Original Article

Effect of chitosan coating enriched with orange peel (*Citrus sinensis*) waste extract on prolonged preservation and chemical and functional properties of frozen *beluga sturgeon* (Huso huso) fillet

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ABSTRACT

Fish is an important part of the diet in both developed and developing countries. It is highly digestible and contains an appropriate combination of essential amino acids such as lysine and methionine, which are essential for good health and nutrition. The health benefits of essential polyunsaturated fatty acids, including omega-3 and omega-6 fats, have stimulated interest in increasing seafood consumption per capita. However, fresh fish is one of the most perishable foods due to the potent action of autolytic enzymes and microbial activity. In this study, chitosan biofilms containing different concentrations of the orangepeel extract (0.5, 1.0, and 1.5%) were prepared and their physical factors including water vapor permeability (WVP), color, and water solubility (WS) were characterized. Enrichment of the chitosan-based coatings with orange peel extracts improved the physical property factors and WVP, color, and WS improved for prolonged preservation of frozen *beluga sturgeon* so that significant differences in these factors were observed between the enriched coatings and control ones ($p<0.05$). The fish fillets were analyzed for total lipid (3.33 ± 0.41%), crude protein (14.90 ± 1.04%), and total volatile basic nitrogen (10.12 ± 1.15 mg N/100 gr) and then subjected to the three treatments as well as blank coatings with 0.0-1.5% levels of orange peel waste extract for 14 days at 4 ^{0}C. The results showed that the biofilm enriched with 1.5% orange peel waste extract was effective in preserving phenolic compounds and maintaining the antioxidant properties of fish fillets during the preservation period ($p<0.05$). The results of this study showed that the chitosan coating enriched with orange peel extract improved physical factors in the coatings preserved the antioxidant compounds in the fish fillets and maintained their shelf life during a two-week refrigeration period.

Keywords: Antioxidants, *Beluga sturgeon*, Chitosan coating, Functional properties, Orange Peel Extract

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1. Introduction

Nowadays, fish is an important part of the diet of the peoplein both developed and developing countries. Due to the high digestibility of protein and an appropriatecombination of essential amino acids such as lysine and methionine, fish is essential for good health and nutrition. The health benefits of essential polyunsaturated fatty acids including omega-3 and omega-6 fats stimulate interest in increasing seafood consumption. The potent action of autolytic enzymes and microbial activity in fresh fish makes it one of the most perishable foods. The use of fresh fish is in high demand among consumers,but an emerging group of " green consumers" want to use natural foods without chemical additives that are microbially healthy and, in appropriate cases, are packaged in an environmentally friendly approach. Extending the shelf life of food products reduces the financial and environmental burden on the food industry (1). Therefore, due to food safety and human health concerns and consumers' desire to use natural compounds, the use of healthy compounds with antimicrobial and antioxidant properties can be considered an effective way to control microbial growth and increase the required shelf life. In addition, the potential toxicological effects of chemical preservatives and synthetic antioxidants have led researchers to develop methods to extend the shelf life of perishable foods based on natural compounds with a broad spectrum of antimicrobial and antioxidant activity. Plants, vegetables, and even some foodextracts, and essential oils are among the natural compounds that have been studied for their antimicrobial and antioxidant properties. Recent studies have shown that the use of these compounds in the food industry can be a good alternative to synthetic chemical preservatives such as benzoate and sorbate, which are commonly used in many foods (2). However, their cost of use and other challenges such as odor intensity and potential toxicity for uncontrolled release of these substances into the food products and inactivation of some valuable compounds due to their undesirable reactions with the final productscaused a reseaonable limitation in their use as food preservatives. It has been shown that plant extracts contain active compounds that act specifically on bacterial cells. It seems that their antimicrobial activity is mainly due to the hydrophobic nature of the active compounds and secondary metabolites. A combination of natural and inexpensive plant waste extracts with special polymers such as chitosan may be a good choice to reduce the dose of these extracts and prevent their undesirable effects (3). Previous studies have shown that the main advantage of this technology is that it maintains the rate of releasing antimicrobial agents into the product where it is most exposed to bacterial attack (4). Chitosan is one of the most important biopolymers ever used to make biofilms and edible coatings. This linear polysaccharide compound is an important renewable resource and is reported to be the second most abundant natural biopolymer in the world after cellulose. The ability of renewable films to carry antioxidant and antimicrobial compounds and other active ingredients to control pathogens and improve the quality and shelf life of seafood in the packaging industry is well documented. Citrus fruits and their wastes are of great value as they are rich source of antioxidants and antimicrobials such as glycosidic flavonoids, coumarin, glycosides, beta- and gamma-sitosterol, polyphenols and essential oils. These fruits and their by-products are widely consumed around the world due to their affordable economic reach, delicious taste, preferred flavor, and consumer awareness of the potential health benefits. Orange peel (*Citrus sinensis L.*) an important source of nutraceuticals such as ascorbic acid, carotenoids, and phenolics is the most widely cultivated worldwide (5). The main aim of this study was to evaluate the effect of chitosan coating enriched with orange waste extract on prolonging the shelf life and chemical and functional properties of frozen *beluga sturgeon* fish fillets.

