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The Combined Effects of Bio-Components and Alginate Coating on Chemical and Sensory Quality of Chill-Stored Rainbow Trout Fillets

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ABSTRACT

The objective of the present study was to reduce lipid oxidation and preserve sensory quality of fish fillets maintained under refrigerator condition. The lactoperoxidase system (LPOS) or Zataria multiflora Boiss essential oil (ZEO) was added individually or in combination in biodegradable coatings. Fish fillets were analyzed for peroxide value (PV), free fatty acid value (FFA), fatty acid profile, thiobarbituric acid reactive substances (TBARS) value, and sensory evaluation. Suppressive effect of ZEO and LPOS was seen in the combined groups; so that PV value ranged from 0.32 (0 day) and 0.41 (4 day) meq/kg to 1.97 meq/kg on day 8. The lowest amount of TBARS (1.54 mg MDA/kg) and FFA (1.84% of oleic acid) was obtained in the samples treated with alginate coating containing LPOS and ZEO 1% at the end of storage. The use of ZEO and natural preservatives such as LPOS is presented as alternatives to chemical preservatives in seafood.

KEYWORDS

Lipid oxidation; sodium alginate; lactoperoxidase system (LPOS); *Zataria multiflora* essential oil; rainbow trout

Introduction

A large amount of foods, especially those containing unsaturated fatty acids, are damaged and deteriorate due to lipid oxidation. This biochemical process is the major factor that causes unfavorable odor and decreases food quality (German et al. 1985). Lipid oxidation also influences taste, texture, and color of the product and declines its shelf life during storage (Maqsood et al. 2012). Lipid oxidation is a very important event leading to the deterioration of foods containing highly unsaturated fats that have received much attention in the last decade (Javan et al. 2015). It is one of the key problems associated with the loss of quality of foods and the reason for undesirable odor, taste, as well as shelf life reductio. During storage, fish and other seafood contain a high level of unsaturated fatty acids that readily deteriorate via peroxidation even at low temperatures (Huang and Weng 1998). Therefore, the measurement of oxidative spoilage level in fish is of particular importance (Shokri et al. 2015). Several approaches are utilized to control or retard the lipid oxidation process and increase the shelf life of fish. Active packaging with refrigeration or freezing is one of the strategies that is used to control lipid oxidation (Ehsani et al. 2020; Noudoost et al. 2015). Since the absorption of synthetic additives can influence and damage the food quality, consumers prefer food products with no chemical additive. Polysaccharides and proteins are a kind of decomposable organic material that can be used as edible coating in refrigeration condition. Edible coatings prevent oxygen and water from permeating the food product and resulting in reduced oxidation and moisture.

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Sodium alginate contains L-guluronic acid and D-mannuronic acid, and it is a sodium salt of alginic acid. Alginate is a polymer extracted from brown algae, and due to its ability as a coagulating and film-forming factor, it can be utilized in the food industry. As a nontoxic, biocompatible, biodegradable, and reproducible biopolymer, alginate is suitable in biology or enzyme delivery and preparation of edible films and coatings. However, when alginate is used as a coating solution, it lacks antimicrobial or antioxidant properties (Sharifi et al. 2017). This macromolecule owns distinct colloidal characters and can develop Ca^{2+} cross-linking and form insoluble polymers when treated with $CaCl_2$ solution (Raeisi et al. 2016).

Substances with antimicrobial properties are able to incorporate into the packaging materials (Ehsani et al. 2019). *Zataria multiflora* Boiss (ZEO) belongs to the *Laminaceae* family and mainly grows in Iran, Pakistan, and Afghanistan. This medicinal plant is largely applied as a spice and food additive. *Zataria multiflora* Boiss is used due to antioxidant, antimicrobial, and antifungal properties, and its major compounds include carvacrol, thymol, and *p*-cymene (Shakeri et al. 2011).

Lactoperoxidase system (LPOS) is a single-chain polypeptide enzymatic system that is naturally found in animal-derived products, such as milk, and human secretions, such as saliva and tears (Molayi et al. 2018). It can be used as a suitable option in combination with other materials for delaying the process of fat oxidation and spoilage due to bacteria in the coatings (Ehsani et al. 2020). LPOS may be utilized as a bactericidal agent for gram-negative bacteria (Jasour et al. 2015). LPO enzyme, thiocyanate ion (SCN−), and hydrogen peroxide (H_2O_2) are the constituents of LPOS. Thiocyanate (SCN_) oxides by H_2O_2 via lactoperoxidase enzyme as catalyst. During this enzymatic oxidation reaction, some intermediates, such as hypothiocyanite (OSCN_) and hypothiocyanous acid (HOSCN) (Kussendrager and Van Hooijdonk 2000), with antimicrobial effects that can oxidize sulphydryl (−SH) groups of microorganisms are produced (Sharifi et al. 2017).

Many studies have investigated the effects of natural antimicrobial (LPOS) in edible coatings such as alginate (Didar et al. 2018; Yousefi et al. 2018), chitosan (Jasour et al. 2015) as well as whey protein (Farshidi et al. 2018; Molayi et al. 2018). The application of some LPOS systems in association with fish also has been studied (Shokri et al. 2015). Although some studies have examined the effects of essential oils such as ZEO in fish products (Khanzadi et al. 2020), the effects of LPOS and ZEO, individually and in combination, have not been evaluated until now. Accordingly, the present study was planned to determine the influences of alginate coating solution incorporated with Lactoperoxidase system and *Zataria multiflora* Boiss essential oil, individually and in combination, to decrease lipid oxidation and maintain sensory attributes in salmon fillets during a 16-day storage at 4 ± 1 °C. The findings of this study will contribute to sustaining nutritional value and quality of salmon fillets during refrigeration.

