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Original Article

Studying Effects of Quince Seed Mucilage Film Strengthened with Tragacanth Gum Containing *Cinnamon* Essential Oil on Microbial, Chemical and Sensory Characteristics of *Rainbow Trout* Fillets

Mohammad Sadegh Mohammadi Resketi[1], Hamidreza Kazemeini[*2]

1- Master's Student in Food Hygiene and Quality Control, Amol University of Special Modern Technologies, Mazandaran, Iran.

2- Assistant Professor, Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Mazandaran, Iran

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ABSTRACT

Background and Objectives: The aim of this study was to investigate the effect of quince seed mucilage film strengthened with tragacanth gum containing cinnamon essential oil on microbial, chemical and sensory characteristics of rainbow trout fillets for 9 d during storage at refrigerator temperature.

Materials and Methods: First, the film and treatments were prepared (control treatment, quince seed mucilage film strengthened with tragacanth gum: film, quince seed mucilage film strengthened with tragacanth gum containing 1% of cinnamon essential oil: film and cinnamon essential oil). Moreover, cinnamon essential oil was analyzed using gas chromatography/mass spectrometry and its antibacterial activity was assessed (MIC and MBC). The prepared treatments were subjected to microbial assessments (total viable counts and total psychrotrophs counts), chemical assessments (pH, PV, TVN and TBARS) and sensory evaluation.

Results: The major composition in cinnamon essential oil was cinnamaldehyde (59.97%). At the end of the storage time, total viable counts and total psychrotrophs counts for the film and cinnamon essential oil treatments were 5.31 and 5.55 log cfu/g, respectively. Quantities of pH, PV, TVN and TBARS for film and cinnamon essential oil treatment were 6.38, 2.99 mEq/kg, 26.09 mg/100 g, 1.39 mg MDA/kg, respectively. In all the microbial, chemical and sensory assessments, a significant difference was observed between the treatment of film and cinnamon essential oil and the control treatment during the storage time ($P < 0.05$).

Conclusions: Results showed that the film and cinnamon essential oil treatment decreased total viable counts and total psychrotrophs counts and prevented increases of pH, PV, TVN and TBARS; therefore, film containing cinnamon essential oil improved microbial, chemical and sensory characteristics of the rainbow trout fillets and increased their shelf life in the refrigerator.

Keywords: Mucilage, Quince seed, Tragacanth gum, Rainbow

Highlights

- The results showed that E-Cinnamaldehyde (59.97%) has the highest amount identified in this essential oil
- The Peroxide value in Film+CEO treatments from day 0 to the end of the study was significantly ($P<0.05$) lower than the Peroxide value in the control treatment.
- The amount of TBARS in samples on day 0 was 0.39 mg MDA/kg, which reached 2.79, 1.99, 1.39 mg MDA/kg for Control, Film, and Film+CEO treatments on the 9th day respectively.
- In parameters of aroma, texture, greed and overall acceptance, the Film+CEO treatment had better scores than the control group and the Film treatment.

***Address for correspondence:** Hamidreza Kazemeini, Assistant Professor, Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, Amol University of Special Modern Technologies, Mazandaran, Iran. E-mail address: msm1376m97@gmail.com

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Introduction

Fish and seafood in the diets are excellent sources of proteins. These food products include a high quantity of fat-soluble and water-soluble vitamins, omega-3 unsaturated fatty acids (UFA) and minerals (1). Rainbow trout include a high nutritional value and its production worldwide has increased since the 1950s. Based on the the statistical yearbook of the Iranian Fisheries Organization (IFO), production of trout in Iran has increased in recent years (1, 2). Maintaining quality of perishable foods such as fish fillets is essential for long-term storage. Fish and seafood are prone to bacterial growth and fat oxidation due to their high quantities of fatty acids (FA) and proteins. Fresh fish is a highly perishable product due to its nutrients, almost neutral pH, presence of autolytic enzymes and high water activity (aw). Factors of fish spoilage are first microbial growth and invasion followed by autolytic enzymes and chemical reactions such as hydrolysis or oxidation (2, 3). One of the reasons for the necessity of increasing shelf life of food products is that they are sold in areas far from their places of production. Various cold storage methods such as high pressure processing, vacuum packaging and modified atmosphere, radiation, natural substances and films/edible coatings (2) are used to improve the long-term shelf life of fresh fish and prevent microbial and chemical changes and preserve sensory characteristics (2, 3).

One of the novel methods in food packaging to increase the shelf life and preserve food quality is use of biopolymer based edible films and coatings (3). The edible coating is a thin layer of liquid biopolymers that are sprayed or immersed directly on the surface of foods and edible films are made into a separate solid layer and then set between or on the food products during packaging. Since the 20th Century, films and coatings have been used to prevent dehydration of vegetables and fruits. It is characterized by preventing penetration of moisture and gas, flavoring, sweetening, improving the color carrier and antimicrobial and antioxidant characteristics. Therefore, oxidative pickling/browning and microbial spoilage can be prevented and organoleptic characteristics of food products can be improved (4).

Quince from Rosaceae family is a fruit with high nutritional value that includes a positive effect on human health but is one of the most important fruit species that is less commonly used. Several studies have shown that quince jams and byproducts such as peels and seeds are good and inexpensive sources of phenolic acids, antioxidants and flavonoids (5). Early studies on quince seeds showed that quince seed mucilage included a mixture of cellulose and water-soluble polysaccharides. Later studies have shown that the major water soluble polysaccharides in quince seed mucilage are glucuronic acid. Another study has shown that quince seed mucilage hydrolysis include arabinosis, gazillose, galactose and glucose in a ratio of 8, 54, 4 and 34. Quince seed mucilage film in various biodegradable edible films includes good characteristics such as hydrophilicity, inhibitory, antioxidant and mechanical characteristics (5).

Plant gum as a natural polymer is able to form films and coatings that include good inhibitory characteristics against the transmission of gases such as oxygen, carbon dioxide and moisture. Other characteristics include low-costs, availability, biocompatibility, chemical ineffectiveness, non-toxicity water binding capacity, gelling and stabilization of emulsion systems which makes it widely used in various industries (6). Tragacanth gum is made from the skin and branches of *Astragalus leguminosae*. Chemical structure of tragacanth gum includes D-galactose, D-galacturonic acid, D-xylose, L-fucose and L-arabinose. This anionic hydrocloid is acid resistant, containing small quantities of proteins and a high water binding capacity. It acts as a thickener, emulsifier, stabilizer suspension agent in various industries (6).

Plant essential oils are natural substances used in food packaging due to their antimicrobial and antioxidant characteristics (7, 8). The CEO is one of the active natural compounds that includes an antimicrobial effect on a wide range of bacteria. This activity is due to the presence of cinnamaldehyde. By interfering in the biological systems of microorganisms, the compound prevents their growth. In addition, cinnamon contains various other compounds such as coumarin and cinnamic acid, which strengthen the antimicrobial and antioxidant characteristics with antifungal characteristics (9).