2. Materials and Methods

2.1. Chemicals and Reagents

All the chemicals and reagents were of analytical grade with the highest purity. All standard solutions of phenolic compound (including ellagic acid, gallic acid, catechol, resorcinol, vanillin, benzoic acid, and ascorbic acid) were prepared from analytical reagent grade chemicals (Sigma-Aldrich, Steinheim, Germany) and ultrapure water obtained by purifying water with the UHQ Elga instrument (Millipore, Le montsur-Lausanne, Switzerland). All solvents used in the chromatographic analyses were HPLC grade and were purchased from Merck Chemical Co. (Darmstadt, Germany).

2.2. Sample Collection and Preparation

Beluga fish with an average weight of 47-50 kg were purchased from a sturgeon farm in Sari City, Mazandaran Province of Iran. The fish were placed on the crushed ice in insulated boxes and transferred to the food chemistry laboratory of the food hygiene department of Islamic Azad University under sterile conditions. The fish were washed with tap water several times and then filleted by hand. The average weight of each fillet was 10.0 g and placed under UV sterilization conditions for 20 minutes.

2.3. Preparation of coating solution

For preparation of the coating solution, 2.0 g of medium molecular weight chitosan powder (Chitosan Poly D-Glucosamine, Sigma-Aldrich, Germany) was added to 100 ml of acetic acid solution (1% v/v) and was then stirred with a magnetic stirrer (IKA, Germany, 1200 rpm) at room temperature for 3 hours. Glycerol (Merck chemical Co., Dortmund, Germany) was then added to the chitosan at a concentration of 0.75% as a plasticizer and stirred at 60 °C for 30 minutes. A chitosan coating solution was filtrated through a Whatman No. 3 filter paper to remove any undissolved particles (6).

2.4. Preparation of orange peel extract

Healthy oranges without any fungal contamination were purchased from Sari Citrus Research Center, peeled, and placed in 96% ethanol for 20 minutes. The peels were then

dried in an oven at 60°C for one hour. To increase the contact area with the solvent, the dried peels were crushed and sieved using a laboratory grinder. In the next step, 50.0 g of the peels were mixed with 300 ml of pure ethanol in a decanter, and after 24 hours at room temperature, the alcoholic phase was separated from the plant material using Whatman No. 1 filter paper. After evaporation of the solvent, the samples were stored at -4°C until further analyses.

2.5. Biofilm Preparation

Orange peel extract was mixed with Tween 80 (0.2%) (Sigma-Aldrich, Germany) as an emulsifier and gently stirred mildly at 40°C for 30 min to obtain a uniform of emulsifier . Separate treatments of orange peel extract in different concentrations (0.0, 0.5, 1.0, and 1.5%) were added to the chitosan solution and well homogenized by Ultra Turrax homogenizer (IKA, Germany) at 20000 rpm for 5 min. Air bubbles were removed by a relative vacuum. The formulated solution was finely sprayed on nylon polymer film (Ultrafine spraying, Techno Sanat, Iran). The prepared biofilms were dried at room temperature for 72 hours and then placed in a desiccator in a dry atmosphere in the presence of magnesium nitrate for 6 hours until all the biofilms were thoroughly dried (7)

2.6. Treatment of fish fillets

The treatments selected for this study include: control test (beluga coated with biofilm-free orange peel extract as a blank sample), treatment 1 (Beluga coated with biofilm enriched with 0.0% of orange peel extract), treatment 2 (Beluga coated with biofilm enriched with 0.5% of orange peel extract), treatment 3 (Beluga coated with biofilm enriched with 1.0% of orange peel extract) and treatment 4 (Beluga coated with biofilm enriched with 1.5% of orange peel extract). The prepared biofilms were categorized into five different groups and were stored in a refrigerator at 4 ± 1 °C. The physicochemical and antioxidant properties of the samples were analyzed on the 1st, 5th, and 14th day of storage. For each treatment, five different batches were randomly considered to obtain better statistical data and analysis (8).

