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ORIGINAL ARTICLE

Effects of Alginate Coating Containing *Zataria multiflora* Essential Oil in the Form of an Emulsion Gel and Nano-emulsion on the Chemical Quality and Sensory Properties of Rainbow Trout Fillet

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KEYWORDS

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Shelf life

ABSTRACT: Fish is often more perishable than most other foodstuffs. Microbial progression, enzymatic activity, and non-enzymatic oxidation of fats reduce the quality of fish and its products. The present study aimed to evaluate the effects of the nano-gel emulsion and emulsion gel of an alginate coating containing *Zataria multiflora* essential oil (ZMEO) on the chemical and sensorial quality of rainbow trout fillet (*Oncorhynchus mykiss*) during 16 days of refrigerated storage (4±1°C). The fish fillets were treated with alginate coating, alginate emulsion with different ZMEO concentrations (E0.25%, E0.5%, and E1%), and nano-emulsion with different ZMEO concentrations (N0.25%, N0.5%, and N1%). Afterwards, the fillets were analyzed for chemical changes (pH, TVB-N, TBARS, PV, FFA, and fatty acid profile) and sensory properties (color, texture, flavor, odor, and overall acceptability) on days zero, four, eight, 12, and 16. Data analysis was performed in SPSS version 21 using ANOVA, Bonferroni post-hoc test, and Dunnett T3 test to assess significant differences at $P<0.05$. The results of chemical analysis showed an increasing trend (pH, TVB-N, TBARS, PV, FFAs, MUFAs, and SFAs) during the storage period (day 16) although the rate was slower in the nano-emulsion treatments. Furthermore, the sensory properties of the samples decreased during the storage period, while using ZMEO in the alginate coating (especially in the nano-emulsion form) showed better results compared to the control. According to the results, the alginate coating containing ZMEO (especially in the nano-emulsion form) could preserve the chemical and sensorial quality of the fresh trout fillets for four days.

INTRODUCTION

Rainbow trout (*Oncorhynchus mykiss*) is from the Salmonidae family and is a high-value species native to cold-water tributaries among European consumers. The quality of rainbow trout has long been considered a major concern of the consumers [1, 2]. Immediately after catching, a set of changes begins in the body of the fish, resulting in a significant reduction in the qualitative properties of the product. Some of the influential factors in the rate of these changes include stress, mechanical damage while hunting, structure and chemical composition of the fish body, breeding season, region, pH, and storage temperature [3].

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Lipid oxidation is controlled by various enzymatic (bacterial and non-bacterial enzymes) and non-enzymatic reactions. When the bacterial activity stops or slows-down by freezing, drying or other methods, lipid oxidation due to the formation of hydroperoxides becomes another cause of the reduced quality and nutritional value of fish [4]. Moreover, changes in lipids (especially in oily fish) lead to the change of taste and smell of fish, which is known as pungency and makes the product unusable [5].

Recently, films and coatings have been widely used for protecting food against the growth of pathogenic and spoilage bacteria with several benefits such as edibility and biodegradability, as well as being non-polluting and non-toxic. In addition, films and coatings could act as carriers of additives and flavorings such as antimicrobial agents [6, 7]. Alginate polysaccharides extracted from brown seaweed (*Phaeophyceae*) are a common gelling agent used in the food industry as a coating [8].

Essential oils could be added to coatings and films as natural additives to increase the compatibility and safety of foods [9-11]. Researches claim that due to the hydrophobic nature of essential oils, they could directly break the cytoplasmic membrane or indirectly bind to the membrane proteins and increase the permeability of the membrane, thereby causing the material to escape from the cytoplasm [12-17]. Among various essential oils, *Zataria multiflora* (Shirazi thyme) essential oil (ZMEO) has received more attention from researchers and the food industry owing to its antioxidant and antimicrobial activities [18-20]. In addition, ZMEO contains plenty of terpenoids, such as thymol, carvacrol, γ-terpinene, and p-cymene [21, 22].

Nanotechnology has become a priority field for today's world trade. Rewardingly, the production of nano-emulsions for plasticization and controlling the release of superoxide compounds (e.g., drugs, colors, essential oils, and vitamins) has attracted great attention in the food industry. Properties such as transparency/semi-transparency, stability, and resistance against sedimentation or becoming creamy have made nano-emulsions suitable for fundamental and applied studies in chemical, health, pharmaceutical, and other fields [23].

To date, numerous researchers have focused on the application of natural antimicrobial compounds in foods. However, only a few studies have considered natural antioxidants for the control of oxidation in fish [24, 25]. Furthermore, there are no comparative studies regarding the effects of ZMEO in the forms of emulsion gel and nano-emulsion on fish [26].

The present study aimed to evaluate the effects of an alginate coating solution containing two forms of ZMEO, nano-gel emulsion, and emulsion gel on controlling oxidation in salmon fillets.

MATERIALS AND METHODS

Preparation of Fish Fillets

Fish weighing 450±25 grams were purchased from a fish farm located in Mashhad city, Iran. After washing, the fish were transferred to the laboratory in a Styrofoam box containing ice. After removing the viscera and bones, 10 grams of the fish fillets were prepared for each treatment.

Preparation of Alginate Coating Solution Containing ZMEO

At this stage, three grams of sodium alginate was dissolved in 100 milliliters of distilled water to provide a 3% alginate solution. After adding glycerol, 2% (v v^{-1}) was added to the solution as a plasticizer at the controlled temperature of 70°C, and the solution was stirred well for 30 minutes to become clear. Calcium chloride was dissolved (2% w v^{-1}) in distilled water, autoclaved at the temperature of 121°C for 15 minutes, and mixed with the alginate solution. Following that, ZMEO was added to the coating solution at the concentrations of 0.25%, 0.5%, and 1% (w v^{-1}). In order to dissolve the essential oil in the coating solution, 0.5% of Tween 80 was used as an emulsifier [27].