Materials and Methods

Preparation of CEO

Cinnamon was purchased from a grocery in the city. The CEO was extracted from cinnamon sticks using Clevenger device and steam distillation method. In each essential extraction, essential oil was prepared from 100 g of plants within 3.5 h (9).

Mucilage preparation

First, quince seeds were soaked with distilled water (DW) for 1 h. Then, this was exposed to microwaves at various times. To achieve as much mucilage as possible, the resulting mixture was set at room temperature (RT) for 1 h and then the resulting mixture was smoothed using cloth net. The achieved mucilage mixture was mixed with twice its volume of 96% ethanol to form a clot and was set at RT for 1 h. The achieved mucilage clots were separated using strainer and transferred to an oven for drying. Drying was carried out at 50 °C for 18–20 h using oven.

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The achieved mucilages were stored separately in a dry cool place for further uses (10).

Preparation of mucilage film

The film solution was prepared by slowly dissolving 1% mucilage and glycerol as a softener at 35% (w/w) based on the weight of seed mucilage under constant stirring at 45 °C ±1 for 15 min. Then, tragacanth gum and CEO (1% v/v) were dissolved in the solution at RT (25 °C) for 1 h using magnetic stirrer. Tween 80 was added as a surfactant in concentrations 0.1–0.2% (w/v). Solution was homogenized at 12000 rpm for 5 min to achieve an emulsion. Emulsion was centrifuged at 3800 rpm for 3 min to remove air bubbles. The film solution was transferred onto glass Petri dishes with Teflon coating (13 cm). The cast pieces were transferred under a fume hood and stored at relative humidity of 37% ±1 and temperature of 25 °C ±1 for 24 h (11).

Preparation of fish fillets and studied treatments

The fish fillet was prepared in one of the sale centers of Amol Bazaar, Amol, Iran. Briefly, 50 g of the fish fillet samples for each treatment were transferred between two films and then transferred into sterile polyethylene bags. Then, various treatments were prepared and stored at 4 °C. Treatment used in this study is shown in Table 1.

Essential oil analysis

Essential oil analysis was carried out using gas chromatography/mass spectrometry (GC/MS). The GC analysis was carried out using Shimadzu GC-14A unit with CR-4AX integrator (Shimadzu, Japan). Moreover, SE30 fused capillary column (25 m × 0.25 mm × 0.25 mm) was used. Carrier gas was nitrogen, flow velocity of gas was 1 ml/min and the splitter included an 1:75 ratio. Oven temperature was set at 110 °C for 3 min and programmed to 220 °C at a rate of 8 °C/min and then isothermal at 220 C for 15 min. Injector temperature was 220 °C, while the detector (FID) temperature was 250 °C. For GC-MS detection, electron ionization system (model 5890/II GC-5971/A MSD, Hewlett-Packard, USA) was used with a Supelcowax 10 column (60 m × 0.25 mm; film thickness, 0.25 mm). Technically, the carrier gas was helium. Mass spectra were recorded at 70 eV. The essential oil was injected without dilution, while SFE samples were diluted in ethanol:hexane (1:1 w/w) solution before the analysis. Identification of the compounds was based on the comparison of their retention times with those of authentic samples (linalool, Fluka, Switzerland; terpinen-4-ol, Roth, Germany) and/or by comparison of their mass spectra with those of data in Wiley and NIST libraries (8, 12).

Antibacterial activity of cinnamon essential oil

A 96-well microplate was used to assess the minimum inhibitory concentration (MIC) of the growth of infection and food poisoning by essential oils. In this method, a mother solution of sterile cinnamon essence with a concentration of 400 mg/ml was first prepared. Consecutive concentrations were prepared from the mother solution. In general, 100 µl of various concentrations of CEO and 10 µl of standard microbial suspension were poured into each of the 96-well microplate wells. Then, lid of the microplate was close and each of the microplates was placed at the appropriate temperature for the growth of microbial strains. After incubation, cloudiness of the house indicated growth of the microbial strain. The first well; in which, turbidity was not observed, was reported as the MIC of microbial growth (12). To assess the minimum bactericidal concentration (MBC), CEO from the well; in which, no color change was observed, was cultured on appropriate culture media for the growth of bacterial strains. After 24 h of incubation for bacterial strains, the first incubation; in which, no colony was observed was considered as the minimum lethal concentration (MLC) of CEO (12).

Total counts

First, 5 g of various parts of the fish meat were weighed under sterile conditions and homogenized with 45 ml of peptone water using Stomacher. Then, serial dilutions were prepared. Pour plate culture was used for plate count agar (Merck, Germany). After cultivation, this was incubated at 37 °C for 48 h. Count of mesophilic aerobic bacteria was expressed as log cfu/g (1).

Table 1. Various treatments in the current study.

Row	Treatments	Description
1	Control	*rainbow trout* fillet packaging without using film and the desired essential oil
2	Film	*rainbow trout* fillet packaging using Quince seed mucilage film
3	Film and CEO	*rainbow trout* fillet packaging using Quince seed mucilage film containing 1% cinnamon essential oil

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Psychrotrophs counts

First, 5 g of various parts of fish meat were weighed under sterile conditions and homogenized with 45 ml of peptone water using Stomacher. Then, serial dilutions were prepared. Pour plate culture was used for plate count agar. After cultivation, this was incubated at 10 °C for 10 d. Psychrophilic bacteria count was expressed as log cfu/g (1).

pH

To assess pH, 5 g of the meat samples were homogenized with 10 ml of DW. Then, pH of the samples was assessed at RT using pH meter (Metrohm, Switzerland) (1).

Peroxide values

First, 150 g of the fish fillets were homogenized with 250 ml of chloroform using filter strainer. After dewatering, the strained solution was heated at 105 °C to assess fat content of the sample. In the peroxide method, 0.3 g of fat sample was mixed with 9.8 (7–3) ml of methanol-chloroform, 0.05 ml of ammonium thiocyanate (10 mM) and 0.05 ml of II iron chloride solution and vortexed. After 5 min of storage at RT, absorbance was read at 500 nm using spectrophotometer (11). After drawing the standard curve, superoxide was calculated as mEq/kg as follows:

$$\frac{(\mathrm{As} - \mathrm{Ab}) \times \mathrm{m} \times \mathrm{mo} \times 2}{55/84}$$

Total volatile nitrogen

Assessment of total volatile nitrogen (TVN) was carried out using Kjeldahl method. Briefly, 10 g of the homogenized sample, 2 g of magnesium oxide and 300 ml of DW were transferred into a laboratory flask and then distilled vapors were collected in a solution of 3% boric acid and methyl red reagent. This was titrated with 5% sulfuric acid and TVN was reported as mg/100 g fish fillets (13).

Thiobarbituric acid reactive substances

In general, 2.5 mg of the sample were poured into a 50-ml balloon; then, 10% trichloroacetic acid solution was added to the volume and mixed for 30 min. This was passed through a red filter and 2 ml of water were dissolved in 2 ml of water for 45 min using heater. This was poured into the test tube and the absorbance was measured at 533 nm to assess the quantity of thiobarbituric acid reactive substances (TBARS) based on mg MDA/kg (13).