2.7. Chemical Analyses

The proximate composition and some chemical spoilage indices such as protein, lipid, ash, peroxide value (PV), thiobarbituric acid (TBA), and total volatile basic nitrogen (TVB-N) were determined in unprocessed beluga fish. Total crude protein was determined by the Kjeldahl method with a multiplier of 6.25 coefficient (9), lipid was determined by the Soxhlet method (10) and the crude ash was determined by mineralization of the samples at 550°C (11). The Lee method was used to determine the amount of PV (12). The TBA was determined by the AOAC method (11). Vapor distillation was used for the determination of TVB-N (11). In each case , the PH was measured using a digital AZ-Metrohm, 780 pH meter (Germany) equipped with a combined glass-calomel electrode on 10 g of homogenized sample in distilled water (1/10 v/v, sample/water) (13).

2.8. Biofilm Water Vapor Permeability

The water vapor permeability (WVP) of biofilms was determined using specific glass vials. At first, 8.0 ml of distilled water was added to create 100% humidity in the vial atmosphere. The biofilm samples were placed on the entrance of the vials and sealed with a rubber O-ring and clamped tightly. The initial weight of all biofilms was recorded using an analytical balance (Sartorius, Germany) and then placed in a desiccator containing silica gel to create a near-zero relative humidity using anhydrous calcium sulfate. Samples were weighed every hour for up to 7 hours. Finally, the weight loss and then the water vapor permeability rate (WVPR) were calculated using the following formula:

$$WVPR = \frac{\Delta m/\Delta t}{A}$$

Where Δm is the weight loss (gr), Δt is the difference between initial and final time (s) and A is the film area (m^2) (14). The the WVP was then calculated and reported as gr/m.s.pa based on the recent calculations:

$$WVP = \frac{WVPR}{P} \times X$$

Where X is the film thickness (m) and P is the vapor pressure of pure water at 25 ^{0}C, assumed to be 3169 pa.

2.9. Water Solubility of Biofilms

One square centimeter (cm^2) of biofilm pieces were accurately separated and weighed before being placed them in a dry desiccator overnight. These biofilm pieces were placed in 100 ml of deionized water and stirred on a magnetic stirrer (IKA, Germany) for 24 hours. The biofilms were then separated from the aqueous phase and placed at 40 °C up to reach constant weight. The solubility value for biofilms was calculated using the following equation:

$$WS = \frac{(W0 - W1)}{W0}$$

Where W0 is the initial weight of the biofilm, W1 is the final weight of the dry biofilm and WS is the value of the water solubility (15).

2.10. Biofilm colorimetry

The colorimetry test on the biofilms was performed by diffuse reflectance spectroscopy (DRS) using a solid state UV spectrometer (Shimadzu, Japan). All the spectra were recorded from 190 to 800 nm and the effect of enrichment with orange peel waste extract on the profile of ultraviolet and visible spectrum was evaluated (16).

2.11. Detection of phenolic compounds by HPLC

A Dionex HPLC apparatus was used to record profile of the phenolic compounds in canned fish fillet samples. This chromatographic system was consisted of a high-pressure pump and a UVD 170U multi-wavelength detector set at 254 nm. A Rehodyne 7725i injector with a 25 µl loop was used to inject the phenolic fish-extracted sample solutions into the analytical column (ODS, 4.6*250 mm, 5 µm). For

the mobile phase, a degassed mixture of methanol and acetic acid (75% in water) was prepared and delivered at a flow rate of 1.0 ml/min. The column was eluted with 100% methanol for 5.0 minutes and the acetic acid phase was increased up to 50% for 7.0 minutes and continued until the end of the elution program. HPLC data were acquired and the area under the curve (AUC) for each phenolic compound was cslculated using Chromeleon ver. 6.60 software. These data were taken as a signal roportional to the concentration of each phenolic compound (17).

2.12. Statistical Analyses

Data analysis was performed using SPSS software version No. 18 (SPSS Inc., Chicago, IL, USA). All the experiments were performed in triplicate, coating treatments were prepared in five different batches, and a completely randomized design was used for statistical analyses. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Duncan's multiplerange test. A *p*-value of less than 0.05 was considered significant.

3. Results

3.1. Proximate Compositions and Chemical Spoilage Indices

The chemical quality of the collected fish samples and their suitability for storage and final consumption were evaluated for proximate compositions and some chemical spoilage indices. The results for the investigated factors are presented in Table 1.

3.2. Water Vapor Permeability

The effects of the filmforming solution enriched with different levels of orange peel extract are shown in Table 2. The obtained results showed that the biofilm coatings with higher levels of orange peel extract have lower WVP than the control treatment, significantly ($p<0.05$).

3.3. Solubility Characteristics

Evaluation of the quality of the treated nylon biofilms showed that the film pieces maintained their integrity after reaching constant weight at 40°C. The results showed that there was no significant difference between the treatment coatings among themselves, while the solubility percent showed a significant difference between the treatments and control coating ($p<0.05$) (Table 3).