Alginate containing the nano-emulsion of ZMEO was prepared from the 3% alginate solution using a DIAX device (DIAX 900, Heidolph, Schwabach, Germany) during six minutes to form a primary emulsion and subjected to an ultrasound device (Sonoplus, Bandelin, Berlin, Germany) at 50% power for six minutes. Following

that, the particle size was measured using a dynamic light scattering (DLS) device (Nano S, Marven, Marven, England) [28]. Finally, the specimens were diluted with deionized water, and the range of special particle size was 100-600 nanometers [29].

Treatment Preparation

The samples were immersed in the coating solutions for 60 seconds, allowed to rest for 30 seconds, and immersed in a 2% calcium chloride solution for 30 seconds. Afterwards, the treatments were packed in aseptic zipper bags and stored at the temperature of 4±1°C for 16 days for further analysis on days zero, four, eight, 12, and 16 (Table 1) [30, 31].

Table1. Different combinations and treatments in the present study

	Treatments		Description
1	CO	Control	Sample without alginate coating
2	ALG	Control with alginate	Sample with alginate coating
3	E0.25%	0.25% emulsion	Sample with alginate coating containing 0.25% ZMEO as a simple emulsion
4	E0.5%	0.5% emulsion	Sample with alginate coating containing 0. 5% ZMEO as a simple emulsion
5	E1%	1% emulsion	Sample with alginate coating containing 1% ZMEO as a simple emulsion
6	N0.25%	0.25% Nano emulsion	Sample with alginate coating containing 0.25% ZMEO as Nano emulsion
7	N0.5%	0. 5% Nano emulsion	Sample with alginate coating containing 0. 5% ZMEO as Nano emulsion
8	N1%	1% Nano emulsion	Sample with alginate coating containing 1% ZMEO as Nano emulsion

Chemical analysis

Total Volatile Basic Nitrogen (TVB-N)

The micro-diffusion method was used to measure the total volatile basic nitrogen (TVB-N) value of the samples on days zero, four, eight, 12, and 16. Briefly, homogenized samples were mixed with magnesium oxide (MgO) and distillated. The distillates were collected in flasks containing an aqueous solution of boric acid (3% v v^{-1}) and an indicator solution; the indicator was produced by dissolving methyl red (0.1 g) and methylene blue (0.1 g) in ethanol (100 ml). Following that, the boric acid solution was titrated by the sulfuric acid solution (0.05 mol l^{-1}). The TVB-N value was calculated based on the amount of consumed sulfuric acid and expressed as mg $100g^{-1}$ of the fish flesh [32].

pH

At this stage, the fish samples (10 g) were homogenized in 100 milliliters of distilled water. Afterwards, the mixtures were filtered through a filter paper, and the pH values of the filtrates were measured using a digital pH meter (Cyberscan PC 510 UK) [33] on days zero, four, eight, 12, and 16.

Measurement of lipid oxidation parameters

Total lipid extraction

Lipid extraction was performed using the method proposed by Bligh and Dyer [34]. For this purpose, the fish fillets were properly mixed using a blender (GSB827, Gosonic, China), and 50 grams of the fish fillet was uniformly mixed with 50 milliliters of chloroform and 100 milliliters of methanol for two minutes. Following that, 50 milliliters of chloroform and 50 milliliters of distilled water were added to the mixture in two steps, remixed, and passed through a Whatman filter paper under vacuum conditions to remove the solid phase from the liquid phase. The filtered liquid was transferred into a decanter and left for several minutes until the appearance of the two phases. The lower clear

phase (chloroform and extracted lipid) was removed by opening the decanter valve, and the solution was re-filtered and transferred to a rotary evaporator device (RV, Heidolph, Germany) to remove the chloroform to obtain the lipid mass [32]. The temperature of the water bath was approximately 40°C to prevent the chloroform from drying completely. The obtained lipid mass was poured into a dark container, which was filled with nitrogen gas, and stored at the temperature of -80°C until performing the lipid oxidation assays [32]; this experiment was carried out on days zero, four, eight, 12, and 16.

Peroxide Value (PV)

The amount of peroxide represents the active oxygen content in milliequivalents (mEq) of iodine per kilogram of fat. The measurement of peroxide value (PV) is possible by the titration of the released iodine from potassium iodide using a sodium thiosulfate solution. Based on the method of International Dairy Federation (IDF), the denominator gives the concentration of Fe^{2+} oxidized to Fe^{3+} in micrograms as the milliequivalents of peroxide instead of the milliequivalents of oxygen [35].

$$PV = (As - Ab) \times 40.86/55.84 \times W \times 2$$

In the formula above, *As* shows the absorbance of the sample, *Ab* is the absorbance of blank, and *W* represents the oil weight.

Thiobarbituric Acid-reactive Substance (TBARS) value

The measurement of thiobarbituric acid-reactive substance (TBARS) was performed using the colorimetric method. For this purpose, 0.2 gram of the extracted lipid was completely dissolved in 25 milliliters of butanol. Following that, five milliliters of the solution and five milliliters of the TBARS reagent (200 mg of TBARS powder in 100 ml of butanol solvent completely dissolved) were sealed in test tubes, completely vortexed and placed in a water bath (WD-23B, Hyse, Korea) at the temperature of 95°C for 120 minutes, and cooled at an ambient temperature. Afterwards, the absorbance of the samples was read using a spectrophotometer (7305-Jenway, UK) at the wavelength of 532 nanometers and compared to the controls (butanol and TBARS). The TBARS value (mg of MDA kg^{-1} of fish fillet) was calculated on days zero, four, eight, 12, and 16 using the following formula [32]:

$$\text{TBARS value} = (As\text{-}Ab) \times 0.25$$

where *As* shows the absorbance of the sample, and *Ab* is the absorbance of blank.