Materials and methods

Chemicals

The essential oil of Zataria *Multiflora* Boiss (ZEO) prepared by steam distillation was provided from the Iranian Institute of Medicinal Plants (Karaj, Iran) and stored under dark condition at 4°C until the usage time. The alginate and LPOS were supplied from Sigma-Aldrich (St. Louis, MO, USA). Thiobarbituric acid was purchased from Merck (Darmstadt, Germany). All reagents were of analytical grade.

Gas chromatography-mass spectrometry analysis of ZEO

The essential oil of *Zataria multiflora* Boiss was analyzed using a gas chromatographer (Agilent 7890A/5975C) equipped with a Chrome-pack CP-Sil 8 CB capillary column (50 m × 250 µm x 0.12 µm) as previously described by Keykhosravy et al. (2020).

Preparation of the LPOS

LPOS solution was prepared according to the procedure described by Jasour et al. (2015). The weight proportion of the LPOS components included: the LPO (1.00), glucose oxidase (0.35), D-(α)-glucose (108.70), potassium thiocyanate (KSCN) (1.09), and H_2O_2, H_2O_2 (2.17). All components (according to 15.5 mg LPO) were dissolved independently in 50 ml phosphate buffer (pH 6.2, 0.2 M). To exacerbate the antimicrobial activity of LPOS, the prepared solution was maintained at 23 ± 2°C for 24 h under constant shaking at 160 rpm using a shaker incubator (GFL 3031, Burgwedel, Germany).

Preparation of alginate coating containing ZEO and LPOS

Firstly, sodium alginate (3 g) was entirely dissolved in 100 ml distilled water via continuous stirring in hot water at 70°C. After adding glycerol (2% V/V) to alginate solution as a plasticizer, the mixture was shaken for 30 min to become clear (Khanzadi et al. 2020). Afterwards, ZEO (0.5% and 1% (w/v)), LPOS (5%), and Tween 80 (50% v/w EO) were added to alginate solution according to Table 1.

Preparation of samples and treatments

Fresh, skinless and boneless fillets of trout with the average weight of 550–600 g were purchased from a local protein market in Mashhad, Iran. The samples were moved to the laboratory of food hygiene and aquaculture in the department of veterinary medicine, Ferdowsi University of Mashhad, Iran using insulated polystyrene ice flasks. After washing the fillets, they were cut into small slices with the dimensions of 2 × 4 × 1 cm and a weight of 10 g (Fan et al. 2009).

Accidental selection of seven groups of fish fillets are presented in Table 1. Ten gram samples were used for each treatment. All samples were treated using different treatments (1 min). Afterwards, they were drained for 2 min followed by being submerged in a solution prepared from dissolving $CaCl_2$ (2% w/v) and 2 g calcium chloride in distilled water. The samples were autoclaved at 121°C for 15 min and for 1 min to induce cross-linking reaction (Khanzadi et al. 2020). Finally, the meat slices were maintained at 4°C, and they were harvested on days 0, 4, 8, 12, and 16 for biochemical analysis.

Total lipid extraction

Total lipid was extracted according to Bligh and Dyer procedure. Briefly, the fish fillets were well-mixed with a blender. The homogenate was mixed uniformly with chloroform and methanol with 1:2:4 ratios for 2 min and re-homogenized with 1:2:3 ratio chloroform and distilled water. When homogenate was filtered completely under suction, the final system was separated into two phases. Afterwards, the lower layer including lipids dissolved in chloroform was collected and transferred to a pear-shaped flask. Evaporation was done with rotary, and the residue was further dried under a stream of nitrogen (Kamkar et al. 2014), sealed, and stored at −80°C until the measurements of the lipid oxidation would be assayed.

Table 1. List of treatments in the present study.

	Treatment	Description
1	CON	without any coating solution
2	ALG	Alginate coating
3	LPOS	Alginate coating containing LPOS
4	ALG+ZEO 0.5%	Alginate coating containing $0.5\%_{(W/V)}$ZEO
5	ALG+ZEO 1%	Alginate coating containing $1\%_{(W/V)}$ZEO
6	LPOS+ZEO 0.5%	Alginate coating containing LPOS and $0.5\%_{(W/V)}$ZEO
7	LPOS+ZEO 1%	Alginate coating containing LPOS and $1\%_{(W/V)}$ZEO

Peroxide value (PV)

Peroxide value was measured according to method Cd 8–53 of AOCS (1998). After dissolving a certain amount of fat (0.1 g) in 25 mL of acetic acid/chloroform (3:2 v/v), saturated potassium iodide (1 mL) was added to the mixture. The flask containing the solution was shaken vigorously and instantly sealed to be kept under dark condition for 10 min. Then, distilled water (20 ml) and 1.5% starch indicator (1 mL) was added to the solution. Completing the mixing process was confirmed by changing the color solution to dark blue. At this step, sodium thiosulfate ($Na_2S_2O_3$) 0.01 N was applied for titration of the mixture until the disappearance of the blue color and appearance of a light-yellow color. At the same time, another test was carried out on a fat-free sample (blank). To calculate PV (milliequivalents of active oxygen/kg of fat) value, the following equation was applied:

$$\mathrm{PV(meq\ of\ active\ oxygen/kg\ of\ fat)} = \frac{(V1 - V2) \times \mathrm{N} \times 1000}{M}$$

V_1= Titration volume of sample
V_2= Titration volume of blank
N= Normality of $Na_2S_2O_3$ solution
M= Sample weigh