Sensory characteristics

Gallas and Contaminase method was used for sensory evaluation. The rainbow trout fillets were assessed by a trained group of nine people during the storage time. Sensory evaluation was carried out for aroma, color, texture and overall acceptability. A scale from 0 to 10 was used in scoring as 10 was the highest score and 0 was the lowest score. A product with a score of less than 6 was reported as an unacceptable product (13).

Statistical analysis

Statistical analysis of data was carried out using SPSS software v.25. First, normality of data was checked using Kolmogorav-Smirnov test and then homogeneity of the variance of data was carried out using Leven test. Repeated measure (ANOVA) was used to compare the average numbers of bacteria between the groups. Moreover, Friedman's statistical test was used for qualitative variables such as sensory evaluation.

Results

Analysis results of the compounds identified in CEO are present in Table 2. Results of the analysis for the chemical composition of CEO prepared by GC/MS led to the identification of 15 chemical compounds with a total rate of 98.01%. Results showed that E-cinnamaldehyde (59.97%) included the highest quantity in this essential oil. Other major components of the essential oil included p-methoxy-cinnamaldehyde (8.22%), α-copaene (5.91%) and Z-cinnamaldehyde (4.25%) as well as other compounds of epi-α-bisabolol, β-calacorene, α-amorphene and benzaldehyde. Furthermore, small quantities of eugenol, cadinol, limonene and other compounds were present.

Antibacterial activity of cinnamon was assessed qualitatively and quantitatively using MIC, MBC values. Results of MIC and MBC of cinnamon essential oil (CEO) are shown in Table 3. The antibacterial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was investigated with MIC of CEO as 4 and 2 mg/ml, respectively, and the MLC of CEO against the two bacteria as 4 mg/ml.

Results of counting aerobic bacteria in treatments of rainbow trout fillets packed with quince seed mucilage film strengthened with tragacanth gum containing CEO during storage at refrigerator temperature for 9 d are reported in Fig. 1. Generally, number of aerobic bacteria in the samples increased during the study. The initial quantity of aerobic bacteria in salmon fillet samples on Day 0 was 3.40 log cfu/g, while Control reached 29.8 log cfu/g on day 9. On Day 9, this number was 25.7 log cfu/g for film treatment and 31.5 log cfu/g for film and CEO treatment. All the treatments of salmon fillets wrapped with antimicrobial film (except that on Day 0) showed a significant difference in the number of aerobic bacteria, compared to the control treatment on all assessed days ($P < 0.05$). The lowest quantity of aerobic bacteria in the

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whole study was linked to film and CEO treatment and the highest quantity of aerobic bacteria was linked to rainbow trout fillet control.

Table 2. Analysis results of the cinnamon essential oil using gas chromatography/mass spectrometry

Number	Compounds	Relative percentage of compounds
1	Eugenol	0.88
2	p-methoxy-Cinnamaldehyde	8.22
3	E-Cinnamaldehyde	59.97
4	Cadinol	0.64
5	Cadina-1,4-diene	0.13
6	Gleenol	0.62
7	Z-Cinnamaldehyde	4.25
8	α-Copaene	5.91
9	epi-α-Bisabolol	2.01
10	Benzaldehyde	3.02
11	β- Calacorene	3.88
12	ß-Oploenone	0.33
13	epi-α-Bisabolol	4.61
14	Limonene	0.32
15	α-Amorphene	3.21
Total	-	98.01

Table 3. Results of the minimum inhibitory concentration and minimum bactericidal concentration of the cinnamon essential oil

Bacteria	MIC(mg/ml)	MBC(mg/ml)
Staphylococcus aureus	4	4
Pseudomonas aeruginosa	2	4

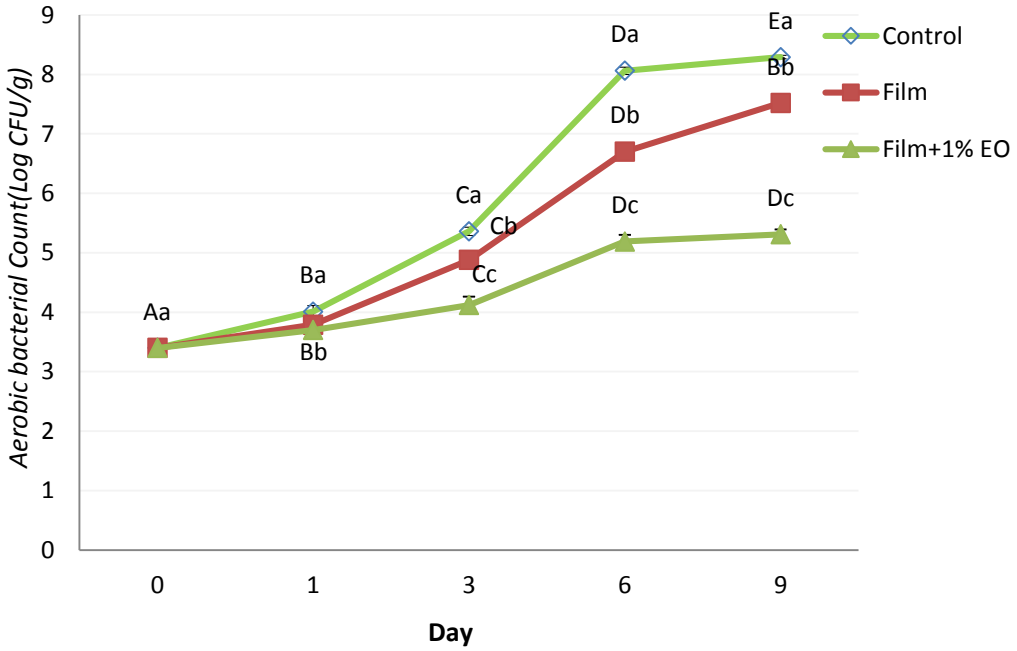

Figure 1. Results of the aerobic bacterial counts (log CFU/g)

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Results of counting psychrophilic bacteria in rainbow trout fillet treatments packed with quince seed mucilage film strengthened with tragacanth gum containing CEO during storage at refrigerator temperature for 9 d are reported in Fig. 2. Number of psychrophilic bacteria in the samples increased during the study. Initial quantity of the psychrophilic bacteria in the samples of rainbow trout fillets (control) on Day 0 was 2.97 log cfu/g, while it reached 8.08 log cfu/g on Day 9. On Day 9, this number was 6.92 log cfu/g for the film treatment and 5.55 log cfu/g for the film and CEO treatment. On Day 1, film and CEO treatment showed a significant difference, compared to the control treatment and film treatment ($P < 0.05$). All treatments of rainbow trout fillets wrapped with antimicrobial films showed a significant difference in the number of psychrophilic bacteria, compared to the control treatment on Days 3, 6 and 9 of the experiment ($P < 0.05$). The lowest quantity of psychrophilic bacteria in the entire study was linked to the treatment of *rainbow trout* fillet packed with Quince seed mucilage film strengthened with tragacanth gum containing CEO and the highest quantity of psychrophilic bacteria was linked to the control rainbow trout fillets.