Measurement of the free fatty Acid content

For this purpose, the oleic acid content was initially measured, followed by the measurement of the free fatty acid content. First, 0.2 gram of the extracted fat was dissolved in 50 milliliters of the solvent (mixture containing equal volumes of 96% ethanol and diethyl ether). At the next stage, 1-2 drops of phenolphthalein (indicator) were added to the solution, and the samples were titrated with 0.1 N sodium hydroxide using the behr Labor-Technique. When the color of the samples turned pink and persisted for about 30 seconds, titration was discontinued; this method was applied on days zero, four, eight, 12, and 16. Following that, the acid content was calculated using the following formula [36]:

$$\text{Acid value} = (56.1\times \text{normality of solution} \times \text{volume of sodium hydroxide used}) / (\text{volume/weight of fat/oil sample used})$$

Finally, the amount of free fatty acids was determined using the following formula:

$$\text{Free fatty acid content (\%)} = \text{acid value} \times 1/2$$

Identification of free fatty acids

To identify the free fatty acids on days zero, four, eight, 12, and 16, the fatty acids of the extracted oil were converted into fatty acid methyl esters by methylation. After dissolving the fatty acids in a hexane solution, they were injected into the gas chromatography (CG) column, and the mean values were obtained from the replications and reported as weight percentages. Briefly, GC analysis was carried out in a gas chromatograph (450-VARIAN, USA),

equipped with a Ph-5 column (30 m × 0.1 mm; film thickness: 0.25 µm). Oven temperature was set at 60ºC for five minutes and increased to 210ºC at the rate of 3ºC min^{-1}, and with the rate of 20ºC min^{-1}, reached 240ºC and was maintained for 8.5 minutes. In addition, the temperature of the injector and detector [37] was set at 280ºC. Helium (purity: 99.999%) was also used as the carrier gas with the linear velocity of 31.5 cm s^{-1}, split ratio of 1:60, and ionization energy of 70 eV [38].

Sensory analysis

The sensory analysis of the taste, color, odor, texture, and overall acceptance of different treatments was performed using a nine-point hedonic scale on days zero, four, eight, 12, and 16. On each day, the samples were completely cooked, and 21 semi-trained examiners conducted the test. To this end, the necessary training was provided, and the examiners were asked to taste and rate each sample on the acceptability of four attributes (i.e., color, smell, texture, and overall acceptability) based on a nine-point scale (Extremely Dislike=1, Extremely Like=9). In addition, qualitative rating was determined based on the information in Table 2 [32].

Statistical analysis

Data analysis was performed in SPSS version 21, and the mean values of the chemical parameters during the 16-day period were compared between different groups using one-way analysis of variance (ANOVA). The pairwise comparison of the groups was also carried out using the Bonferroni post-hoc test. Moreover, the sensory parameters of the treatments were compared using the Kruskal-Wallis test, and the pairwise comparison of the experimental groups with the control group was performed using the Mann-Whitney U test and the Bonferroni correction. Considering the multiplicity of the pairwise comparisons, the P-value of less than 0.01 was considered significant in all the statistical analyses.

RESULTS AND DISCUSSION

Several studies have reported the use of essential oils as an antioxidant to increase the shelf life and delay the oxidation of fats [39]. Reduction of lipid oxidation could significantly prolong the shelf life of fish and affect the rancidity, color, odor, and taste of fish due to the decreased formation of free radicals. Furthermore, using 250 and 500 ppm of thyme essential oil in cobia fillet (perciform marine fish) kept at freezing temperatures has been reported to delay the oxidative spoilage of fats [40]. In the present study, the antioxidant properties of thyme essential oil indicated protective effects against fat oxidation in various treatments.

TVB-N

TVB-N is a product derived from bacterial growth and endogenous enzyme activities, and its content is considered an indicator of fish spoilage [41]. According to the information in Table 2, the range of 14.0 mg/100 grams of TVB-N was observed in the raw fillets at baseline (day 0). Studies have shown that the normal range of TVB-N concentration is 5-20 mg $100g^{-1}$ of fresh fish fillet [42].

In the present study, the amount of TVB-N in all the treatments was similar on the first day, while it increased until day 16. This is consistent with the previous findings in this regard, indicating the increased index of TVB-N during refrigerated storage [43]. The highest and lowest values were observed in the control samples (53.50±3.40 mg $100g^{-1}$) and the essential oil groups (1% nano-emulsion; 25.80±4.30 mg $100g^{-1}$), respectively, which implied the double protective effects of the nano-emulsion against endogenous enzyme activities [42].

Despite signs of corruption, the sensory and microbial findings indicated that TVB-N had an acceptable level in all the groups. This is consistent with the previous studies reporting that rainbow trout had lower TVB-N levels than the maximum limits on day 16 of storage at the temperature of 4°C [44]. Since TVB-N is mainly produced due to the bacterial decomposition of fish meat, the production of volatile nitrogen decreased due to the lower growth of microorganisms in the treatments containing the essential

oil. In 2017, some studies investigated the effects of sunflower oil with various concentrations of the nano-emulsion form of ZMEO (0.5% and 1%) on the physical, chemical, and sensory properties of rainbow trout fillets during storage at the temperature of 4±1°C. The obtained results showed that antioxidant effects increased with the higher concentration of the essential oil, which is consistent with the results of the present study [42].

Table 2. Changes in mean± SD of TVB-N (TVB-N, mg N 100 g^{-1} tissue) of trout fillets treatments during 16 days of storage at 4±1°C.

Samples / Days	0	4	8	12	16
CO	11.90 ± 1.40 ^{a,A}	14.70±1.40 ^{a,c,A,B}	21.70±1.40 ^{a,A,C}	29.70±2.40 $^{a,b\ ,B,C}$	53.50±3.40 ^{a,C}
ALG	14.7±1.40 ^{b,A}	17.50±1.40 ^{b,A,B}	18.90±2.68 $^{a,b\ A,B}$	38.50±7.00 ^{b,B,C}	53.20±3.23 $^{a\ ,C}$
E 0.25%	14.00±0.00 ^{b,A}	16.80±0.00 ^{b,A,B}	18.90±1.40 ^{a,b,A,C}	23.80±4.85 $^{a,b,c\ ,B,C}$	42.50±15.33 ^{a,b,C}
E 0.5%	14.00±0.00 ^{b,A}	15.40±1.62 ^{a,b,c,A}	18.20±1.62 $^{b\ ,A,B}$	22.40±4.96 $^{a,c,\ B}$	33.60±0.00 ^{a,b,B}
E 1%	14.00±0.00 ^{b,A}	16.73±1.15 ^{a,b,A,B}	18.90±1.40 $^{a,b\ A,C}$	21.70±4.20 $^{a,c,\ B,C}$	30.80±7.58 $^{b,\ ,C}$
N 0.25%	14.00±0.00 ^{b,A}	14.70±1.40 ^{a,c,A}	16.50±0.00 ^{b,A,B}	21.00±4.85 ^{c,B}	28.00±0.00 ^{b,B}
N 0.5%	14.00±0.00 ^{b,A}	14.70±1.40 ^{a,c,A}	16.80±0.00 ^{b,A,B}	18.90±1.40 ^{c,B}	28.00±0.00 ^{b,B}
N 1%	14.00±0.00 ^{b,A}	14.00±0.00 ^{c,A}	16.80±0.00 ^{b,A,B}	18.20±1.62 ^{c,B}	25.80±4.30 ^{b,B}