Thiobarbituric acid reactive substance (TBARS) value

$TBAR_S$ value was assessed using a colorimetrically based procedure described by Kirk and Sawyer (1991). The resulting data were expressed as mg of malondialdehyde (MDA) per kg of meat (Gharibzahedi and Mohammadnabi 2017). Briefly, a certain amount of fat (200 mg) was added into a 25 mL volumetric flask and after dissolving in 1 mL of 1-butanol, it was diluted to volume and mixed. Thiobarbituric acid (TBA) reagent was prepared via dissolving 200 mg of TBA reagent in 100 mL 1-butanol, and 5 ml of the reagent was added to the same volume of the mixture. After vortexing the test tubes, they were maintained in a water bath at 93 ± 2 °C for 2 h and then allowed to reach the room temperature. The sample absorbance (A_s) was determined at 532 nm using an UV – Vis spectrophotometer (Milton Roy, NY, USA). A meat-free blank sample was prepared for background subtraction, and its absorbance (A_b) was determined. The TBA values were calculated as follows:

$$\mathrm{TBA} = (\mathrm{A_s} - \mathrm{A_b}) \times 0.25$$

Free fatty acid (FFA) value

Free fatty acids, expressed as percentage of oleic acid, were determined based on the protocol used by Kirk and Sawyer (1991). First, 0.2 g of fat was dissolved in 50 mL of solvent (1:1 ratio of ethanol 96% and diethyl ether). Phenolphthalein indicator (1–2 drops) was added to the solution and tittered with sodium hydroxide 0.1 N. After changing the solution color to light pink and persisting for about 30 s, the titration was finished. The contents of oleic acid and free fatty acids were determined according to the following equations:

$$\mathrm{Acid\ value} = \frac{V \times \mathrm{N} \times 56.1}{M}$$

N: Normality of the solution
V: Volume of sodium hydroxide used
M: Sample weight

$$\mathrm{FFA}(\%) = \mathrm{Acid\ value} \times \frac{1}{2}$$

Preparation of fatty acid methyl esters (FAME) and gas chromatography analysis

Transesterification of fatty acids (FA) was conducted to prepare fatty acid methyl esters, which can be identified using gas chromatography. Methanolic KOH and *n*-heptane were applied to catalyze transmethylation reaction (Ehsani et al. 2018). A certain amount of extracted lipid (0.1 g) was put in a vial, and *n*-heptane (1100 µL) and 2 mol L^{-1} methanolic KOH (100 µL) were added. Afterwards, the mixture was vortexed until completely dissolving the lipid. Then, the vial was warmed up at 70°C for 15 min, and when it reached the ambient temperature, it was centrifuged at 1792 ×g for 10 min. The sample was allowed to be separated in two layers, and the upper layer containing FAME and the *n*-heptane layer were harvested. The FA profile of fish fillets was determined by gas chromatography (Agilent Technologies Inc., Santa Clara, CA, USA), equipped with a flame ionization detector and a cyanopropyl-phenyl capillary column (CP7489, CP-Sil 88 for FAME 100 m × 0.25 mm inner diameter × 0.2 μm film thickness). Ultra-high-purity nitrogen (99.9999%) was applied as a carrier gas at a flow rate of 1 mL min^{-1}. Column temperature was planned as follows: the initial temperature was held at 100 °C for 2 min and raised to 182 °C at a rate of 30 °C min^{-1}. Then, it re-increased to 220 °C at a rate of 2 °C min^{-1} and continued for 5 min. Afterwards, the temperature increased to 230 °C at a rate of 3 °C min^{-1} and continued for 3 min. The planed temperatures for the injector and detector were 230 and 300 °C, respectively. For each analysis, the injected sample size was 1 μL. FAs were identified and quantified by comparing the retention time of each FA with that of an external commercial standard FAME mixture (GLC-68d; Nu-Chek Prep., Waterville, MN, USA) under the same run conditions.

Sensory evaluation

Panel members were first trained with basic sensory evaluation techniques and product characteristics, and finally 21 female and male judges in the range of 27–45 years old member were selected from staff, DVM, and Ph.D. students of Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Iran. They were educated about the product and its features. Sensory attributes of the samples included color (acceptability of shade and homogeneity), odor (olfactory sensation on breathing the headspace volatiles), taste (response of taste bud on chewing), texture (required energy to masticate and stability on melting), and overall satisfactoriness (resemblance in comparison to commercial products). The judges were asked to wash their mouths with water during product evaluation. A 9-point hedonic scale in the range of 1–9 was applied for sensory evaluation. In this scale, 1 corresponded to "dislike extremely", while 9 indicated a "like extremely" response (Sani et al. 2017). Furthermore, the scale 1–3.9, 4–6.9, and 7–9 indicated the levels of unacceptability, temperate suitability, and high suitability, respectively. According to the shelf life parameters, sensory attributes below score 4 showed that the product was rejected by the judges (Fan et al. 2009). Fresh fish fillets were defined as control sample.

Statistical analysis

In this work, all experiments were carried out in triplicate. SPSS V21.0 software (SPSS, Inc., Chicago, IL, USA) was used to analyze the data statistically. To evaluate significant differences at $P < .05$ level, repeated measure analysis of variance followed by Dunnette T3 tests were applied. Distribution of sensory score among groups was compared using non-parametric Friedman test. Pairwise comparison was performed using Willcoxon signed rank test. Due to the multiple testing of the data, the significance level was considered significant when $P < .01$.