3.4. Biofilm Colorimetry

The results of the colorimetric test using diffuse reflectance spectroscopy showed an obvious increase in the absorption band at 280 nm. Increasing the concentration of the orange peel extract from zero up to 1.5% in biofilm coatings, caused a dominant increase in absorption intensity from zero to 0.225 nm in the recorded spectrum (Figure 1). This colorimetry assay was repeated three times and the standard deviation for color absorption was less than 3.0 percent for each biofilm.

3.5. Preservation of Phenolic Compounds and Antioxidant Property

The results for control of phenolic compounds levels in frozen beluga sturgeon samples during storage period which were embedded in different treatments and control biofilm coatings are presented in Table 4. Seven phenolic compounds including ellagic acid, gallic acid, catechol, resorcinol, vanillin, benzoic acid, and ascorbic acid were detected in beluga fish fillets by HPLC analysis and recorded their variation level was recorded by integrating area under the curve (AUC) for each phenolic compound peak in the chromatogram. As can be seen from the data presented in table 4, the HPLC signals for all phenolic compounds showed a slight decrease after initial storage in biofilm coatings, except for vanillin which was not detected in the experiments after the first control in 5^{th} day and ellagic acid,which was detected only in the 1.5% treatment biofilm coating in the 5^{th} day and not detected in other treatment and control biofilm coatings in this evaluation. As can be seen in Table 4, there was a significant difference between the recorded AUC signals in the treatments and control biofilm coatings at each day for ascorbic acid, gallic acid, resorcinol, catechol, and benzoic acid throughout the storage time ($p<0.05$). Also, the data in this table clearly shows the decrease in the HPLC signal of the control sample on different days which were taken under the experiment. The obtained results clearly show the inappropriateness of the coating without chitosan and orange peel extract for the preservation of the main detected phenolic compounds (except for gallic acid after 5 days from first coating by the biofilm) and maintaining antioxidant property of fish fillets during the period of 14 days even in the condition of storage at refrigerator temperature. The obtained results also showed that the effect of orange peel extract in the biofilm coating, especially in 1.5% level on the preservation of phenolic compounds was significantly attributed to gallic acid, ascorbic acid, and benzoic acid with maintaining higher level of these phenolic compounds. The obtained data was also showed that AUC in each chromatogram had a direct proportional increase with percentage of orange peel extract up to 1.5%,which indicates the improvement in preservation of phenolic compounds in the biofilm coatings with high levels of orange peel extract. Evaluation of the results obtained after five days from the initial preservation showed that each increase in the amount of orange peel extract from zero to 0.5%, 1.0% and 1.5% was effective in preserving ascorbic acid, gallic acid, resorcinol, catechol and benzoic acid by 5.7, 8.8, 11.2, 1.5 and 10.3 times, respectively. The similar results were recorded for after analyses of data in 14^{th} day, the effectiveness of increase in orange peel extract were 9.2, 25.2, 36.7, N.D. and 10.0 times, respectively for the mentioned phenolic compounds in comparison with the control coating film. Based on this study, chitosan-based biofilm coatings enriched with orange peel extract were prepared with improved favorable water vapor permeability, color, and maintaining water solubility.

Table 1: Proximate composition and chemical spoilage analyses of unprocessed *beluga* fish fillet (Mean ±SE)

Chemical Parameter	Determined value	Chemical Parameter	Determined value
Total lipid (%)	3.33 ± 0.41	Crude ash (%)	11.42± 0.15
Total crude protein (%)	14.90 ± 1.04	PV (meq/Kg)	0.93± 0.02
TVB-N (mg N / 100 gr)pH	10.12 ± 1.15 6.35 ± 0.10	TBA (MDA/Kg sample)	0.91± 0.01

*(For all analyses the results reported as mean ± standard deviation, n=5)

Table 2: Effect of orange peel extract levels on WVP of the chitosan coatings

Measure No.	Δt (h)	Level of orange peel extract			
		0%	0.5%	1.0%	1.5%
	0	0	0	0	0
1	1	0.001	0.001	0.001	0
2	2	0.002	0.002	0.002	0
3	3	0.003	0.003	0.003	0
4	4	0.003	0.003	0.003	0
5	24	0.016	0.016	0.013	0.012
6	48	0.031	0.032	0.024	0.017
7	53	0.034	0.033	0.027	0.020
WVP (gr /m s pa × 1012)		3.10^{a}	3.0^{6a}	2.50^{a}	1.80^{b}*
R^2		0.9995	0.9978	0.9980	0.9751

***** Different letters, show a statistically significant different between treatments (P<0.05).

Table 3: Solubility levels of the biofilm coatings with various orange peel extract

Orange peel extract in biofilm formulation (%)	Initial weight (g)	Final weight (g)	Solubility value	Solubility (%)
0.0	0.0140	0.0139	0.0071	0.71aA*
0.5	0.0195	0.0192	0.0153	1.53bB*
1.0	0.0305	0.0298	0.0229	2.29bB*
1.5	0.0415	0.0401	0.0337	3.37bB*

* Statistical analyses for the effect of orange peel extract in biofilm composition were performed separately for each extract level using Duncan's multiple range test (number of replications = 3). Solubility values with similar small letters don't have a significantly different at p <0.05 with the previous treatment sample. Solubility values with different capital letters showed significantly different at p <0.05 with the blank sample.