Same uppercase and lowercase letters indicate no significant differences within a row and column, respectively ($p > 0.05$).

pH

According to the information in Table 3, the initial pH of the fillets was 6.00±41.01 in all the treatments, which is consistent with the previous findings in this regard [45, 46]. During the storage period, the pH trend increased, which is also in line with a study regarding the effects of oregano essential oil on salmon [47]. At the end of the storage period, the highest pH was observed in the control group (6.76±0.05), while the nano-emulsion treatments had the lowest pH value (6.46±0.04). In all the treatments, the pH value was lower than the recommended maximum pH of fish (6.8-7) [48]. In addition to the production of volatile compounds in the control group, the primary products of fat oxidation (e.g., hydroperoxides and aldehydes with alkaline properties) were produced and increased the pH of the product [49]. The lower pH of the emulsion gel and nano-emulsion treatments during 16 days of storage could be attributed to factors such as the antimicrobial and antioxidant properties of the essential oil [50].

Table 3. Changes in the pH of the trout fillet treatments during 16 days of storage at 4±1°C (mean± SD).

Samples / Days	0	4	8	12	16
CO	6.41±0.01 ^{a,A}	6.46±0.02 ^{a,A,B}	^{a,A,C} 6.51±0.04	6.69±0.03 $^{a\ ,B,C}$	^{a,C} 6.76±0.05
ALG	6.41±0.01 ^{a,A}	^{a,A,B} 6.42±0.01	^{a,b,A,B} 6.44±0.02	^{a,b,B,} 6.62±0.04	6.67±0.03 ^{a,b,B}
E 0.25%	6.41±0.01 ^{a,A,B}	^{a,A,} 6.41±0.01	6.44±0.02 ^{a,c,A,C}	6.48±0.03 $^{a\ ,B,C}$	6.64±0.04 ^{a,c,C}
E 0.5%	6.41±0.01 ^{a,A}	6.41±0.01 ^{a,A}	6.44±0.01 ^{b,c,A,B}	6.44±0.02 ^{b,c,A,B}	6.63±0.04 ^{a,d,B}
E 1%	6.41±0.01 ^{a,A}	6.41±0.01 ^{a,A}	6.41±0.01 ^{b,c,A}	6.44±0.02 ^{c,A,B}	6.49±0.03 ^{b,c,d,B}
N 0.25%	6.41±0.01 ^{a,A}	6.41±0.01 ^{a,A}	6.41±0.01 ^{b,c,A,B}	6.44±0.02 ^{c,A,B}	6.47±0.05 ^{c,d,B}
N 0.5%	6.41±0.01 ^{a,A}	6.41±0.01 ^{a,A}	6.41±0.01 ^{c,A}	6.44±0.02 ^{c,A,B}	6.47±0.04 $^{d,\ B}$
N 1%	6.41±0.01 ^{a,A}	6.41±0.01 ^{a,A}	6.41±0.01 ^{c,A}	6.43±0.02 ^{c,A,B}	6.46±0.04 ^{d,B}

Same uppercase and lowercase letters indicate no significant differences within a row and column, respectively ($p > 0.05$).

PV

Hydro peroxides are the primary products of auto-oxidation, which do not contribute to the formation of flavor and oxidative degradation. In the present study, the amount of peroxide was low at the initial stages; this is known as the slow oxidation period and is affected by cellular compounds as oxidation inhibitors. These compounds have a limited lifetime and are ultimately oxidized, which led to the rapid increase of the peroxide value in our samples [51]. Table 4 shows the changes in the peroxide value (meq kg $^{-1}$) of the trout fillet treatments during 16 days of storage at the temperature of 4±1°C.

According to the current research, PV increased during the storage period, and the increase was more significant in the control samples compared to the treatments. In the alginate coating, emulsion gel, and nano-emulsion groups, increased PV was less significant due to the antioxidant properties of ZMEO and the protective effects of the alginate against oxidation [52]. Among different treatments with various compositions of coating, the alginate coating prepared by the combination of ZMEO nano-emulsion proved to be more effective in delaying lipid oxidation. According to a study regarding the effects of alginate coating containing thyme essential oil, treatment samples had lower PV compared to the controls [53].

Table 4. Changes in mean± SD of Peroxide Value (meq kg^{-1}) of trout fillets treatments during 16 days of storage at 4±1°C.

