, which are shaped by both bacterial and endogenous enzymes. In the treated samples of our study, the values of TVN were lower than in other groups, which may be attributed to the extended lag phase of the spoilage microorganisms as a result of competitive inhibition by LAB and at the same time effect of LAB acidification (Ndaw *et al.*, 2008). Ibrahim & Salha (2009) (Ibrahim & Desouky, 2009) reported that the combined

coating of LAB in tilapia fillets had reduced TVN values. Sudalayandi & Manja (2011) described that out of 7 different species of LAB tested for quality indices reduction, *L. helveticus*, *L. lactis*, and *Pediococcus acidilactici* (*P. acidilactici*) effectively monitored TVN content (Sudalayandi, 2011). Vacuum packaging together with low-temperature preservation is also reported to have the ability to inhibit the growth of spoilage bacteria (Goussault & Leveau, 2006). Similar findings have been reported from Turkey on Bonito fish (Sardasarda) fillets packaged with chitosan film (Alak, 2010).

Oxidative rancidity is one of the most significant factors that effect on the acceptability of the fish throughout storage and processing. As general rule of Standard is that the level of PV in seafood should not exceed 10–20 meq/kg of fat (Lakshmanan, 2000). The control fillet samples of our study harbored the limit of acceptance of PV on day 10 of storage ( $4.20 \pm 0.00$  meq/kg). In the presence of *L. lactis* subsp. *lactis* under vacuum, the sample treated with 2% and 4% supernatant and also those treated with pure bacteria harbored the limit of acceptability for PV on day 15 ( $7.40 \pm 0.11$ ,  $3.66 \pm 0.52$  and  $4.20 \pm 0.07$  meq/kg, respectively). Nath *et al.* (2014) reported that the control fillets crossed the limit of acceptance of PV on day 6 of storage ( $14.11 \pm 0.37$  meq/kg), while fillet groups treated with *L. sakei* under vacuum packaging crossed the limit of acceptability for PV on day 9 ( $18.78 \pm 0.10$  meq/kg). As far as we know, the present research reported the lowest content of PV value in *O. mykiss* fillet samples treated with 4% supernatant of *L. lactis* subsp. *lactis* under vacuum packaging. A probable reason for this finding is the effects of low pH (which occurred by the activity of *L. lactis* subsp. *lactis*) on prevention from the production of PV in fish fillet samples.

We found that the levels of pH in all studied groups have increased during the maintenance period, while the amounts of increase in the fish fillet samples treated with 4% supernatant of the *L. lactis* subsp. *lactis* bacteria were lower

than other studied groups. This finding is mainly due to the activity of *L. lactis* subsp. *lactis* and production of acidic products. The pH value should not be above than 4.00–4.50 for soaked fish for products' safety. Acidic conditions make the tissue cathepsins much more active resulting in the degradation of some muscle proteins into peptides and amino acids. These components give the marinade its characteristic flavor and texture. Nath *et al.* (2014) reported that in the presence of *L. sakei* in aerobic or anaerobic (vacuum packaged) conditions all the samples exhibited a lowering of pH values from the initial level of 7.8 which was similar to our findings. Likewise, reductions in pH values were seen in several investigations on marinated anchovies (Sen & Temelli, 2003), sardines (Kilinc & Cakli, 2004), and Pacific saury (Sallam *et al.*, 2007).

TBA test measures malonaldehyde (MDA) produced due to the oxidation of fatty acids with three or more double bonds, and it measures other TBARS such as 2-alkenes and 2, 4-alkadienals. TBA is also usually applied as a quality marker in the fish industry and it has a close association with the sensory properties of fish fillets (Bogdanović *et al.*, 2012). So far, no data have been reported to investigate the effects of *L. lactis* subsp. *lactis* on TBARS content of the vacuum-packaged *O. mykiss* fillets. We found that the fish fillet samples treated with pure *L. lactis* subsp. *lactis* and also those treated with 4% supernatant of *L. lactis* subsp. *lactis* had the lower contents of TBARS. A probable reason for this finding is the effects of low pH (which occurred by the activity of *L. lactis* subsp. *lactis*) on preventing TBARS production in fish fillet samples. Low pH is probably induced by the production of some kinds of acids especially lactic acid, acetic acid, and citric acid due to the activity of *L. lactis* subsp. *lactis* guarantee the higher antioxidant effects which decrease the content of TBARS in fish fillet samples. Furthermore, the lower TBARS values of some treatments might result from the direct microbial utilization of MDA and other TBARS or result from reactions

between TBARS and the amine compounds produced by bacterial metabolism. (Payap & Ommee, 2007). The increase in TBARS values in all treatments is probably due to the induced denaturation of muscle protein, leading to the release of from heme iron, a potential pro-oxidant in the muscle system (Greene & Cumuze, 1981; Payap & Ommee, 2007). Previous research showed that a TBARS value of at least 2.0 mg malonaldehyde/kg is essential for the perception of rancid taste and odors (Greene & Cumuze, 1981). We found that the contents of TBARS in fish fillet samples treated with pure *L. lactis* subsp. *lactis* and also 4% supernatant of *L. lactis* subsp. *lactis* on day 15 of the maintenance period were lower than 1.0 mg malonaldehyde/kg which could indirectly guarantee high scores given to the taste and odor of these treatments.