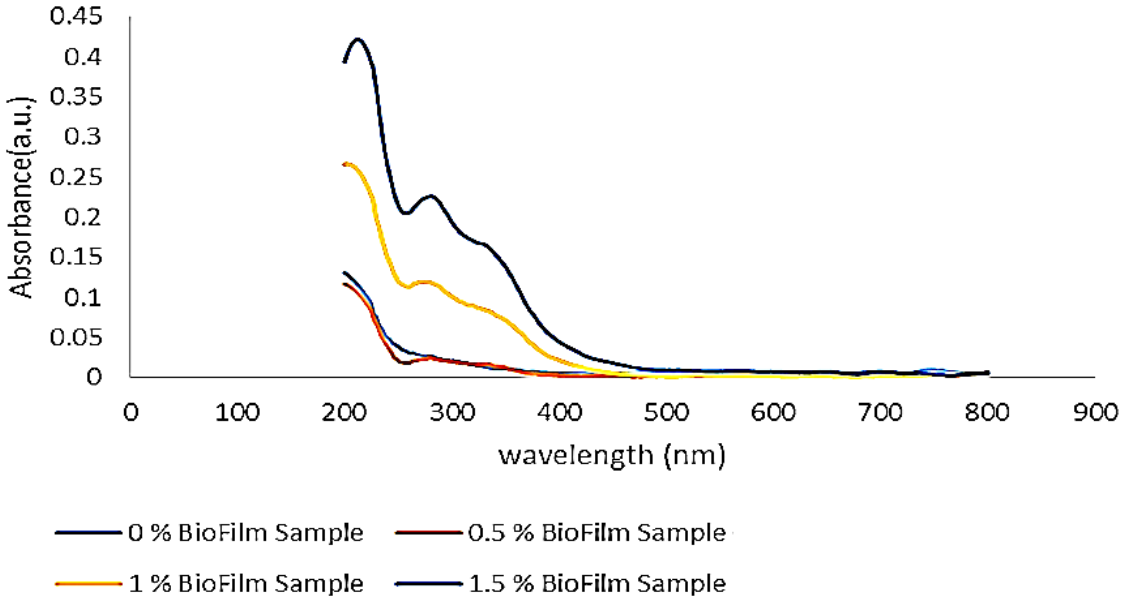

Figure 1: The color spectrum for control and treated biofilm coatings with orange peel extract.

Table 4: Detection of phenolic compounds in fish fillets after coating with control and treated biofilms up to 14 days by HPLC analysis

Analysis Day	Treatment or Control	Phenolic Compounds						
		ascorbic acid	Gallic acid	resorcinol	catechol	Ellagic acid	vanillin	benzoic acid
1st Day	Control	RT= 2.83 AUC=723.9A**	RT=3.40 AUC=479.2A	RT= 4.59 AUC=248.1A	RT=6.07 AUC=208.7A	RT= 7.36 AUC=7.9	RT=11.53 AUC=126.2	RT=12.59 AUC=123.9A
5th Day	Control	RT= 2.84 AUC=40.4aB*	RT=3.41 AUC=53.6aA	RT= 4.51 AUC=18.0aB	RT=5.99 AUC=125.9aB	N.D.	N.D.	RT=12.55 AUC=11.1aB
	0%	RT= 2.81 AUC=31.7a	RT=3.35 AUC=295.1b	RT= 4.49 AUC=19.0a	RT=5.93 AUC=157.5a	N.D.	N.D.	RT=12.40 AUC=19.9b
	0.5%	RT= 2.83 AUC=46.7a	RT=3.37 AUC=309.2b	RT= 4.54 AUC=148.0a	RT=5.84 AUC=167.0a	N.D.	N.D.	RT=12.47 AUC=105.0b
	1.0%	RT= 2.81 AUC=180.0b	RT=3.44 AUC=446.7b	RT= 4.55 AUC=180.1b	RT=5.99 AUC=169.7b	N.D.	N.D.	RT=12.50 AUC=111.0b
	1.5%	RT= 2.88 AUC=232.8b	RT=3.38 AUC=471.9b	RT= 4.50 AUC=201.2b	RT=5.99 AUC=197.0a	RT= 7.39 AUC=5.1	N.D.	RT=12.55 AUC=114.0b
14th Day	Control	RT= 2.81 AUC=22.7aB	RT=3.30 AUC=16.3aB	RT= 4.45 AUC=5.2aB	N.D.	N.D.	N.D.	RT=12.60 AUC=8.9aB
	0%	RT= 2.91 AUC=18.0b	RT=223.24 AUC=17.2a	RT= 4.47 AUC=126.2a	N.D.	N.D.	N.D.	RT=12.60 AUC=59.9b
	0.5%	RT= 2.99 AUC=21.1b	RT=3.34 AUC=294.0b	RT= 4.78 AUC=132.3b	N.D.	N.D.	N.D.	RT=12.60 AUC=68.1b
	1.0%	RT= 2.93 AUC=154.7b	RT=3.34 AUC=311.0b	RT= 4.73 AUC=172.3b	N.D.	N.D.	N.D.	RT=12.60 AUC=79.5b
	1.5%	RT= 2.90 AUC=209.9b	RT=3.38 AUC=411.0b	RT= 4.61 AUC=189.1b	N.D.	N.D.	N.D.	RT=12.60 AUC=89.5b