Results of assessing the effect of quince seed mucilage film strengthened with tragacanth gum containing CEO during 9 d of study at refrigerator temperature on pH of rainbow trout fillet samples are present in Table 4. As seen in the table, this increased in all groups and no significant difference was observed between the treatments on Day 0 ($P < 0.05$). Based on the results, pH of the control increased from 6.21 on Day 0 to 7.01 on the last day of the study. On Day 9, pH of the control treatment included a significant difference ($P > 0.05$) between the control and other treatments. Moreover, pH on Day 9 was 6.73 for film treatment and 6.38 for film and CEO treatment. A significant difference was seen in pH of the salmon samples packed with quince seed mucilage film strengthened with tragacanth gum containing CEO, compared to the control treatment samples on Days 3, 6 and 9 of the study, except for Days 0 and 1 ($P > 0.05$). On Day 1, film and CEO treatment showed a significant difference, compared to the control treatment and film treatment. During the study, the lowest pH difference was linked to rainbow trout fillet treatment packaged with quince seed mucilage film strengthened with tragacanth gum containing CEO and the highest pH difference was linked to control rainbow trout fillet samples.

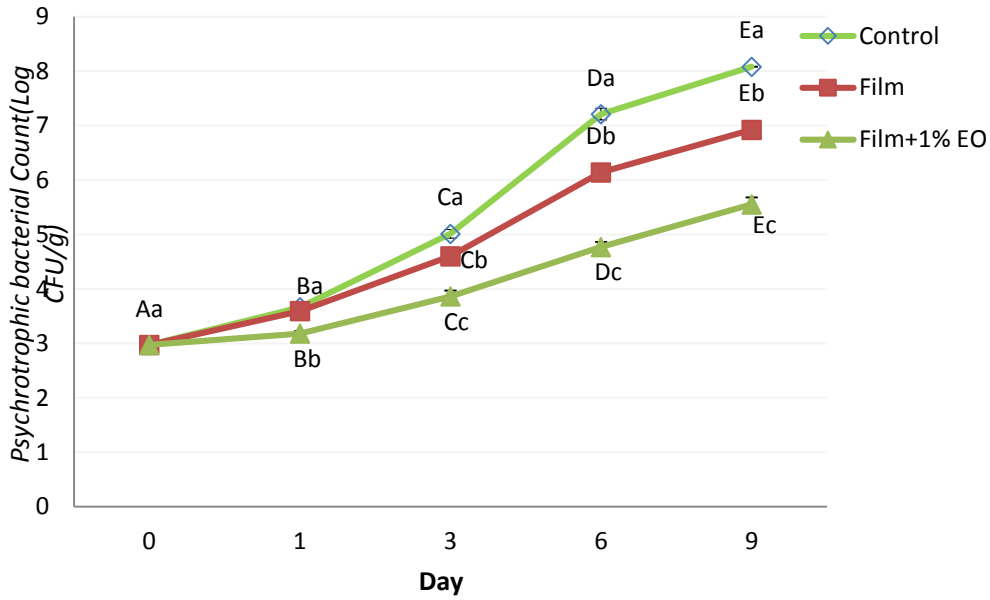

Figure 2. Results of the psychrotrophic bacterial counts (log CFU/g)

Table 4. Average pH changes in various treatments (mean ±SD).

Treatment	Storage time (days)				
	0	1	3	6	9
Control	6.21± 0.01 [a]	6.34± 0.00 [a]	6.70±0.04 [a]	6.81±0.01 [a]	7.01±0.03 [a]
Film	6.21±0.01 [a]	6.33± 0.01 [a]	6.42±0.00 [b]	6.50±0.02 [b]	6.73±0.03 [b]
Film and CEO	6.21±0.01 [a]	6.15± 0.03 [b]	6.19±0.02 [c]	6.30±0.00 [c]	6.38±0.00 [c]

Non-similar lower case English letters indicate significant differences between treatments in the same day ($p<0.05$).

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Results of the assessment of peroxide in rainbow trout fillet treatments packed with quince seed mucilage film strengthened with tragacanth gum containing CEO during storage at refrigerator temperature for 9 d are present in Table 5. Based on the results, a significant difference ($P < 0.05$) was observed in the peroxide value of the control, compared to all other treatments. This increased in all treatments. The peroxide value of control was 0.60 mEq/kg on Day 0 and reached 4.83 mEq/kg on the last day. Results from the comparisons between the control and film and CEO treatment included a significant difference ($P < 0.05$). The peroxide value of film treatment increased to 4.02 mEq/kg on the last day and film and CEO treatment to 2.99 mEq/kg on the same day. The peroxide value in film and CEO treatments from Day 0 to the end of the study was significantly ($P < 0.05$) lower than that in the control treatment. A significant difference was seen in the quantity of peroxide value of rainbow trout samples packed film and CEO treatment on Days 3, 6 and 9 of the study, except for Days 0 and 1 ($P > 0.05$). On Day 1, film and CEO treatment showed a significant difference, compared to the control treatment and film treatment.

Results of assessing volatile basic nitrogen (VBN) substances in treatments of rainbow trout fillets packed with quince seed mucilage film strengthened with tragacanth gum containing CEO during storage at refrigerator temperature for 9 d are reported in Table 6. The VBN content in the samples increased during the study. The initial quantity of VBN substances in rainbow trout fillet samples on Day 0 was 44.9 mg/100 g, which reached 40.51 mg/100 g for the control on Day 9. On Day 9, this number was 36.22 mg/100 g for film treatment and 26.02 mg/100 g for film and CEO treatment. All the treatments of rainbow trout fillets wrapped with antimicrobial films in all days, except Day 0, showed a significant difference in the quantity of VBN substances, compared to the control treatment ($P > 0.05$). The lowest quantity of VBN in the entire study was linked to the treatment of rainbow trout fillet packed with quince seed mucilage film strengthened with tragacanth gum containing CEO and the highest quantity of VBN was linked to the control treatment.

Results of TBARS assessment in treatments of rainbow trout fillets packed with quince seed mucilage film strengthened with tragacanth gum containing CEO during storage at refrigerator temperature for 9 d are reported in Table 7. This increased in the samples during the study. The quantity of TBARS in rainbow trout fillet samples on Day 0 was 0.39 mg MDA/kg, which reached 2.79 mg MDA/kg for the control on Day 9. On Day 9, this number was 1.99 mg MDA/kg for film treatment and 1.39 mg MDA/kg for film and CEO treatment. The lowest quantity of TBARS in the entire study was linked to the treatment of rainbow trout fillets packed with quince seed mucilage film strengthened with tragacanth gum containing CEO and the highest quantity was linked to the control treatment. On Day 1, film and CEO treatment showed a significant difference, compared to the control treatment and film treatment. Rainbow trout fillet samples were stored at refrigerator temperature for sensory evaluation until Day 6. Results of sensory evaluation of various treatments on Day 6 during storage at 4 °C are shown in Fig. 3. On Day 9, samples could not be assessed. Based on the results, no significant difference was seen in sensory characteristics of the samples on Day 0 of the study ($P < 0.05$). The studied characteristics decreased significantly over time in all groups ($P < 0.05$). For aroma, texture, taste and overall acceptance, film and CEO treatment included better scores than those the control group and film treatment did. For the appearance characteristics, control group included the highest score.