Samples	Days				
	0	**4**	**8**	**12**	**16**
CO	2.03±0.09 ^{a,A}	2.27±0.45 ^{a,A}	3.78±1.49 ^{a,A,B}	4.55±0.04 ^{a,A,B}	5.76±0.15 ^{a,B}
ALG	2.03±0.19 ^{a,A}	2.31±0.16 ^{a,A,C}	3.65±1.32 ^{a,A,D}	4.28±0.02 ^{a,B,C,D}	5.82±0.13 ^{a,B,D}
E 0.25%	1.63±0.09 ^{a,c,A}	2.26±0.11 ^{a,d,A,C}	2.46±0.21 ^{a,d,A}	4.60±0.83 ^{a,d,B,C,D}	5.77±0.17 ^{a,B,D}
E 0.5%	0.93±0.13 ^{a,c,A}	1.28±0.13 ^{a,c,A,C}	1.69±0.06 ^{a,c,A,D}	2.73±0.08 ^{a,c,B,C,D}	5.61±0.02 ^{a,c,B,D}
E 1%	0.89±0.17 ^{b,c,A}	1.28±0.07 ^{a,c,A,C}	1.92±0.06 ^{a,c,A,D}	2.33±0.17 ^{a,c,B,C,D}	4.31±0.83 ^{a,c,B,D}
N 0.25%	0.87±0.17 ^{b,c,A}	1.20±0.15 ^{b,c,d,A,C}	1.63±0.21 ^{b,c,d,B,C}	1.51±0.21 ^{b,c,d,A,B,C}	2.38±0.37 ^{b,c,B}
N 0.5%	0.84±0.07 $^{b,c,\ A}$	1.13±0.24 ^{b,c,A,C}	1.31±0.18 ^{b,c,A,D}	1.37±0.15 ^{b,c,B,C,D}	1.87±0.92 ^{b,c,B,D}
N 1%	0.81±0.06 ^{b,c,A}	1.10±0.18 ^{b,c,A,C}	1.21±0.8 ^{b,c,A,D}	1.31±0.06 ^{b,c,B,C,D}	1.87±0.04 ^{b,c,B,D}

Same uppercase and lowercase letters indicate no significant differences within a row and column, respectively ($p > 0.05$).

In the current research, the TBARS index was also used to evaluate the fat oxidation rate in the fish samples as an indicator of secondary oxidation products [54, 55]. Notably, TBARS does not represent the true rate of fat oxidation due to the reaction of malondialdehyde with other compounds [56]. In our study, TBARS had an increasing trend in all the samples, which could be associated with the oxidation of fats and production of volatile compounds in the presence of oxygen (Table 5) [57]. On the other hand, the low TBARS value in the alginate group could be attributed to oxygen interference, while the low TBARS in the treatments coated with sodium alginate containing ZMEO may be due to the antioxidant effects of the essential oil and the protective effects of alginate against oxidation. The TBARS values of all the treatments were below the permissible limits of storage (2 mg of MDA kg^{-1}), which is consistent with another research conducted on a European snake [54].

Table 5. Changes in mean± SD of TBARS (mg MDA kg^{-1}) value of trout fillets treatments during 16 days of storage at 4±1°C

Samples/ Days	0	4	8	12	16
CO	0.10±0.01 ^{a,A}	0.11±0.01 ^{a,A}	0.11±0.01 ^{a,A,B}	0.14±0.01 $^{a, A, B}$	0.25±0.06 ^{a,B}
ALG	0.09±0.01 ^{a,c,A}	0.10±0.01 ^{a,e,d,A,C}	0.11±0.01 ^{a,e,A,B}	0.14±0.01 ^{a,e,B,C}	0.17±0.04 ^{a,d,B}
E 0.25%	0.08±0.01 $^{a,c, A}$	0.10±0.01 ^{a,e,c,A}	0.10±0.0 ^{a,d,e,A}	0.13±0.05 ^{a,d,e,A}	0.15±0.04 ^{a,d,A}
E 0.5%	0.08±0.01 ^{a,c,d,A}	0.09±0.01 ^{a,b,A,B}	0.09±0.01 ^{a,c,A,B}	0.10±0.01 $^{a,d,e ,A,B}$	0.11±0.01 $^{a,c,d ,B}$
E 1%	0.08±0.01 ^{a,b,c,A}	0.09±0.01 ^{b,e,A,C}	0.09±0.01 $^{c,e,A, B}$	0.09±0.01 ^{b,c,e,B,C}	0.10±0.01 ^{b,d,B}
N 0.25%	0.08±0.01 ^{a,b,c,A}	0.09±0.01 ^{b,c,d,A}	0.09±0.01 ^{b,c,d,A}	0.09±0.01 ^{b,c,d,A}	0.10±0.01 ^{b,d,A}
N 0.5%	0.07±0.02 ^{b,c,A}	0.09±0.01 ^{b,c,A,C}	0.09±0.01 ^{b,c,A,B}	0.09±0.01 ^{b,c,d,B,C}	0.10±0.01 ^{b,c,B}
N 1%	0.08±0.01 $^{b,d, A}$	0.07±0.01 ^{b,A,C}	0.09±0.01 ^{b,c,A,B}	0.09±0.01 ^{b,c,B,C}	0.09±0.01 ^{b,B}

Same uppercase and lowercase letters indicate no significant differences within a row and column, respectively ($p > 0.05$).

Free Fatty Acids (FFAs)

The formation of free fatty acids (FFAs) is essential to the development of food corruption [58]. This is due to the prooxidant effects of FFAs on lipid substances, the catalytic effects of carboxyl groups, and the formation of free radicals. In the present study (Table 6), changes were not the same in the FFAs of the study groups during the study period. Over time, FFAs increased in all the treatments, while the increase in the control group was more significant compared to the other treatments. On the last day of storage, the highest level of FFAs was observed in the control group (range: 4.69±4.05-21.0±04.01), and the lowest level of FFAs was observed in the treatment containing 1% nano-emulsion (range: 4.21±0.1-5.61±0.01). In addition, the alginate group had the closest level of FFAs to the control samples, which indicated the less significant effect of the coating alone on the levels of FFAs compared to the emulsion gel and nano-emulsion treatments [44]. In a study conducted by Hamza et al. (2011), the effects of magnesium alginate containing ZMEO were investigated, and FFAs were reported to increase in the treatments containing lower concentrations of the essential oil; this is in line with the results of the present study [53].