Results and discussion

Change in PV

Oxidative spoilage of fish slices was determined via PV and $TBAR_S$ assessment (Ehsani et al. 2019). Table 2 presents the PV values of meat slices. The PV values of the control groups were

found to be 0.42 (meq kg-1) at the beginning of the experiment. As the storage period increased, PV value was uninterruptedly augmented, reaching 16.60 (meq kg-1) at the end of experiment. The recommended limit for PV is 10 meq kg- for good-quality fish oils (Hazards 2010). In the present study, PV values of treatments containing ZEO and LPOS during chilled storage were in the acceptable range. As Table 2 demonstrates, the PV values measured on days 0 and 4 were similar. On the 8th day of the experiment, when suppressive effect of ZEO and LPOS occurred, PV was increased. The same pattern was reported by Ehsani et al. (2019). Although this rising trend continued ($P < .05$) in both coated samples and control groups, the ascending gradient in coated meat slices containing ZEO or LPOS was lower than in the control. The finding of this work was confirmed by previous studies that defined the antioxidant properties of EO and LPOS films (Ehsani et al. 2019; Yousefi et al. 2018).

Changes in TBARS

Lipid oxidation level was evaluated using $TBAR_S$ value representing malondialdehyde (MDA) content. MDA is the secondary product of the lipid oxidation process and results from poly-unsaturated lipid degradation (Ehsani et al. 2019). Its concentration during food processing and storage is used as criteria to control the quality of products (Farshidi et al. 2018). $TBAR_S$ assessment is based on the colorimetric detection of the malondialdehyde production forming due to reactive oxygen species. The reaction of malondialdehyde with thiobarbituric acid results in a colored substance (Saravani et al. 2019). $TBAR_S$ values in high qualified and good qualified fish meats have been proposed to be lower than 3 and 5 mg MDA/kg, respectively. $TBAR_S$ values ≥5 mg MDA/kg are considered as the threshold to identify off-odors and off-taste for humans (Kilinc et al. 2009). As shown in Table 2, $TBAR_S$ value of trout samples at the beginning of the storage duration was almost similar (0.48 mg MDA/kg as mean). $TBAR_S$ values in the control groups quickly reached 2.86 ± 0.48 mg MDA/kg after 4 days, indicating the maximum enhancement. $TBAR_S$ values throughout the storage duration were determined to be less than 5 mg MDA/kg in all groups except CON. In CON and all the treatments, $TBAR_S$ values showed a rising trend until the 8th and 12th day of the storage and after that a decreasing trend was observed between the days of 12 to 16. The enhancement of $TBAR_S$ value is associated with the production of secondary lipid oxidation substances, whereas breaking the formation of MDA due to tertiary degradation decreases this value. The observed trend in this study was comparable to previous research reported in chicken breast meat (Chouliara et al. 2008) and fish fillets (Jasour et al. 2015). As Aubourg (1993) suggested, due to the interaction of MDA with several macro-molecules such as nucleosides, nucleic acid, proteins, amino acids, and other ingredients of fish flesh, $TBAR_S$ level may not reflect the actual degree of lipid peroxidation can interact. According to the data indicated in Table 2, the $TBAR_S$ values in the samples treated with LPOS were affected less than CON treatment. This finding was in agreement with other studies reported by Shokri et al. (2015) and Jasour et al. (2015). It is assumed that activated LPOS inside the alginate coating solution may not influence the $TBAR_S$ value. Although it resulted in the minor elevation of $TBAR_S$ production, the more negative effect of coatings on this value was obtained in LPOS +ZEO 1% and ALG+ZEO 1%.

Changes in FFA

Lipid-based food products are mainly spoiled and degraded due to lipolysis process (Yousefi et al. 2018). In the present study, FFA content of the fish fillets was determined to evaluate the progress of lipid hydrolysis. The FFA content as a main factor in the formation of the off-flavor and undesirable taste of fish meat products is indicated in Table 2. However, the FFA production may not directly result in decreasing the nutritional quality of meat foods; it affects the quality of meat via induction of proteins denaturation and lipid oxidation. In this work, FFA production was not changed significantly

Table 2. Changes in the main chemical spoilage parameters (PV [meq active oxygen kg $^{-1}$ of fat], TBARs [mg malonaldehyde/100 g of sample] and FFA [% of oleic acid]) of samples during 16 days of storage[1].