Table 5. Average peroxide value changes in various treatments as mEq/kg (mean ±SD).

Treatment	Storage time (days)				
	0	1	3	6	9
Control	0.60±0.01 [a]	0.92±0.01 [a]	1.78±0.02 [a]	2.81±0.01 [a]	4.83±0.02 [a]
Film	0.60±0.01 [a]	0.88±0.03 [a]	1.59±0.03 [b]	2.60±0.01 [b]	4.02±0.04 [b]
Film and CEO	0.60±0.01 [a]	0.73±0.00 [b]	1.11±0.04 [c]	1.85±0.03 [c]	2.99±0.01 [c]

Non-similar lower case English letters indicate significant differences between treatments in the same day ($p<0.05$).

Table 6. Average changes of minimum bactericidal concentration in various treatments as mg/100 g (mean ±SD).

Treatment	Storage time (days)				
	0	1	3	6	9
Control	9.44±0.03 [a]	10.31±0.01 [a]	16.33±0.01 [a]	28.24±0.00 [a]	40.51±0.02 [a]
Film	9.44±0.03 [a]	10.16±0.00 [b]	15.02±0.03 [b]	25.10±0.02 [b]	36.22±0.01 [b]
Film and CEO	9.44±0.03 [a]	9.98±0.02 [c]	12.11±0.04 [c]	18.08±0.03 [c]	26.09±0.03 [c]

Non-similar lower case English letters indicate significant differences between treatments in the same day ($p<0.05$).

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Table 7. Average changes of thiobarbituric acid reactive substances in various treatments as mg MDA/kg (mean ±SD).

Treatment	Storage time (days)				
	0	1	3	6	9
Control	0.39±0.01 [a]	0.89±0.01 [a]	1.51±0.00 [a]	2.02±0.00 [a]	2.79±0.01 [a]
Film	0.39±0.01 [a]	0.88±0.02 [a]	1.32±0.02 [b]	1.76±0.00 [b]	1.99±0.01 [b]
Film and CEO	0.39±0.01 [a]	0.61±0.02 [b]	1.09±0.00 [c]	1.16±0.01 [c]	1.39±0.02 [c]

Non-similar lower case English letters indicate significant differences between treatments in the same day ($p<0.05$).

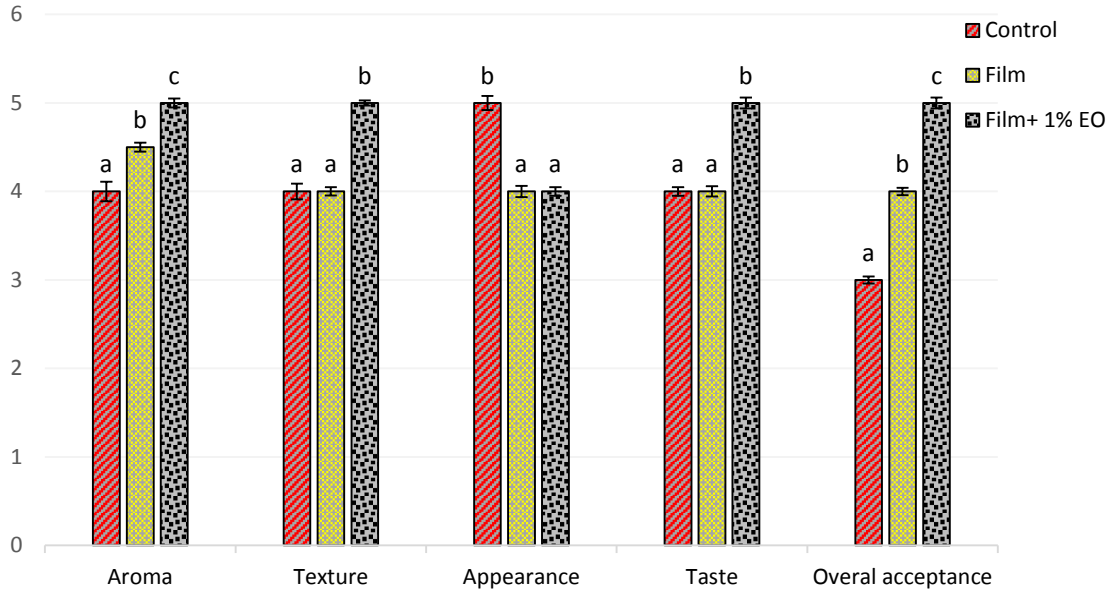

Figure 3. Results of the sensory evaluation in various treatments during storage

Discussion

Cinnamon is one of the most important spices used daily by people worldwide. Cinnamon primarily contains essential oils and other derivatives such as cinnamaldehyde, cinnamic acid and cinnamate. In addition to include antioxidant, anti-inflammatory, anti-diabetic, antimicrobial, anti-cancer, lipid-decreasing and anti-cardiovascular disease (CVD) characteristics, cinnamon has been reported to include activity against neurological disorders such as Parkinson's and Alzheimer's diseases (14). Based on the analysis of chemical compounds in CEO, 15 chemical compounds with a total rate of 98.01% were identified in this study. Results showed that cinnamaldehyde with 59.97% was the highest quantity detected in this essential oil. There are several reports on the antioxidant characteristics of CEO (15, 16). Results achieved from this study were similar to results of a study by Ojagh et al (2010), who stated that cinnamaldehyde was the most identified compound in CEO (60.41%). Linalool, ortho-methoxycinnamaldehyde, beta-caryophyllene and eugenol were other compounds that were identified in results of the analysis of CEO compounds (17). One of the most important significant characteristics of the essential oils is their hydrophobicity. The compounds enter the fat part of the bacterial and mitochondrial cell membranes and eventually lead to their disruption (18). In another study, Singh et al. (2007) reported that cinnamaldehyde was the major component of cinnamon barks and its pungent flavor and aroma were due to the presence of cinnamaldehyde and oxygen absorption (16). In other similar studies, Błaszczyk et al. (2021), who included the chemical compounds of various parts of cinnamon in their studies, stated that the most compound in cinnamon bark was linked to cinnamaldehyde (14). Quantity of these compounds can be changed based on various factors such as plant species, age, cultivation and processing conditions, harvest season, climatic conditions, parts used for essential oil extraction and essential oil extraction methods. In other reports, it was stated that the two compounds in CEO, cinnamaldehyde with the quantity of 55–67% and eugenol with the quantity of 5–18%, were the major antibacterial compounds and other compounds such as caryophyllene and cinnamyl acetate were present in lesser quantities (19).