Table 6. Changes in mean± SD of FFA (%) of trout fillets during 16 days of storage at 4±1°C

Samples/ Days	0	4	8	12	16
CO	4.69±4.05 ^{a,A}	16.34±4.05 ^{a,A,B}	21.04±0.01 $^{a ,B}$	21.0±04.01 ^{a,B}	21.0±04.01 ^{a,B}
ALG	4.69±4.05 ^{a,A}	16.34±4.05 ^{a,A}	18.70±4.05 ^{a,A}	21.0±04.01 ^{a,A}	21.0±04.01 ^{a,A}
E 0.25%	6.08±0.62 ^{a,b,A}	7.48±0.81 ^{a,d,A,B}	7.48±0.81 ^{a,d,A,B}	11.69±04.05 ^{a,b,A,B}	14.0±03.01 ^{a,c,B}
E 0.5%	6.08±0.81 ^{a,b,A}	6.08±1.62 ^{a,c,A}	6.55±0.81 ^{a,c,A}	6.55±0.81 ^{a,b,A}	11.69±04.05 ^{a,b,A}
E 1%	5.14±0.81 ^{a,b,A}	5.61±0.01 ^{a,c,A}	5.61±0.01 ^{b,c,d,A}	6.55±0.81 ^{a,b,A,B}	8.89±0.81 ^{a,b,B}
N 0.25%	4.21±0.01 ^{b,A}	5.14±0.81 ^{b,c,d,A}	5.61±0.01 ^{b,c,d,A,B}	6.88±0.81 $^{b ,A,B}$	8.42±0.40 $^{b,c ,B}$
N 0.5%	4.20±0.11 ^{b,A}	4.21±0.01 ^{b,c,A,B}	5.61±0.01 ^{b,c,d,A,B}	5.61±0.01 $^{b ,A,B}$	6.80±0.81 $^{b, B}$
N 1%	4.20±0.1 $^{b, ,A,B}$	3.74±0.81 ^{b,c,A}	4.68±0.81 ^{b,c,A,B}	5.61±0.01 ^{b,c,B}	5.61±0.01 $^{b ,B}$

Same uppercase and lowercase letters indicate no significant differences within a row and column, respectively ($p > 0.05$).

Fatty acid profile of the treatments

According to our findings, the fat content and changes in the fatty acids of the fish samples differed significantly between and within the species. The composition of the fatty acids of fish could also be affected by factors such as size, age, species, environmental factors (e.g., temperature), salt content, and seasonal changes in the living environment. In the present study, 19 fatty acids were detected, and monounsaturated fatty acids, polyunsaturated fatty acids (PUFAs), and saturated fatty acids (SFAs) were analyzed; notably, the chromatograms are not available (Table 7).

According to another study, increased oxidation in rainbow trout during storage is due to the reduction of antioxidant levels in the fish meat tissues. PUFAs are oxidized significantly easier than SFAs [59]; therefore, the food products containing higher levels of omega-3 fatty acid are more prone to oxidation. In a study, the oxidation of fatty acids was observed to decrease in the treatments containing ZMEO due to antioxidant properties [53]. In addition, increased unsaturated fatty acids and SFAs in the emulsion gel (0.25%, 0.5%, and 1%) and nano-emulsion treatments (0.25%, 0.5%, and 1%) were less significant compared to the control and alginate groups. On the other hand, decreased omega-3 and omega-6 were less significant in the emulsion gel (0.25%, 0.5%, and 1%) and nano-emulsion treatments (0.25%, 0.5%, and 1%) compared to the control group.

Table 7. Changes in fatty acids (mean± SD) of trout fillets during 16 days of storage at (4±1°C).

	Samples	Day0	Day16
MUFA	CO	$26.99\pm1.98^{a,B}$	$40.88\pm0.86^{a,B}$
	ALG	$28.70\pm1.39^{a,B}$	$38.10\pm3.50^{a,B}$
	E 0.25%	$31.14\pm1.80^{b,B}$	$35.21\pm1.16^{b,B}$
	E 0.5%	$29.68\pm0.16^{b,B}$	$39.54\pm0.39^{b,B}$
	E 1%	$29.89\pm0.16^{b,B}$	$33.32\pm0.62^{b,B}$
	N 0.25%	$30.46\pm1.06^{b,B}$	$32.66\pm0.30^{b,B}$
	N 0.5%	$30.61\pm0.40^{b,B}$	$31.65\pm0.69^{b,B}$
	N 1%	$30.61\pm0.40^{b,B}$	$31.65\pm0.59^{b,B}$
PUFA	CO	$40.67\pm2.62^{a,B}$	$32.72\pm0.01^{a,B}$
	ALG	$40.68\pm0.58^{a,c,B}$	$32.74\pm0.76^{a,c,B}$
	E 0.25%	$42.30\pm1.33^{c,d,B}$	$37.85\pm0.56^{c,d,B,a}$
	E 0.5%	$42.61\pm2.31^{c,d,B}$	$39.18\pm0.33^{c,d,B,a}$
	E 1%	$41.80\pm0.12^{c,d,B}$	$35.84\pm2.55^{c,d,B,a}$
	N 0.25%	$41.08\pm1.45^{c,d,B}$	$38.06\pm0.64^{c,d,B}$
	N 0.5%	$40.85\pm0.69^{b,d,B}$	$38.73\pm0.14^{b,d,B}$
	N 1%	$40.85\pm0.69^{b,d,B}$	$38.73\pm0.14^{b,d,B}$
SFA	CO	$22.61\pm1.32^{a,B}$	$25.10\pm0.27^{a,B}$
	ALG	$21.79\pm0.66^{a,B}$	$25.89\pm0.19^{a,B}$
	E 0.25%	$21.01\pm1.03^{b,B}$	$23.56\pm0.39^{b,B}$
	E 0.5%	$22.91\pm0.77^{b,B}$	$23.42\pm0.73^{b,B}$
	E 1%	$22.28\pm0.15^{b,B}$	$22.71\pm0.49^{b,B}$
	N 0.25%	$21.54\pm0.37^{b,B}$	$22.44\pm0.32^{b,B}$
	N 0.5%	$21.37\pm0.30^{b,B}$	$21.62\pm0.16^{b,B}$
	N 1%	$21.37\pm0.30^{b,B}$	$21.62\pm0.16^{b,B}$
W3	CO	$11.82\pm0.45^{a,B}$	$6.78\pm0.34^{a,B}$
	ALG	$10.37\pm1.38^{a,c,B}$	$7.08\pm0.45^{a,c,B}$
	E 0.25%	$11.41\pm0.10^{b,B}$	$9.96\pm0.25^{b,B}$
	E 0.5%	$10.84\pm0.45^{b,B}$	$9.81\pm0.27^{b,B}$
	E 1%	$10.62\pm0.53^{b,c,B}$	$8.26\pm0.91^{b,c,B}$
	N 0.25%	$11.25\pm0.44^{b,B}$	$10.43\pm0.34^{b,B}$
	N 0.5%	$11.35\pm0.72^{b,B}$	$10.84\pm0.21^{b,B}$
	N 1%	$11.35\pm0.72^{b,B}$	$10.84\pm0.12^{b,B}$
W6	CO	$23.85\pm1.67^{c,d,B}$	$21.69\pm0.46^{c,d,B}$
	ALG	$24.92\pm1.60^{c,a,B}$	$21.33\pm0.46^{a,c,B}$
	E 0.25%	$24.93\pm0.80^{c,d,B}$	$22.38\pm0.36^{c,d,B}$