		Treatments						
Attributes	Sampling Time (Day)	CON	ALG	LPOS	ALG+ZEO 0.5%	ALG+ZEO 1%	LPOS+ZEO 0.5%	LPOS+ZEO 1%
PV	0	0.42 ± 0.08^{aC}	0.42 ± 0.13^{aC}	0.22 ± 0.04^{bC}	0.44 ± 0.08^{aD}	0.36 ± 0.11^{abD}	0.20 ± 0.10^{bD}	0.18 ± 0.08^{bD}
	4	0.58 ± 0.13^{abC}	0.62 ± 0.14^{aC}	0.34 ± 0.11^{bcdC}	0.46 ± 0.15^{abcD}	0.44 ± 0.08^{abcdD}	0.26 ± 0.11^{cdD}	0.20 ± 0.07^{dD}
	8	5.76 ± 0.20^{bB}	6.06 ± 0.11^{aB}	6.00 ± 0.15^{abB}	$1.98 \pm 0.08b^{cC}$	2.02 ± 0.08^{cC}	1.96 ± 0.11^{cC}	1.92 ± 0.16^{cC}
	12	16.90 ± 0.10^{aA}	16.92 ± 0.13^{aA}	16.88 ± 0.19^{aA}	5.06 ± 0.16^{bB}	5.14 ± 0.11^{bB}	5.00 ± 0.20^{bB}	5.22 ± 0.13^{bB}
	16	16.60 ± 0.51^{aA}	17.22 ± 0.80^{aA}	17.14 ± 0.70^{aA}	9.62 ± 0.22^{bA}	8.50 ± 0.37^{cA}	9.52 ± 0.19^{bA}	8.46 ± 0.27^{aC}
TBARs	0	0.52 ± 0.05^{aD}	0.53 ± 0.05^{aC}	0.47 ± 0.04^{aC}	0.50 ± 0.05^{aC}	0.45 ± 0.00^{aB}	0.47 ± 0.05^{aC}	0.48 ± 0.05^{aC}
	4	2.86 ± 0.48^{aC}	2.53 ± 0.25^{aB}	2.63 ± 0.16^{aB}	1.63 ± 0.13^{bB}	1.26 ± 0.20^{bA}	1.65 ± 0.14^{bB}	1.17 ± 0.09^{bB}
	8	5.26 ± 0.39^{aB}	4.90 ± 0.58^{aA}	4.98 ± 0.65^{aA}	2.08 ± 0.19^{bA}	1.44 ± 0.23^{bA}	2.00 ± 0.17^{bA}	1.36 ± 0.17^{bA}
	12	5.98 ± 0.38^{aA}	4.33 ± 0.30^{aA}	4.49 ± 0.27^{bA}	1.99 ± 0.16^{cA}	1.39 ± 0.23^{deA}	1.90 ± 0.10^{cdA}	1.33 ± 0.25^{eA}
	16	5.87 ± 0.28^{aAB}	4.51 ± 0.20^{bA}	4.47 ± 0.25^{bA}	2.09 ± 0.12^{aC}	1.55 ± 0.23^{dA}	2.11 ± 0.09^{CA}	1.54 ± 0.20^{dA}
FFA	0	0.65 ± 0.08^{aE}	0.60 ± 0.09^{aE}	0.57 ± 0.08^{aE}	0.60 ± 0.07^{aE}	0.57 ± 0.08^{aD}	0.58 ± 0.06^{aE}	0.59 ± 0.07^{aD}
	4	1.14 ± 0.16^{abD}	1.20 ± 0.07^{aD}	1.23 ± 0.17^{aD}	0.96 ± 0.09^{bcD}	0.90 ± 0.02^{cC}	0.84 ± 0.08^{cD}	0.89 ± 0.40^{cC}
	8	1.60 ± 0.07^{aC}	1.66 ± 0.06^{aC}	1.67 ± 0.08^{aC}	1.40 ± 0.12^{bC}	1.30 ± 0.07^{bB}	1.36 ± 0.11^{bC}	1.30 ± 0.10^{bB}
	12	2.21 ± 0.21^{aB}	2.16 ± 0.12^{aB}	2.13 ± 0.07^{aB}	1.61 ± 0.11^{bB}	1.41 ± 0.10^{bB}	1.58 ± 0.05^{bB}	1.39 ± 0.13^{bB}
	16	3.63 ± 0.15^{aA}	3.59 ± 0.09^{aA}	3.62 ± 0.05^{aA}	2.78 ± 0.04^{bA}	1.94 ± 0.06^{cA}	2.73 ± 0.08^{bA}	1.84 ± 0.08^{cA}

[a-d]The different uppercase letters in the same row indicate significant differences ($P < .05$).
[A-D]The different lowercase letters in the same column indicate significant differences ($P < .05$).
[1]Date are expressed as mean ± *SD* ($n = 5$).

at the beginning of the experiment, but during the storage it was enhanced in all treatments. FFA contents of three treatments, including CON, ALG, and LPOS, were enhanced progressively. Oleic acid percentage was enhanced almost sixfold, increasing from 0.57% to 0.65% to 3.59–3.63% at the final day of storage. The LPOS+ZEO 1% treatment had the lowest amount of FFA, equal to 1.84% of oleic acid. The higher the level of ZEO (1%), the better effect on preventing the increased level of FFAs compared to LPOS, ALG, and CON groups. Ehsani et al. (2014) investigated the effect of ZEO and sodium acetate on shelf life of trout burger and showed that the FFA contents in the samples treated with ZEO decreased as compared to other samples. Also, Taheri et al. (2013) showed the minimum content of FFA was obtained in the fish fillets treated with 500 ppm ZEO. A strong correlation was also observed between FFA content and storage time, and with increasing the storage duration, the FFA content was elevated as well.

Fatty acid composition

Tables 3 and 4 demonstrate the major fatty acids of the fish fillets. Among all identified fatty acids, quantitatively and qualitatively, fish fillet lipid fractions were predominantly composed of saturated fatty acids (SFA), including palmitic (C16:0) and stearic acid (C18:0), monounsaturated fatty acids (MUFA), like oleic acid (C18:1 ω-9) and palmitoleic acid (C16:1 ω-7), and polyunsaturated fatty acids (PUFA), such as linoleic acid (C18:2 ω-6 cis), alpha-linolenic acid (C18:3 ω-3), eicosapentaenoic acid (C20:5 ω-3), and docosahexaenoic acid (C22:6 ω-3). During the sample storage, percentage of SFA and MUFA increased, while PUFA contents decreased. Therefore, the PUFA/SFA declined during storage, which was similar to another study (Ehsani et al. 2018). The polyene index (PI), the ratio of PUFA to SFA, is used as an indicator to determine lipid oxidation during storage. The omega (ω)-6 and ω-3 PUFAs, which are the most preferred fatty acids for nutritional purposes, were maximum at the beginning of the experiment. In all groups except CON and ALG, PUFAs and MUFAs constituted the highest level of fatty acids at the end of storage. Similarly, in rainbow trout treated with chitosan coating incorporated with cinnamon oil, SFA, and PUFA percentage declined and the contents of MUFA and ω-6 fatty acid remained unchanged during the storage period (Ojagh et al. 2014).

Table 3. Main muscle FA composition (% of total FAs) in trout fillets at day 0 refrigerated storage (4 ± 1 ◦C).