N.D. = Not detected in HPLC test, AUC= Area under the curve in the chromatogram, RT= Retention Time
* Different small letters, show a statistically significant difference between treatments and control on each day (P<0.05).
** Different capital letters, show a statistically significant difference between control samples in different days (P<0.05).

4. Discussion

Beluga sturgeon fish is an important marine food source candidate for human nutrition due to its nutrient composition. The mean value of TVB-N in the examined samples was 10.12 mg N/100 g while 25-35 mg N/100 g is usually considered as the upper limit of acceptability for human consumption. The first proximate analyses showed that the fish samples can be considered as a suitable lipid and protein nutrient medium for consumers. These results are compatible with those of Bongiorno et al. (18), who considered *sturgeon* as a source of proteinwith a high lipid content. In addition, the mean pH of samples was determined to be 6.35 in the raw sample, which is in accordance with the standard pH value, which should be between 6.00-6.50 for fresh fish. The upper limit of acceptability was considered as 6.80-7.00 (19, 20). In addition, the peroxide value (PV) and 2-thiobarbituric acid (TBA) values were 0.93± 0.02 meq/Kg and 0.91± 0.01 MDA/Kg, respectively, which were by far lower than recommended limits. Recently, Shahhoseini et al. evaluated the antioxidant effects of Pullulan edible coating with *Nasturtiumn officinale* extract on the chemical deterioration of fresh beluga sturgeon fillet during storage in a refrigerator temperature and showed that pullulan coating as well as 1000 ppm watercress extract significantly postponed lipid oxidation by decreasing PV and TBA production in the sample matrix and it had the lowest total volatile basic nitrogen and pH (p<0.05). These results are consistent with the findings of this study and indicate the effectiveness of the coating material on the shelf life of beluga sturgeon fillets. The obtained results showed that the biofilm coatings with higher concentrations of orange peel extract had lower WVP than the control treatment, significantly (p<0.05). The lower WVP for biofilms with higher concentrations of orange peel extract concentrations seems to be a consequence of the high viscosity of the film-forming solutions. Spraying the orange peel extract at higher concentrations onto the nylon polymeric substrate, resulted in the formation of stronger gels. This in turn led to the removal of all air bubbles in the permeability test, resulting in a lower WVP and improved preservation of the embedded product. Similar results have been reported in previous studies showing that polymeric solutions with significantly higher viscosity had lower WVP. According to the results, any differences in orange peel extract concentration resulted in obvious changes in the permeability properties of the resulting biofilm coatings. Water solubility, especially for fish packaging, is an important property of foodcoating biofilms. In industrial applications, water insolubility may be required to improve product integrity and water resistance. However, in some cases it may be beneficial to improve the water solubility of the film with natural compounds prior to final product consumption (21). Natural orange peel extract contains several water soluble ,so increasing their concentration in the biofilm formulation may increase their solubility (22). The lower solubility values ofor the control treatment without orange peel extract in the biofilm formulation may be attributed to chitosan and its waterinsolubility properties. In a similar study, Gao et al. reported that the presence of water-soluble tea polyphenols could increase the water solubility of chitosan and corn starch-based films and the higher hydrophilicity of tea polyphenols may be responsible for the greater interaction between the film matrix and water. In their study, the gradual increase in the concentration of tea polyphenols resulted in an increase in the water solubility of the blended film which is consistent with the results of the current study. Watersoluble derivatives of chitosan have also been reported to be effective in food, paint, and water treatment applications. This result confirmed the homogeneity of the orange peel extract on the surface of the biofilms in all cases enriched with orange peel extracts. Lemes et al. reported that there are some interactions between the chitosan and the compounds of the methanolic fraction of *Euphorbia umbellate* extract (23). The increase in the concentration of the extract added to the membranes resulted in a sharp change in the c color of the membranes to a darker brown color. Some other studies showed chitosan membranes incorporated with extracts containing phenolic compounds showed similar behavior. This may be due to the interaction of the amine groups in the chitosan and carboxylic groups in the extract compounds. It has been shown that phenolic compounds (such as gallic acid) contain carboxylic groups and could interact with chitosan in a similar manner (24). These results