E 0.5%	25.18±1.29^{c,d,B}	24.40±0.63^{c,d,B}
E 1%	24.69±0.83^{c,d,B}	21.63±0.44^{c,d,B}
N 0.25%	24.76±0.38^{c,d,B}	23.82±0.58^{c,d,B}
N 0.5%	24.55±0.41^{b,d,B}	23.98±0.42^{b,d,B}
N 1%	24.55±0.41^{b,d,B}	23.98±0.42^{b,d,B}

Same uppercase and lowercase letters indicate no significant differences within a row and column, respectively ($p > 0.05$).

Sensory analysis

During the storage of the fish fillet samples, bacterial growth and chemical changes producing volatile compounds may affect sensory properties. Table 8 shows the results of the sensory analysis of the trout fillets during refrigerated storage. Accordingly, the color index in all the treatments was higher than the control group, which is consistent with another study regarding the effects of thyme oil on the storage of salmon fillets [44]. Furthermore, the clarity of color in the alginate group compared to the control samples could be attributed to the presence of hydrophilic molecules in the sodium alginate structure, which forms a viscous solution with a clear gel and a pale-colored gel. Sodium alginate could also preserve the color and increase the shelf life of the product compared to the control samples [60].

In the present study, the texture of the samples was acceptable in all the treatments on day zero, while it significantly decreased after 16 days (P<0.05). The texture analysis of different treatments showed the highest score in the nano-emulsion treatments, indicating the positive effect of the ZMEO nano-emulsion coating on texture, which resulted in a higher sensory score compared to the control treatment. Our findings were compared to the results of a study regarding the effects of thyme oil in the nano-emulsion form on salmon [42], and consistency was observed.

According to the results of the present study, the odor index had the lowest value in the control samples compared to the other treatments. This is in line with the results of a study regarding the effects of thyme oil on the sensory properties of fish fillets [61]. On the other hand, the flavor index had the highest value in the nano-emulsion treatments due to the high concentration of the essential oil and its impact on the taste of the samples. Notably, the flavor test in our study was not performed on days four, eight, and 12 of storage due to the possibility of corruption. Our findings in this regard are consistent with another study investigating the effects of thyme on the flavor score [44]. Accordingly, the flavor index had a high score in the treatments containing the nano-emulsion of essential oils [42].

In the current research, the overall acceptance rate of the fillets in the nano-emulsion treatments was significantly higher than the emulsion gel groups (P<0.05). Therefore, it could be concluded that the nano-emulsion of the ZMEO and its combination with the alginate coating increased the sensory evaluation scores of these treatments compared to the controls. However, adding low concentrations of the essential oil caused no significant difference with the other treatments in this regard and only increased the sensory scores compared to the control samples. A remarkable finding of the present study is that the rate of changes was lower in the essential oil treatments, and the sensory evaluation scores were also better during the storage period.

Table 8. Sensory evaluation scoring system based on the nine-point hedonic scale.