	Day 0						
	CON	ALG	LPOS	ALG+ZEO 0.5%	ALG+ZEO 1%	LPOS+ZEO 0.5%	LPOS+ZEO 1%
SFA							
C16:0	0.70 ± 16.02	1.53 ± 15.77	0.19 ± 15.56	0.36 ± 15.77	1.28 ± 15.09	0.14 ± 14.99	0.54 ± 14.53
C18:0	0.72 ± 4.70	0.72 ± 4.30	0.22 ± 4.63	0.86 ± 3.89	0.42 ± 4.39	0.30 ± 4.76	0.22 ± 4.23
Σ SFA	1.87^{d} ±22.27	1.21^{bcd} ±21.65	1.39^{cd} ±21.71	1.68^{bc} ±21.30	1.12^{ab} ±20.77	1.46^{bc} ±21.25	1.52^{a} ±20.28
MUFA							
C16:1 ω9	0.54 ± 2.95	0.42 ± 2.88	0.05 ± 2.95	0.60 ± 3.31	0.34 ± 2.36	0.40 ± 2.60	0.05 ± 2.55
C18:1 ω9	3.11 ± 30.33	2.12 ± 30.01	0.09 ± 30.19	2.42 ± 30.37	1.02 ± 30.62	0.93 ± 30.27	1.67 ± 30.77
Σ MUFA	1.12^{a} ±33.79	1.56^{a} ±33.53	1.02^{a} ±33.70	0.04^{a} ±34.30	1.04^{a} ±33.57	1.03^{a} ±33.44	0.08^{a} ±33.89
PUFA							
C18:2 ω6	3.50 ± 30.10	2.65 ± 30.38	0.10 ± 30.02	1.36 ± 30.48	1.78 ± 30.75	1.46 ± 30.42	1.09 ± 30.65
C18:3 ω3	1.56 ± 4.19	1.56 ± 4.13	0.06 ± 4.02	1.11 ± 4.25	0.06 ± 4.02	0.13 ± 4.10	0.06 ± 4.02
C20:5 ω3	0.28 ± 1.02	0.28^{c} ±1.02	0.24 ± 1.66	0.25 ± 0.67	0.25^{a} ±0.67	0.24^{ab} ±0.69	0.24^{ab} ±0.76
C22:6 ω3	1.25 ± 3.18	1.25 ± 4.18	0.35 ± 3.57	1.07 ± 4.20	1.07 ± 4.24	0.35 ± 4.57	0.41 ± 4.57
Σ PUFA	1.98^{a} ±43.03	2.02^{b} ±44.07	1.73^{b} ±43.60	1.60^{a} ±43.96	1.01^{a} ±43.81	1.11^{a} ±43.44	2.01^{a} ±43.68
Σ ω-3 PUFA	0.94^{a} ±8.62	0.84^{b} ±9.56	0.88^{b} ±9.45	0.93^{b} ±9.36	0.92^{ab} ±9.13	0.85^{b} ±9.58	0.95^{b} ±9.57
Σ ω-6 PUFA	1.04^{a} ±34.41	1.18^{ac} ±34.51	1.04^{a} ±34.15	1.02^{a} ±34.6	1.32^{a} ±34.68	1.03^{a} ±33.86	1.01^{b} ±39.11
PI	0.54^{a} ±1.93	0.51^{a} ±2.03	0.44^{a} ±2.00	0.32^{a} ±2.06	0.42^{b} ±2.10	0.43^{a} ±2.04	0.51^{b} ±2.15

[a-d]The different lowercase letters in the same row indicate significant differences (P< .05).
[1]Date are expressed as mean ± SD (n = 3).

Table 4. Main muscle FA composition (% of total FAs) in trout fillets at day 16 refrigerated storage (4 ± 1 ◦C).

	Day 16						
	CON	ALG	LPOS	ALG+ZEO 0.5%	ALG+ZEO 1%	LPOS+ZEO 0.5%	LPOS+ZEO 1%
SFA							
C16:0	0.49 ± 16.21	0.33 ± 17.08	0.33 ± 17.93	0.99 ± 17.80	0.51 ± 16.53	0.72 ± 16.49	1.56 ± 15.90
C18:0	0.15 ± 6.07	0.32 ± 6.47	0.35 ± 6.30	0.64 ± 5.93	0.17 ± 4.99	1.41 ± 4.90	0.25 ± 4.93
Σ SFA	1.67^{b} ±25.24	1.17^{c} ±27.31	1.39^{c} ±26.94	1.68^{c} ±27.26	1.12^{b} ±24.70	1.46^{b} ±24.65	0.52^{a} ±23.80
MUFA							
C16:1 ω7	0.05 ± 3.66	0.73 ± 3.83	0.60 ± 3.70	0.54 ± 3.50	0.12 ± 3.67	0.69 ± 3.19	3.40 ± 3.55
C18:1 ω9	1.21 ± 34.63	4.2 ± 33.23	3.71 ± 33.73	1.82 ± 28.20	2.80 ± 31.23	2.50 ± 31.37	2.08 ± 32.33
Σ MUFA	1.42^{a} ±38.85	1.66^{ab} ±37.85	1.02^{a} ±38.15	1.04^{c} ±36.50	1.44^{a} ±35.67	2.03^{d} ±35.42	0.08^{b} ±36.96
PUFA							
C18:2 ω6	0.56 ± 26.90	2.12 ± 25.95	1.37 ± 25.23	2.62 ± 25.75	1.07 ± 27.67	3.07 ± 27.37	0.71 ± 27.25
C18:3 ω3	0.15 ± 3.13	0.60 ± 4.00	0.46 ± 3.90	1.11 ± 3.90	0.00 ± 3.60	0.42 ± 3.70	0.14 ± 3.30
C20:5 ω3	0.00^{bc} ±0.40	0.10^{c} ±0.30	0.10^{c} ±0.30	0.31 ± 0.67	0.06^{a} ±0.37	0.21^{ab} ±0.57	0.07^{ab} ±0.45
C22:6 ω3	0.06 ± 1.77	0.57 ± 1.50	0.57 ± 1.50	0.40 ± 2.87	0.35 ± 3.47	0.35 ± 3.57	0.14 ± 3.30
Σ PUFA	1.68^{ab} ±35.61	2.32^{a} ±34.66	1.03^{a} ±33.85	0.60^{cb} ±36.21	1.51^{b} ±38.30	0.01^{b} ±38.14	2.11^{b} ±37.30
Σ ω-3 PUFA	0.64^{a} ±5.47	0.64^{a} ±5.95	0.68^{a} ±5.86	0.03^{bc} ±7.30	1.02^{bc} ±7.54	0.76^{c} ±7.94	0.55^{b} ±7.15
Σ ω-6 PUFA	1.04^{a} ±30.14	1.68^{ac} ±28.71	1.04^{b} ±27.99	0.02^{a} ±28.91	1.22^{ad} ±30.76	0.03^{a} ±30.20	1.21^{a} ±30.15
PI	0.54^{a} ±1.41	0.41^{bc} ±1.26	0.44^{bc} ±1.25	0.32^{bd} ±1.32	0.32^{b} ±1.55	0.43^{b} ±1.54	0.41^{b} ±1.56