are consistent with a recent previous report in which the antioxidant properties of plant-based coatings were attributed to their phenolic compounds. In general, the biofilm coating enriched with 1.5% orange peel extract after five days of initial storage of *beluga sturgeon* fillet at refrigerator temperature was 32%, 98%, 81%, 94%, and 92% effective in preserving the major phenolic compounds which were identified in the fillet (ascorbic acid, gallic acid, resorcinol, catechol, and benzoic acid, respectively). These results were 29%, 85%, 76%, N.D and 72% for preservation of major phenolic compounds and antioxidant properties in Huso huso fillet compared to control coating after 14 days of initial storage at 4°C. The enriched biofilm had the preservative properties for antioxidants and has an excellent role in extending the shelf life of frozen sturgeon fish fillets. The formation of chitosan-based biofilm coatings enriched with favorable orange peel extract was imparted with beneficial water vapor permeability, color, and maintenance of water solubility. This study also showed that the enriched biofilm had the preservative properties for antioxidants and can impart the desired flexibility and did not significantly affect the permeability of WVP biofilms. Based on the results obtained, the effect of orange peel extract in all coating treatments was significant on preserving phenolic compounds (p<0.05) and the treatment enriched with 1.5% orange peel extract showed the best performance in preserving phenolic compounds at 4°C. The results of the study also showed that chitosan biofilm coating with orange peel extract has an excellent role in extending the shelf life of frozen sturgeon fish fillets and preserving phenolic compounds. Due to the proven antioxidant and antibacterial activity, this extract can be used as a suitable alternative to chemical preservatives to increase the shelf life of the frozen fish fillet. It can be concluded that orange peel extract as a natural food additive with the potential to serve as a useful alternative to synthetic antioxidants can be used to improve the safety and quality of fish and fish products.

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Authors' Contribution
B.S, A.M, B.A.A, N.R. Acquisition of data: B.S. and B.A.A. Analysis and interpretation of data: B.S, A.M. and B.A.A. Drafting of the manuscript: B.S, A.M, B.A.A, N.R. Critical revision of the manuscript for important intellectual content: B.S, A.M. and B.A.A. Statistical analysis: B.S, A.M. and B.A.A. Administrative, technical, and material support.

Ethics
The current research study was ethically reviewed and approved by the Islamic Azad University, Science and Research Branch, in accordance with the university's guidelines for ethical research practices. All experimental procedures were carried out with the utmost respect for the principles of ethical research, ensuring the welfare and safety of the participants**.**

Conflict of Interest
The authors declare that they have no competing financial interests or personal relationships that could potentially influence the outcome of this research study. The study was not funded by any company or for profit organization. The authors maintain complete independence in their research and conclusions.

Data Availability
The data that support the findings of this study are available on request from the corresponding author.

References

1. Giannoglou M, Dimitrakellis P, Efthimiadou A, Gogolides E, Katsaros G. Comparative study on the effect of cold atmospheric plasma, ozonation, pulsed electromagnetic fields and high-pressure technologies on sea bream fillet quality indices and shelf life. Food Engineering Reviews. 2021;13(1):175-84.
2. Akbari-Adergani B, Eskandari S, Bahremand N. Determination of sodium benzoate and potassium sorbate in “doogh” samples in post market surveillance in Iran 2012. Journal of Chemical Health Risks. 2013;3(1):65-71.
3. Khorram B, Motalebi A, Razavilar V. Use of chitosan to shelf life extension of Siberian