There are several methods to investigate the effect of antimicrobial agents. In general, a certain concentration of a microbial agent should be assessed to inhibit or destroy the microorganisms. The MIC is the lowest concentration of an antibiotic or inhibitor agent that inhibits growth of

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microorganisms. (20). Researchers have expressed MIC as an indicator to detect antimicrobial activity and announced strategies to assess characteristics such as diffusion and dilution (21). In this study, MIC of CEO for *S. aureus* and *P. aeruginosa* was assessed as 4 and 2 mg/ml, respectively, and the MLC for the two bacteria was assessed as 4 mg/ml. In a similar study, Zhang et al. (2016) stated that CEO showed effective antibacterial activity against food spoilage and pathogenic bacteria (*Escherichia coli* and *Staphylococcus* spp.). The MIC of CEO against *E. coli* and *S. aureus* was 1 mg/ml and the MLC of CEO against *E. coli* and *Staphylococcus* spp. was 4 and 2 mg/ml, respectively. In general, *S. aureus* was more sensitive to CEO than that *E. coli* was (22). The CEO is one of the active natural compounds that includes antimicrobial effects on a wide range of Gram-negative and Gram-positive bacteria. This activity is due to the presence of cinnamaldehyde. By interfering in the biological systems and cell membrane of microorganisms, the compound prevents their growth. In addition, cinnamon contains various other compounds such as coumarin and cinnamic acid, which strengthen the antimicrobial and antioxidant characteristics as well as antifungal characteristics (22). In other studies, Elcocks et al. (2020) detected that cinnamon bark essential oil includes great antibacterial activity against Gram-negative and Gram-positive bacteria, as well as bacteriostatic and bactericidal effects against *P. aeruginosa* [(0.125% (w/w)] and showed other microorganisms (23). Based on the reports on the antibacterial activity of CEO in previous studies, it can be concluded that in the present study, CEO can include a significant antibacterial effect on the two strains.

Fish meat includes ingredients for the growth of microbes and hence fish is prone to spoilage. Therefore, presence of bacteria can be one of the reasons for decreasing the quality of fish during storage. Based on the decision of The International Commission on Microbiological Specifications for Foods, the limit value of total bacteria for fish is 7 log cfu/g. In this study, count of aerobic bacteria showed that preserving of the maximum possible quantity was reported in the control sample up to Day 3, in film treatment up to Day 6, and in film and CEO treatment up to Day 9. They did not violate the highlighted acceptance. The low value of bacterial count (3–4 log cfu/g) demonstrated good quality of fish fillets on Day 0. It has been reported in previous studies that resulted of this study were similar to them (11, 24). In a similar study, Ojagh et al. (2010) on rainbow trout stated that the total count of aerobic bacteria during storage for the samples coated with chitosan and samples coated with chitosan enriched with CEO was less than the permissible limit, while the control sample on Day 8 was greater than the limit (24). Rangberian et al. (2018) stated that in the control, quantity of total aerobic bacterial load reached 7.43 log cfu/g sample on Day 8 of storage, while in samples packed with film containing the essential oil, this parameter was lesser, compared to the coatings. This indicated antibacterial effect of CEO in the film (25). In fact, it could be concluded that by adding CEO to the films, antibacterial characteristics were created (24). Jouki et al. reported that the aerobic bacterial count of rainbow trout fillets packaged with quince seed mucilage film was higher on Day 12 and days later.

In this study, it was detected that the best performance of quince seed mucilage film contained 2% of thyme essential oil (11). In fact, quince seed mucilage film acts as a barrier against oxygen transfer and ultimately prevents growth of aerobic bacteria. In their studies, Jouki et al. showed that the oxygen permeability of quince seed mucilage film was high, showing that this film was a good oxygen barrier (5, 11). In a similar study, Volpe et al. (2015) stated that the total microorganism of uncoated count of rainbow trout fillets was 4.02 log cfu/g on Day 0, which increased to 8.88 log cfu/g on Day 9 of storage. In rainbow trout fillets containing coating and those containing coating and essential oil, the value increased 5.02 and 4.82 log cfu/g, respectively (26). Results of this study were similar to those of the present study. Noshad et al. (2020) detected that quince seed mucilage was effective in decreasing aerobic bacteria in shrimps during storage, similar to the present study (21). In another study by Pilmal et al. (2018) on silver carp finger fish, the researchers revealed that treatment with 2% chitosan coating, 1.5% CEO, and 2% chitosan coating and 1.5% CEO helped decreases in growth of aerobic bacteria, compared to the control sample (27). In the study of Raeisi et al. (2020), quantity of aerobic bacteria on Day 9 of storage of fillets coated with chitosan containing 0.2% of *Zataria multiflora* essential oil, fillets coated with alginate containing 0.2% of *Z. multiflora* essential oil and those coated with chitosan containing 0.2% of *Mentha piperita* essential oil had a slight increase (28). Results of this study were similar to those of film and CEO treatment in the present study.

Most of the microbial spoilage of fish and linked products stored at refrigerator temperature are caused by cold aerobic Gram-negative bacteria such as *Pseudomonas*, *Altromonas*, *Schwanella* and *Flaviobacteria* (29). In the present study, the lowest psychrotrophic bacterial count belonged to film and CEO treatment as 5.55 log cfu/g, which was less than the maximum acceptable value of 7 log cfu/g They did not violate. In fish spoilage, bacteria usually play a major role in deamination of free amino acids (FAA) and creation of non-volatile nitrogenous compounds, leading to a decrease in the nutritional value of fish and creating unpleasant aroma and taste (30). In a similar study, Taghizadeh

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Andevari and Rezaei (2012) aimed to investigate the effect of gelatin coating with CEO on rainbow trout fillets and stated that the treatment with coating containing essential oil included the lowest count of psychrophilic bacteria on Day 20 of storage (24.8 log cfu/g), compared to the control treatment (9.27 log cfu/g) and gelatin treatment (9.72 log cfu/g) (31). Cinnamon antimicrobial characteristic includes preventing growth and activity of microorganisms due to the presence of compounds such as cinnamaldehyde, which act together with the flow of electrons in the biological systems of microorganisms and reacts with nitrogenous compounds (protein and nucleic acid) (32). In another study by Raisi et al. (2020) on quantity of psychrophilic bacteria on Day 9 of storage of fillets coated with chitosan containing 0.2% Shirazi thyme essential oil, fillets coated with alginate containing 0.2% Shirazi thyme essential oil and fillets coated with chitosan containing 0.2% of Shirazi peppermint essential oil included a slight increase (28). Results of this study were similar to those of the treatment of fruit seed mucilage film strengthened with ketira gum containing 1% CEO in the present study. In another similar study, Pilmal et al. (2018) investigated silver carp finger fish and stated that the lowest number of psychrophilic bacteria on Day 18 of storage was linked to chitosan and cinnamon essence treatment (6.24 log cfu/g) (27).