		Days				
	Samples	**0**	**4**	**8**	**12**	**16**
Color	CO	8.53±0.52 ^{a,A}	8.48±0.51 ^{a,A}	8.48±0.51 $^{a, A}$	8.38±0.87 ^{a,A}	7.33±0.86 ^{a,B}
	ALG	8.62±0.50 ^{a,c,A}	8.52±0.51 ^{a,A}	8.43±0.51 ^{a,A}	8.24±0.44 ^{a,A}	7.48±0.75 ^{a,B}
	E 0.25%	8.62±0.50 ^{a,c,A}	8.57±0.48 ^{a,A}	8.53±0.51 ^{a,A}	8.48±0.51 ^{a,A}	7.53±0.51 $^{a ,B}$
	E 0.5%	8.76±0.44 ^{a,b,A}	8.67±0.48 ^{a,A}	8.67±0.48 ^{a,A}	8.48±0.51 ^{a,A}	7.62±0.50 $^{a ,B}$
	E 1%	8.81±0.40 ^{b,c,A}	8.71±0.46 ^{a,A}	8.71±0.46 ^{a,A}	8.43±0.51 ^{a,A}	7.67±0.48 ^{a,B}
	N 0.25%	8.91±0.30 ^{b,A}	8.71±0.46 ^{a,A}	8.71±0.46 ^{a,A}	8.48±0.51 $^{a ,A}$	7.76±0.44 ^{a,B}
	N 0.5%	8.91±0.30 ^{b,A}	8.71±0.46 ^{a,A}	8.71±0.46 ^{a,A}	8.52±0.51 $^{a, A}$	7.81±0.40 $^{a, B}$
	N 1%	8.95±0.22 ^{b,A}	8.71±0.46 ^{a,A}	8.71±0.46 ^{a,A}	8.67±0.48 ^{a,A}	7.81±0.40 $^{a ,B}$
Texture	CO	8.43±0.01 ^{a,A}	8.24±0.83 ^{a,A}	7.41±0.79 $^{a, C}$	5.86±1.11 ^{a,B}	5.14±0.73 ^{a,B}
	ALG	8.33±0.48 ^{a,c,A}	8.29±0.78 ^{a,A}	8.10±0.89 ^{b,c,A}	5.91±0.77 ^{a,B}	5.19±0.87 ^{a,B}
	E 0.25%	8.75±0.44 ^{d,A}	8.38±0.84 ^{a,A}	7.57±0.81 $^{a,c ,e,C}$	5.95±0.81 ^{a,B}	5.71±0.85 $^{a, B}$
	E 0.5%	8.62±0.50 ^{d,A}	8.43±0.68 ^{a,A}	7.38±1.12 $^{a,d, C}$	6.05±0.73 $^{a, B}$	5.76±0.77 ^{a,B}
	E 1%	8.86±0.26^{a,b,d,A}	8.52±0.68 ^{a,A,D}	7.57±0.93 $^{a,c ,C,D}$	6.05±0.87 ^{a,B}	5.90±1.04 $^{a, B}$
	N 0.25%	9.00±0.00 ^{b,A}	8.57±0.51 ^{a,A,D}	7.81±1.03^{b,d,e,C,D}	6.10±0.94 $^{a, B}$	5.95±1.26 $^{a ,B}$
	N 0.5%	9.00±0.00 ^{b,A}	8.62±0.50 ^{a,A,D}	8.10±0.89 ^{b,C,D}	6.14±0.66 $^{a ,B}$	5.95±1.26 $^{a, B}$
	N 1%	9.00±0.00 ^{b,A}	8.76±0.44 ^{a,A}	8.29±0.85 ^{b,A}	6.14±0.66 ^{a,c,B}	5.95±1.32 $^{a ,B}$
Odor	CO	7.76±0.99 ^{a,A}	1.00±14.36^{a,c,B}	1.00±0.00 $^{a ,B}$	1.00±0.00 $^{a ,B}$	1.00±0.00 $^{a ,B}$
	ALG	7.14±1.01 ^{a,A}	1.19±0.40 ^{c,e,B}	1.00±0.00 $^{a ,B}$	1.00±0.00 $^{a ,B}$	1.00±0.00 $^{a ,B}$
	E 0.25%	7.57±1.16 ^{b,A}	1.67±0.48 $^{c ,e,B}$	1.00±0.00 $^{a ,C}$	1.00±0.00 $^{a ,C}$	1.00±0.00 $^{a ,C}$
	E 0.5%	8.24±0.77 ^{b,A}	1.71±0.46^{a,d,e,B}	1.00±0.00 $^{a ,C}$	1.00±0.00 $^{a ,C}$	1.00±0.00 $^{a ,C}$
	E 1%	8.32±0.73 ^{b,A}	1.81±0.41 ^{a,B}	1.00±0.00 $^{a ,C}$	1.00±0.00 $^{a ,C}$	1.00±0.00 $^{a ,C}$
	N 0.25%	8.76±0.44 ^{b,A}	1.86±0.36 ^{b,d,B}	1.00±0.00 $^{a ,C}$	1.00±0.00 $^{a ,C}$	1.00±0.00 $^{a ,C}$
	N 0.5%	8.62±0.50 ^{b,A}	1.86±0.36 ^{b,B}	1.00±0.00 $^{a ,C}$	1.00±0.00 $^{a ,C}$	1.00±0.00 $^{a ,C}$
	N 1%	8.81±0.40 ^{b,A}	1.90±0.30 ^{b,B}	1.00±0.00 $^{a ,C}$	1.00±0.00 $^{a ,C}$	1.00±0.00 ^{a,c}
Overall acceptance	CO	8.53±0.52 ^{a,A}	7.91±0.63 $^{a, B,C}$	8.33±0.73 $^{a ,A,C}$	7.29±0.46 ^{a,C}	6.29±0.78 ^{a,D}
	ALG	8.48±0.51 ^{a,A}	7.62±0.81 ^{a,B}	8.33±0.48 ^{a,A}	7.48±0.51 ^{a,c,C}	6.33±0.80 ^{a,D}
	E 0.25%	8.62±0.50 ^{a,c,A}	7.67±0.80 $^{a, B}$	8.38±0.74 ^{a,A}	7.62±0.59 ^{c,d,B,C}	6.48±0.51 ^{a,D}
	E 0.5%	8.62±0.50 ^{a,c,A}	7.71±0.85 ^{a,B}	8.38±0.50 ^{a,A}	7.67±0.48 ^{b,c,d,B,C}	6.52±0.51 ^{a,D}
	E 1%	8.76±0.44 ^{b,c,A}	7.76±0.77 ^{a,B}	8.38±1.07 ^{a,A}	7.71±0.46 ^{b,c,d,B,C}	6.57±0.51 ^{a,D}
	N 0.25%	8.86±0.36 ^{b,A}	7.81±0.75 ^{a,B}	8.67±0.48 ^{a,A}	7.76±0.44 $^{b, c,d,B,C}$	6.62±0.50 ^{a,D}
	N 0.5%	8.91±0.30 ^{b,A}	7.86±0.91 ^{a,B}	8.71±0.46 ^{a,A}	7.81±0.40 $^{b ,B,C}$	6.67±0.48 ^{a,D}
	N 1%	8.95±0.22 $^{b, ,A}$	7.91±0.83 ^{a,B}	8.76±0.44 ^{a,A}	8.00±0.01 ^{b,B,C}	6.71±0.46 ^{a,D}

Same uppercase and lowercase letters indicate no significant differences within a row and column, respectively ($p > 0.05$).

CONCLUSIONS

According to the results, the use of alginate coatings containing ZMEO in the form of nano-emulsion compared to emulsion gel at the same concentrations could enhance the antioxidant effects on the fish fillet stored in refrigerator conditions through decreasing oxidative stress indices. Furthermore, the nano-emulsion of ZMEO increased the shelf life of the fish fillets for four days and positively affected their sensory properties. Given the high

production rate of this cold-water fish in Iran, using the nano-emulsion form of ZMEO in coatings as a natural preservative could enhance the meat quality and extend the storage period of fish fillets.

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Conflicts of interest

None declared.

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