[a-d]The different lowercase letters in the same row indicate significant differences ($P < .05$).
[1]Date are expressed as mean ± *SD* ($n = 3$).

Sensory evaluation

The sensory evaluation of coated-fish fillets using several types of solutions is shown in Table 5. Different parameters of meat, such as color, odor, taste, and texture, were used to evaluate the overall acceptance. As the storage period prolonged, the sensory scores of fish slices decreased significantly during the storage period ($P < .05$). According to the data presented in Table 5, the treatments of CON and ALG batch were rejected at the 12th day due to acquiring a score below the acceptability level for odor parameter. At the same time, other batches were evaluated as moderate and high acceptable, respectively. At the end of the storage period, all treatments except those containing LPOS and ZEO had an off-odor. The obtained scores for color and texture were acceptable for all groups during the study period (16 day). However, the control samples were discolored at day 16 and obtained moderate score (Table 5). The CON, ALG, LPOS, ALG+ZEO 0.5%, and ALG+ZEO 1% received an unacceptable score of overall acceptability at the end of the storage period (Table 5). High concentrations of EOs negatively affected the sensory attributes of meat samples, especially their flavor (Shahinfar et al. 2017). The application of ZEO enhanced the sensory scores of chicken meat and improved its shelf life during chilled storage (Bazargani-Gilani et al. 2015). Odor, color, and overall acceptability are the main sensory characteristics of foods influencing consumer acceptance. Edible coatings, particularly those containing LPOS/EO, increased the organoleptic values of seafood (Jasour et al. 2015; Song et al. 2011). The sensory score results seem to be related to biochemical composition of meat samples (Bazargani-Gilani et al. 2015; Ojagh et al. 2010). Formation of secondary metabolites such as aldehydes, ketones, hydrocarbons, alcohols, and esters during the prolonged storage may cause taste deterioration (Ghaderi-Ghahfarokhi et al. 2017).

Conclusion

In this study, the oxidative stability of rainbow trout fillets was maximized with alginate-loaded coating that contained *Zataria multiflora* Boiss and lactoperoxidase system via decreasing lipolysis process. According to the results of lipid oxidation and sensory evaluation, the fresh quality of control meat slices was preserved until the 4th day of storage. Nevertheless, it was prolonged by day 8 in all treated samples and by day 12 in combination groups. Also, alginate coating containing LPOS and ZEO 1% had the best oxidative status among the other groups. Moreover, treatment containing LPOS or ZEO resulted in good sensory properties until the end of storage period. Therefore, application of

Table 5. Sensory evaluations of trout fillet during 16 days of storage [1].