Normally, pH is one of the factors that can be changed during the storage time of fish, which can be used as an indicator of fish freshness. Based on the results of this study, the initial pH in the control treatment during the first days of storage was 6.21, which reached 7.01 on the last day of storage, indicating growth of bacteria in the sample. Increase in pH during the study could be due to the breakdown of proteins, which caused production of alkaline compounds such as ammonia and amines. In the control group, further nitrogenous compounds were produced because there was further bacterial contaminations; similar to the study results by Fan et al. (2009). In the present study, the lowest pH was linked to the film and CEO treatment (pH 6.38) on the last day of the study. Decrease in pH was due to the presence of lactic acid, which was possibly transferred to the sample (33). In a study by Volpe et al. (2015), pH level of uncoated fish fillets reached 6.50 to 7.13 after 15 d, while pH of coated fish fillets, and coated fish fillets and essential oil was 6.82 that changed to 6.75. These results were due to the decrease of bacterial activity by the coating and essential oil and in presence of the coating, preventing spoilage by oxygen (26). Results of this study were similar to results of the present study; however, pH was slightly lower in the present study. In another study, Noshad et al. (2020) showed that the initial pH of shrimps was 6.97. The pH of shrimp in all samples increased during storage at 4 °C. Increase in pH of the shrimps coated with mucilage seeds and mucilage coatings containing green tea extract was less than that of control shrimps; hence, treatment with mucilage seed coating containing 20% green tea extract showed the lowest pH (21). Results of this study were similar to those of the current study. In another study, Mahjoob and Ataye Salehi (2019) on veal meat stated that the lowest pH on Day 9 of storage with films was 0.4% of CEO (34). During the start of product storage, pH value decreased due to the breakdown of glycogen and formation of mineral acids such as lactic acid and ultimately prevented the growth of microorganisms (34).

Rainbow trout are sensitive to oxidative spoilage due to several UFAs. During the storage of the product, oxidation causes numerous problems on the quality of the product, especially its taste and aroma (35). Fat oxidation is a major problem in fresh fish and other seafood products. Mostly, peroxide value (PV) is the primary oxidation product of fats. The higher the degrees of unsaturation of fats, the more ready the substance is for oxidation (35). It is suggested that the maximum acceptable quantity of peroxide for optimal fish quality is 10–20 mEq/kg (36). In the present study, after 9 d of storage at refrigerator temperature, the PV index increased for all treatments and this increase was further intense in the control treatment, reaching from 0.6 to 4.83 mEq/kg. Increase in PV was due to the activity of psychrophilic bacteria, especially *Pseudomonas* spp. In fact, psychrophilic bacteria can produce lipase and phospholipase enzymes during the storage of foods in refrigerator conditions and thus increase the quantity of short-chain fatty acids (SCFA). This type of FAs is sensitive to oxidation and eventually hydroperoxide is produced in foods (24). In the present study, quantity of peroxide in the film, and film and CEO treatments respectively increased to 4.02 and 2.99 mEq/kg on the last day. In a similar study, Sallem et al. (2007) stated the quantity of peroxide as 1.12–1.23 mEq/kg. On the last day of storage, this value was 4.23 for the control sample and 2.43 and 3.14 mEq/kg for the sodium citrate and sodium acetate treatments, respectively. Storage time included a significant effect on peroxides for each of the control and treated samples. In all samples, levels less than the recommended acceptable levels of 10–20 mEq/kg were reported (1). It could be reported that the increase in PV index of the treatment with essential oil was due to the antioxidant characteristics and preservation of fish fats from oxidation (37).

In this study, peroxide value in film and CEO treatments from Day 0 to the end of the study was significantly ($P < 0.05$) lower than that in the control treatment. In another study, Ojagh et al. (2010) investigated the effect of coating on the peroxide changes of fish fats and reported that the initial peroxide level in the sample varied 0.29–1.14 mEq/kg. Peroxide values of

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control and coated samples increased significantly with storage time. At the end of the storage time (Day 16), significant differences in peroxide were observed between the control (4.23 mEq/kg) and each of the samples coated with chitosan, and chitosan and cinnamon essence with lower values of 3.43 as 4.60 mEq/kg. Results of this study showed that chitosan coating was effective in delaying peroxide production in salmon fillets stored in refrigerator (4 °C ±1) (24). In the present study, it was shown that film and CEO treatment included a greater effect in postponing peroxide production, compared to chitosan coating. In addition, film treatment included a greater effect than that chitosan coating and CEO did. Fatty fish are highly vulnerable to oxidation, causing quality problems such as rancidity and bad aroma (sourness). There may be changes in texture, color and nutritional values even during storage at sub-zero temperatures. Various reactions are involved in fat oxidation, which may be enzymatic or non-enzymatic. Microbial enzymes or intracellular and digestive enzymes of the fish play roles in this reaction (36). In a similar study, Mahjoob and Ataye Salehi (2019) reported the highest quantity of peroxide in the control sample. At the end of the study (Day 12), the lowest quantity was linked to the samples with 0.3% CEO film on veal (34). It could be concluded that CEO included an inhibitory effect due to its phenolic compounds such as eugenol and cinnamic aldehyde. Presence of these compounds includes the ability to sensitize the phospholipid bilayer membrane and ultimately lead to an increase in permeability and release of important parts of the cell inside such as iron, ATP, nucleic acid and AAs (18).

To assess quantity of spoilage in marine products, TVN is used as an indicator of spoilage in meats. Majorly, TVN is composed of ammonia and amines. By increasing activity of bacteria and enzymes, this index increases and causes an unpleasant taste in fish. The maximum acceptable level of TVN content is 25 mg/100 g of fish meat sample (24). For fish fillets, a limit of TVN quantity of up to 27 mg/100 g has been announced (36). In this study, TVN materials in the samples generally increased. For the control treatment on the last day of storage, this quantity was 40.51 mg/100 g. For the film treatment, this was 36.22 mg/100 g (greater than the set standard) and for the film and CEO treatment, this was 26.02 mg/100 g. During the storage time, increase in the quantity of TVN could be linked to the activities of spoilage bacteria. In a similar study, Jouki et al. (2014) stated the initial TVN values of 8.23 mg of nitrogen per 100 g of fish. On the last day of the study (Day 18), samples packed with grain mucilage film contained 2% thyme essential oil in a significantly lower quantity (17.4 mg of nitrogen per 100 g of fish), compared to the samples packed with grain mucilage film and the controls (36.4 and 43.2 mg of nitrogen per 100 g of fish). The lower quantities of TVN in the samples of seed mucilage film containing 2% thyme essential oil might be attributed to the antimicrobial characteristics of thyme essential oil. In this study, TVN content in the samples packed with grain mucilage film containing thyme essential oil and oregano essential oil was significantly lower than that in the samples packed with grain mucilage film. This could be attributed to the rapid decrease of the bacterial population or decreased capacity of bacteria to oxidative dispose of non-protein nitrogenous compounds or the two, possibly due to the effect of oregano thyme essential oil on fish fillets (11). Compared to the present study, oregano and thyme essential oil included a greater effect than that CEO did against the increasing quantity of TVN. Similar to results of this study, Mahjoob and Ataye Salehi (2019) stated that the highest TVN values were linked to the control group and then the samples covered with films without essential oils. The lowest TVN value was linked to the samples treated with CEO (34). Reason for the low TVN in treated samples included increases of bacterial population and the ability of bacteria to oxidize for the separation of amine from non-volatile nitrogenous compounds or a combination of the two (33). In a study of Ojagh et al., the initial values of TVN (mg of nitrogen per 100 g of fish) of the control sample and coated samples were 9.33–12.13. The TVN values of control and coated samples increased significantly with storage time. At the end of the storage time (Day 16), samples coated with chitosan and CEO reached a significantly lower quantity of VBN (14.23), compared to samples coated with chitosan and the control, which were 22.86 and 93.42, respectively. Since TVN is majorly produced by the bacterial decomposition of fish meats, the higher total bacterial counts of uncoated against coated samples could account for the higher TVN values of uncoated samples (24). In other similar studies, Volpe et al. (2015) showed that TVN increased during 15 d of storage in all groups. In rainbow trout fillets without coating and rainbow trout fillets containing coating, TVN was greater the acceptable limit after 15 d. In rainbow trout fillets containing coating and essential oil, the final TVN value was less than 25 mg of nitrogen per 100 g of fish. These results showed that although carrageenan coating was able to decrease the rate of TVN production; however, presence of essential oil was important to control the production of TVN (26). In a study of Noshad et al. (2020), quince seed mucilage was effective in decreasing TVN values in shrimps during storage; similar to results of the present study (21).