Sensory evaluation showed that fish fillet samples treated with pure and also 4% supernatant of *L. lactis* subsp. *lactis* harbored the highest scores of odor, flavor, texture, and color. This finding is mainly due to the effects of *L. lactis* subsp. *lactis* on contents of TVN, TBARS, pH, and PV. Similar findings have been reported by (Soltanian *et al.*, 2011; Boulares *et al.*, 2013; Chanarat *et al.*, 2014; Adilla *et al.*, 2017 and Rahmatipoor *et al.*, 2017).

## Conclusion

The present research is the first report of the effects of *L. lactis* subsp. *lactis* on the shelf life

of *O. mykiss* fillets. Results showed that using 4% supernatant and also pure *L. lactis* subsp. *lactis* bacteria caused a significant decrease in the contents of TBARS, TVN, pH, and PV values of *O. mykiss* fillets. Contents of the majority of studied chemical parameters were lower than the allowed limit reported by the standard organizations (Tables 1-4). Additionally, levels of protein destruction were studied by the SDS-PAGE in *O. mykiss* fillet samples treated with 4% supernatant and also pure *L. lactis* subsp. *lactis* bacteria were significantly lower than other studied groups. Furthermore, the mean scores given to the odor, flavor, texture, and color parameters were higher in the *O. mykiss* fillet samples treated with 4% supernatant and also pure *L. lactis* subsp. *lactis* bacteria. Therefore, soaking of *O. mykiss* fillet samples on 4% supernatant of *L. lactis* subsp. *lactis* and  $10^6$  CFU/g of pure bacteria is recommended as efficient method to extend the shelf life of *O. mykiss* fillet samples. However, further studies are required to analyze other chemical, sensory, and microbiological aspects of *O. mykiss* fillet samples treated with other LAB and their metabolites.

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## مقاله پژوهشی

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# اثرات سوپرناتانت باکتری *Lactococcus lactis* (L. lactis) subsp. lactis بر ماندگاری فیله قزل‌آلای رنگین‌کمان (*Oncorhynchus mykiss*) بسته‌بندی شده در خلاء

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## چکیده

تحقیق حاضر جهت بررسی اثرات لاکتوباسیلوس لاکتیس زیرگونه لاکتیس بر ماندگاری فیله قزل‌آلای رنگین‌کمان بسته‌بندی شده در خلاء انجام شد. فیله های ماهی تهیه و به ۵ گروه تیمار مختلف شامل کنترل (آب مقطر)، باکتری خالص و ۲ تیمار غوطه‌ور شده در مایع رویی ۲٪ و ۴٪ و  $10^6$  CFU/g *L. lactis* زیرگونه لاکتیس طبقه‌بندی شدند. مقادیر pH، تیوباربتوریک اسید (TBARS)، نیتروژن فرار کل (TVN) و مقدار پراکسید (PV) فیله‌ها در روزهای ۰، ۵، ۱۰ و ۱۵ دوره نگهداری در دمای ۴ درجه سانتی‌گراد بررسی شد. بیان و تخریب پروتئین با استفاده از الکتروفورز ژل سدیم دودسیل سولفات-پلی آکریل آمید (SDS-PAGE) آنالیز شد. ارزیابی ارگانولپتیک با استفاده از پنج ارزیاب حسی خبره انجام شد. مقادیر TBARS، TVN، pH و PV در طول دوره نگهداری افزایش یافته است ( $P < 0.05$ ). افزایش غلظت مایع رویی باعث کاهش معنی‌داری در مقادیر TBARS، TVN، pH و PV شد ( $P < 0.05$ ). بیشترین و کمترین مقادیر TBARS، TVN، pH و PV در روز ۱۵ به ترتیب مربوط به گروه‌های شاهد ( $3/367 \pm 0/04$  میلی‌گرم بر کیلوگرم) و باکتری خالص ( $0/70 \pm 0/02$  میلی‌گرم بر کیلوگرم)، کنترل ( $87/20 \pm 6/40$  میلی‌گرم بر کیلوگرم) وزن بدن) بود. و ۴٪ مایع رویی ( $40/0 \pm 79/61$  mgN/100g)، باکتری خالص ( $6/23 \pm 0/04$ ) و ۴٪ مایع رویی ( $5/44 \pm 0/07$ ) و شاهد ( $12/0 \pm 22/01$ ) meq/kg و ۴٪ مایع رویی ( $3/08 \pm 0/06$ ) meq/kg بود. تخریب پروتئین در نمونه‌های فیله تیمار شده با باکتری خالص و ۴ درصد مایع رویی کمتر بود. بالاترین امتیاز بو، طعم، بافت و رنگ به فیله‌های تیمار شده با ۴ درصد مایع رویی ( $8/00 \pm 0/00$ )، باکتری خالص ( $7/00 \pm 0/40$ )، باکتری خالص و ۴ درصد مایع رویی ( $7/75 \pm 0/28$ ) و باکتری خالص ( $8/50 \pm 0/28$ ) به دست آمد. خیساندن فیله‌های *O. mykiss* روی ۴٪ مایع رویی و  $10^6$  CFU/g از Subsp. *L. lactis* می‌تواند عمر مفید فیله‌های قزل‌آلای رنگین‌کمان را افزایش دهد.

واژه‌های کلیدی: باکتری اسید لاکتیک، بسته‌بندی در خلاء، فیله ماهی، ماندگاری، *Oncorhynchus mykiss*

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