		Treatments						
Attributes	Sampling Time (Day)	CON	ALG	LPOS	ALG+ZEO 0.5%	ALG+ZEO 1%	LPOS+ZEO 0.5%	LPOS+ZEO 1%
Odor	0	8.00 (7, 9)^{a}	9.00 (8, 9)^{b}	8.00 (7, 9)^{a}	9.00 (7, 9)^{a}	8.00 (6, 9)^{ac}	9.00 (7, 9)^{a}	8.00 (7, 9)^{ac}
	4	6.00 (5, 8)^{a}	9.00 (8, 9)^{b}	8.00 (5, 8)^{c}	9.00 (7, 9)^{b}	9.00 (6, 9)^{b}	9.00 (8, 9)^{b}	9.00 (8, 9)^{b}
	8	5.00 (4, 6)^{a}	6.00 (5, 6)^{d}	8.00 (5, 9)^{c}	7.00 (6, 8)^{bc}	8.00 (6, 9)^{bc}	8.00 (6, 9)^{bc}	9.00 (6, 9)^{e}
	12	1.00 (1, 4)^{a}	2.00 (1, 4)^{c}	6.00 (4, 9)^{b}	6.50 (6, 8)^{be}	8.00 (4, 9)^{b}	8.00 (6, 9)^{bd}	7.00 (6, 9)^{bd}
	16	1.00 (1, 2)^{a}	1.00 (1, 4)^{a}	5.00 (4, 8)^{b}	6.00 (4, 7)^{b}	6.00 (4, 8)^{b}	6.00 (4, 8)^{b}	6.00 (4, 8)^{b}
Color	0	9.00 (8, 9)^{a}	9.00 (7, 9)^{a}	9.00 (8, 9)^{a}	9.00 (8, 9)^{a}	9.00 (9, 9)^{a}	9.00 (9, 9)^{a}	9.00 (9, 9)^{a}
	4	9.00 (8, 9)^{a}	9.00 (6, 9)^{a}	9.00 (8, 9)^{a}	9.00 (7, 9)^{a}	9.00 (8, 9)^{a}	9.00 (9, 9)^{a}	9.00 (8, 9)^{a}
	8	8.00 (7, 9)^{a}	9.00 (7, 9)^{a}	9.00 (8, 9)^{a}	9.00 (7, 9)^{a}	9.00 (7, 9)^{a}	9.00 (7, 9)^{a}	9.00 (8, 9)^{b}
	12	8.00 (6, 9)^{a}	7.5.00 (6, 9)^{a}	8.00 (7, 9)^{b}	8.00 (6, 9)^{a}	8.00 (6, 9)^{a}	9.00 (8, 9)^{b}	9.00 (8, 9)^{b}
	16	6.00 (4, 8)^{a}	7.00 (5, 8)^{b}	7.5.00 (7, 9)^{bc}	8.00 (6, 8)^{b}	8.00 (7, 9)^{bc}	9.00 (7, 9)^{bc}	8.00 (7, 9)^{bc}
Texture	0	9.00 (7, 9)^{a}	9.00 (8, 9)^{a}	9.00 (7, 9)^{a}	9.00 (7, 9)^{a}	9.00 (8, 9)^{a}	9.00 (7, 9)^{a}	9.00 (8, 9)^{a}
	4	9.00 (7, 9)^{a}	9.00 (6, 9)^{a}	9.00 (6, 9)^{a}	9.00 (6, 9)^{a}	9.00 (6, 9)^{a}	9.00 (6, 9)^{a}	9.00 (6, 9)^{a}
	8	6.50 (5, 9)^{a}	8.00 (6, 9)^{b}	8.00 (5, 9)^{ab}	6.00 (5, 8)^{ac}	7.00 (5, 9)^{ac}	8.00 (5, 9)^{ab}	7.50 (5, 9)^{ac}
	12	5.00 (4, 6)^{a}	6.50 (5, 9)^{b}	6.00 (5, 8)^{ab}	6.00 (5, 8)^{a}	5.00 (4, 8)^{ab}	6.00 (5, 8)^{b}	6.00 (5, 9)^{b}
	16	4.00 (3, 6)^{a}	5.50 (3, 7)^{a}	4.50 (3, 7)^{a}	4.00 (3, 7)^{a}	4.00 (3, 6)^{a}	6.00 (3, 7)^{a}	6.00 (3, 7)^{a}
Taste	0	9.00 (7, 9)^{ac}	9.00 (7, 9)^{a}	9.00 (6, 9)^{ac}	8.00 (7, 9)^{ac}	6.00 (5, 9)^{bc}	8.00 (7, 9)^{ad}	7.00 (6, 9)^{bc}
	4	7.00 (5, 7)^{a}	8.00 (6, 9)^{b}	7.00 (5, 9)^{a}	8.00 (6, 9)^{b}	7.00 (5, 9)^{a}	8.00 (6, 9)^{b}	6.50 (6, 9)^{a}
	8				NT[2]			
	12				NT			
	16				NT			
Overall acceptability	0	9.00 (6, 9)^{a}	9.00 (6, 9)^{a}	9.00 (6, 9)^{a}	9.00 (6, 9)^{a}	9.00 (4, 9)^{a}	9.00 (8, 9)^{a}	9.00 (6, 9)^{a}
	4	7.50 (5, 9)^{a}	9.00 (6, 9)^{a}	9.00 (5, 9)^{a}	9.00 (6, 9)^{a}	9.00 (6, 9)^{a}	9.00 (6, 9)^{a}	9.00 (6, 9)^{a}
	8	6.00 (4, 8)^{a}	6.50 (5, 8)^{a}	8.00 (5, 9)^{ab}	6.00 (3, 8)^{ac}	7.50 (5, 9)^{a}	8.00 (4, 9)^{ac}	8.00 (5, 9)^{ac}
	12	4.00 (4, 5)^{a}	5.50 (3, 6)^{ac}	4.50 (3, 9)^{ae}	4.00 (3, 7)^{be}	5.00 (3, 8)^{be}	5.00 (3, 7)^{ae}	6.00 (3, 9)^{a}
	16	2.00 (2, 4)^{a}	3.00 (3, 5)^{a}	3.50 (2, 7)^{ab}	2.50 (2, 6)^{c}	3.00 (2, 6)^{ab}	4.00 (3, 8)^{ab}	5.00 (3, 7)^{a}

$^{a-c}$The different lowercase letters in the same row indicate significant differences ($P < .01$).
[1]Date are expressed as median ± min, max (n = 3).
[2]NT: Non-Test.

Zataria multiflora Boiss essential oil and lactoperoxidase system is highly recommended in the food industry as an approach to preserve the nutritional quality of food products.

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Authors' contributions

Saeid Khanzadi: Conceptualization, Funding acquisition, Methodology, Project administration, Supervision, Writing – review & editing

Mohammad Hashemi: Conceptualization, Methodology, Project administration, Resources, Visualization, Roles, Writing – review & editing

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Kobra Keykhosravy: Methodology, Writing-original draft

Practical application

The spoilage risk of fresh fish products is higher than other foods. Fish muscle spoils because of biological reactions such as the oxidation of lipids, the action of endogenous enzymes, and the metabolic activities of microorganisms that end in a short shelf life. The oxidation of lipids not only reduces or retards, but also inhibits by edible coatings. Edible coatings formed from bioactive compounds would effectively provide possibility of active compounds onto surface of minimally processed foods. Therefore, application alginate coating containing *Zatariamultiflora Boiss* essential oil (ZEO) and lactoperoxidase system is recommended in food industry especially for fishery industry to increase the chemistry and sensory quality of fish fillets.

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