The TBARS is an indicator of fat oxidation, which is based on the quantity of malondialdehyde. Through the oxidation of hydroperoxides, malondialdehyde is formed into compounds such as aldehyde and ketone (38). In this study, quantity of TBARS during storage in the treatment

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of rainbow trout fillets packed with quince seed mucilage film strengthened with tragacanth gum containing CEO was lower than that of the other two groups, which could be linked to the antioxidant function of CEO in decreasing fat oxidation. In a similar study, Jouki et al. (2014) investigated salmon fillets (18 d of storage) and stated that the quantity of TBARS was lower in grain mucilage film samples containing 2% oregano essential oil (0.49 mg MDA/kg) than that in the control samples or those packaged with quince seed mucilage film (0.89 and 0.95 mg MDA/kg, respectively) (11). It is suggested that the maximum acceptable quantity of TBARS for the optimal quality of fish is 5 mg MDA/kg of sample, while up to 8 mg MDA/kg of sample can be consumed. In this study, the quantity in all treatments was within the permissible range on Day 9 (39). In the current study, this value increased generally in samples and the quantity of TBARS in the rainbow trout fillet samples of the control group was 0.39 mg MDA/kg at the beginning of the study, reaching 2.79 mg MDA/kg on the last day. Increase in the quantity of TBARS was due to the increase in the quantity of free iron and peroxides in the muscles as well as creation of aldehydes (resulting from secondary products of decomposing hydroperoxide) (40). In a similar study, Mahjoob and Ataye Salehi (2019) stated that the lowest values were linked to veal samples packed with films containing 0.2 and 0.3% of CEO (34). In another similar study, Ojagh et al. (2010) stated that treatments coated with chitosan and CEO showed lower quantities of TBARS during the storage time, compared to the control samples. In fact, it could be concluded that CEO possibly included a synergistic effect. In most plant extracts, the antioxidant activity has been attributed to their ability to break the free radical chain by donating hydrogen (24). In Raeisi et al. study (2020), the researchers stated that the initial quantity of TBARS varied 0.17–0.20 mg MDA/kg. On Day 9 of storage, the best performance was linked to rainbow trout fillets coated with chitosan containing 0.2% *Z. multiflora* essential oil (28). It could be reported that in the control sample, increase in the quantity of TBARS, as well as the high quantity of peroxide, might indicate food spoilage (28).

Taste and appearance are two important factors in meat products, which can include a great effect on the overall acceptance of the consumer and the shelf life of the product (41). Results of the assessment of aroma, texture and taste in this study showed that the film and CEO treatment included better scores than that the control groups and the film treatment did, which increased the acceptability. For appearance characteristics, the control group included the highest acceptance score. Increasing the quantity of spoilage during product storage could lead to a decrease in its taste and appearance score. Oxidation of fat is a factor that leads to changes in the appearance of fats (41). In a similar study, Taghizad Andvari and Rezaei (2012) demonstrated that salmon fillets coated with CEO were acceptable for sensory characteristics such as texture, aroma, color and overall acceptance until Day 15 of storage (31). Effect of the antimicrobial and antioxidant characteristics of CEO can increase the storage time and preserve quality of the product as well as creating a protective layer against the air (24). In another study, Jouki et al. (2014) investigated sensory characteristics of trout fillets and reported that grain mucilage film containing oregano essential oil or thyme essential oil included no significant negative effects on the organoleptic acceptance of rainbow trout fillets based on sensory evaluation (11). The major problem for the use of essential oils as food preservatives (especially those releasing strong odors such as oregano or thyme) is that when these are added in sufficient quantities to produce antimicrobial effects, they produce negative organoleptic effects as well. Adding essential oils to the edible film matrix can be a solution to this problem. In another study, Ojagh et al. (2010) stated that due to high fat oxidation and microbial growth, control samples (uncoated) of rainbow trout fillets showed spoilage, which appeared smelly, slimy and discolored after 8 d of storage. Antioxidant, antimicrobial and gas barrier effects have been demonstrated with the coating, which minimizes oxidative effects and extends product shelf life while maintaining the quality. Addition of CEO to chitosan coating significantly increased the beneficial effects on color and overall acceptance of fish in the final days (11); similar to the present study. In a study, Khodayi et al. (2020) aimed to investigate the effect of edible coating containing a type of natural preservative on the sensory characteristics of a fish breed. Based on the findings, it was detected that this coating preserved most of the sensory characteristics of the fish (38).

Conclusion

In all rainbow trout fillet samples, it was detected that samples treated with quince seed mucilage film strengthened with tragacanth gum containing CEO could increase their shelf life during 9 d of storage in refrigerator conditions. These included a favorable effect on the microbial and chemical characteristics during the storage time to decrease the total count of aerobic bacteria, cold-oriented bacteria and the MIC and MBC indices of *S. aureus* and *P. aeruginosa* as well as decreasing the values of pH, PV, TVBN and TBARS indicators. For sensory characteristics, treated samples included good and acceptable grades; therefore, quince seed mucilage film strengthened with tragacanth gum containing CEO could increase the shelf life of rainbow trout fillets and could be used in the food industry for active packaging of food products, especially fish meats.

Author contributions

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Mohammad Sadegh Mohammadi Resketi collected the samples, carried out the experiments, HamidReza Kazemeini analyzed data and contributed to writing and revising the manuscript.

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Financial disclosure

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