sturgeon (Acipenser baeri) caviar. Iranian Journal of Fisheries Sciences. 2022;21(3):726-40.
4. Kargar M, Ahari H, Kakoolaki S, Mizani M. Synthesis of Nano silver and copper by chemical reduction method to produce of NanoTiO2 composites (based on Ag & copper) antimicrobial nanocoated packaging to increase the shelf life of caviar (Huso huso fish fillets). Iranian Journal of Fisheries Sciences. 2021;20(1):13-31.
5. Dulta K, Koşarsoy Ağçeli G, Thakur A, Singh S, Chauhan P, Chauhan P. Development of alginate-chitosan based coating enriched with ZnO nanoparticles for increasing the shelf life of orange fruits (Citrus sinensis L.). Journal of Polymers and the Environment. 2022:1-14.
6. Alparslan Y, Baygar T. Effect of chitosan film coating combined with orange peel essential oil on the shelf life of deepwater pink shrimp. Food and bioprocess technology. 2017;10(5):842-53.
7. Chhatariya HF, Srinivasan S, Choudhary PM, Begum SS. Corn starch biofilm reinforced with orange peel powder: Characterization of physicochemical and mechanical properties. Materials Today: Proceedings. 2022;59:884-92.
8. Ojagh SM, Rezaei M, Razavi SH, Hosseini SMH. Effect of chitosan coatings enriched with cinnamon oil on the quality of refrigerated rainbow trout. Food chemistry. 2010;120(1):193-8.
9. Acar Ü, Kesbiç OS, Yılmaz S, Gültepe N, Türker A. Evaluation of the effects of essential oil extracted from sweet orange peel (Citrus sinensis) on growth rate of tilapia (Oreochromis mossambicus) and possible disease resistance against Streptococcus iniae. Aquaculture. 2015;437:282-6.
10. López-Bascón M, De Castro ML. Soxhlet extraction. Liquid-phase extraction: Elsevier; 2020. p. 327-54.
11. Horwitz W. Official methods of analysis of AOAC International. Volume I, agricultural chemicals, contaminants, drugs/edited by William Horwitz: Gaithersburg (Maryland): AOAC International, 1997;2010.
12. Ghorbanzade T, Akhavan-Mahdavi S, Kharazmi MS, Ibrahim SA, Jafari SM. Loading of fish oil into β-cyclodextrin nanocomplexes for the production of a functional yogurt. Food Chemistry: X. 2022;15:100406.
13. Silva-Pereira MC, Teixeira JA, Pereira-Júnior VA, Stefani R. Chitosan/corn starch blend films with extract from Brassica oleraceae (red cabbage) as a visual indicator of fish deterioration. LWT-Food Science and Technology. 2015;61(1):258-62.
14. Zelinka SL, Glass SV, Boardman CR. Improvements to water vapor transmission and capillary absorption measurements in porous materials. Journal of Testing and Evaluation. 2016;44(6):2396-402.
15. Sharma M, Sharma R, Jain DK, Saraf A. Enhancement of oral bioavailability of poorly water soluble carvedilol by chitosan nanoparticles: Optimization and pharmacokinetic study. International journal of biological macromolecules. 2019;135:246-60.
16. Ahari H, Naeimabadi M. Employing nanoemulsions in food packaging: Shelf life enhancement. Food Engineering Reviews. 2021;13(4):858-83.
17. Mehmood T, Khan MR, Shabbir MA, Zia MA. Phytochemical profiling and hplc quantification of citrus peel from different varieties. Prog Nutr. 2018;20:279-88.
18. Bongiorno T, Foglio L, Proietti L, Vasconi M, Lopez A, Pizzera A, et al. Microalgae from biorefinery as potential protein source for siberian sturgeon (A. baerii) aquafeed. Sustainability. 2020;12(21):8779.
19. Chen YW, Cai WQ, Shi YG, Dong XQ, Bai F, Shen SK, et al. Effects of different salt concentrations and vacuum packaging on the shelf-stability of Russian sturgeon (Acipenser gueldenstaedti) stored at 4° C. Food Control. 2020;109:106865.
20. Vilkova D, Chèné C, Kondratenko E, Karoui R. A comprehensive review on the assessment of the quality and authenticity of the sturgeon species by different analytical techniques. Food Control. 2022;133:108648.
21. dos Santos Caetano K, Lopes NA, Costa TMH, Brandelli A, Rodrigues E, Flôres SH, et al. Characterization of active biodegradable films based on cassava starch and natural compounds. Food packaging and shelf life. 2018;16:138-47.

22. Gaff M, Esteban-Decloux M, Giampaoli P. Bitter orange peel essential oil: A review of the different factors and chemical reactions influencing its composition. Flavour and fragrance journal. 2020;35(3):247-69.
23. Lemes BM, Novatski A, Ferrari PC, Minozzo BR, Justo AdS, Petry VE, et al. Physicochemical, biological and release studies of chitosan membranes incorporated with Euphorbia umbellata fraction. Revista Brasileira de Farmacognosia. 2018;28:433-43.
24. de Farias BS, Junior TRSAC, de Almeida Pinto LA. Chitosan-functionalized nanofibers: A comprehensive review on challenges and prospects for food applications. International journal of biological macromolecules. 2019;123:210-20.