Shelf life extension of *Oncorhynchus aguabonita* fillets based on *Trachyspermum copticum* essential oil nanoemulsion coating during storage at 4°C

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ABSTRACT

This study investigated the effects of *Trachyspermum copticum* essential oil nanoemulsion (TCEO-NE) coating on chemical, microbial and sensory changes of *Oncorhynchus aguabonita* fillets during storage at 4 °C. The components of *T. copticum* EO were identified using gas chromatography-mass spectrometry. TCEO-NE was prepared by ultrasonic method and its properties were determined. Fresh *O. aguabonita* fillets were immersed in TCEO-NE and stored at 4 °C. Chemical (pH, TVB-N, peroxide and TBARS), microbiological (total aerobic mesophilic bacteria, psychrotrophs, Enterobacteriaceae, lactic acid bacteria and *Staphylococcus aureus*) and sensory analyses (color, odor and taste) of fish fillets were evaluated on days 0, 2, 4, 6, 8 and 11. Thymol, limonene, and alpha-terpinene were the major compounds in *T. copticum* EO. The droplet size of TCEO-NE was 127.6 nm and PDI was 0.210. The control group exceeded the peroxide limit on day 6, while TCEO-NE 500.00 and 666.66 did so on day 8. The TBARS value in fish fillets was 0.57 mg MDA/kg on day zero which reached 4.76 mg MDA/kg in the control group and 2.90 mg MDA/kg in TCEO-NE 666.66 after 11 days at 4 °C. Aerobic mesophilic count in the control group exceeded the permissible level on day 6 and, in TCEO-NE 500.00 and 666.66, on day 8, therefore the shelf life of fish fillets was improved by two days. On the grounds of the favorable properties of TCEO-NE and its positive effects on chemical, microbial and sensory changes in fish fillets, it can be used as a natural food additive.

Introduction

Fish and their products are rich sources of protein and contain large amounts of minerals, unsaturated fatty acids, water and fat-soluble vitamins, which make such products highly perishable with restrictions in storage even under refrigeration. Microbial and chemical spoilage in fish products occurs more quickly than in other animal products such as chicken and red meat (26, 67).

Various methods such as cold and freezing, heating and canning, drying, salting and adding preservatives can be used to prevent microbial and chemical spoilage in meat (17, 37, 78). Synthetic preservatives are used as a complementary method to increase the shelf life of food at low temperatures (16). Due to the increasing demand for food production, there is an urgent need to find and use safe food additives to improve food quality and shelf life.

Plant essential oils (EOs) are one of the food additives which increase the shelf life and improve the sensory properties of food by reducing or eliminating pathogens and preventing fat oxidation (72). EOs are secondary metabolites produced by aromatic plants. These substances are liquid and volatile compounds which are soluble in fats and organic solvents with lower density than water (12). The antimicrobial and antioxidant properties of EOs are well-known for many centuries (22, 74, 76). Substances such as carvacrol, eugenol and thymol belong to the phenolic group. The higher the number of phenolic groups, the greater the antimicrobial properties

of EOs (11). EOs can replace synthetic antioxidants and increase their shelf life by adding them to high-fat and perishable foods (46).

T. copticum is an annual plant of Apiaceae family that naturally grows in arid and semi-arid lands of India, Iran, Egypt, Afghanistan, Pakistan and Europe. The EO of this plant is rich in phenolic compounds such as thymol, cymene, β-pinene, γ-terpinene and sabinene (77). Chemical compounds of this EO have been identified previously (40, 45, 47, 49). Many traditional medicine books mentioned the antimicrobial and medicinal properties of *T. copticum*. Today, in traditional medicine, it is used to improve recovery weakness, cough, stomach pain, rheumatism, and also in treatment of various microbial infections and even for the treatment of abdominal tumors. Above all, *T. copticum* is also used as a flavoring and aromatizing agent in foods (8, 14).

EOs are hydrophobic compounds with very low solubility in water and are mostly soluble in non-polar and semi-polar solvents, alcohols, oils and waxes. They are sensitive to light, heat and air and easily oxidize, due to their molecular structure's double bonds and hydroxyl, aldehyde and ester (62). Another disadvantage of using EOs in food is the creation of a special taste and smell that the consumers may not like. The use of nanoemulsions of EOs allows for more solubility in water, increases the stability and protection of volatile compounds and improves the antimicrobial and antioxidant effects by boosting cell absorption and reducing the adverse impacts of EOs (18). Antimicrobial properties and food shelf life extension of essential oil nanoemulsions have been proved previously (64).

Golden trout (*Oncorhynchus aguabonita*) belongs to the Salmonidae. This fish is often referred to as the California golden trout and the native river trout, which live on the tributaries of the Kern River. Golden trout was chosen in this study due to its high consumption by the population in Iran, very little literature on this fish and the presence of unsaturated fatty acids and being more prone to oxidative spoilage (42).

This study aimed to investigate the effects of *T. copticum* EO nanoemulsion (TCEO-NE) coating on chemical, microbial and sensory properties of *O. aguabonita* fillet stored at 4 °C.

Materials and Methods

Preparation of EO and determination of its components: The EO of *T. copticum* was purchased on the market. The components of *T. copticum* EO were identified using gas chromatography (Thermoquest 2000, Manchester, UK) connected to a mass spectrometer (MSD5973). This device has an Hp5 capillary column and the data were obtained under the following conditions: initial temperature of 50 °C, the final temperature of 265 °C and injection temperature of 250 °C. Helium gas was used as a carrier gas at a rate of 1.1 ml/min with a separation ratio of 1:100. The mass spectrometer has an ionization energy of 70 electron volts and an interface temperature of 250 °C (54).

Preparation of nanoemulsion: The ultrasonication emulsification method has been used to prepare a variety of essential oil nanoemulsions (10, 69, 70). Generally, oil in water nanoemulsions are very stable and can only be separated into two phases quickly using unique methods such as electric fields (34, 35). To make TCEO-NE at room temperature, first, the weight of *T. copticum* EO, double-distilled water, and a mixture of some surfactants (tween 80/ span 80) were calculated and were mixed together. It was transferred to an ultrasonic device to apply ultrasound and produce the nanoemulsion (model UP400S, maximum power 400 w, frequency 20 kHz, Hielscher, Germany). TCEO-NE was prepared at concentrations of 500.00 and 666.66 µl/ml with an ultrasound time of 300 seconds, an ultrasound cycle of 0.75% and an ultrasound intensity of 208 w/cm2 by ultrasound apparatus. These concentrations were chosen according to another study by the authors regarding the antibacterial effect of this nanoemulsion studied by disc diffusion assay, determination of MIC and MBC and bacterial growth kinetics (33).

Determination of nanoemulsion properties: The average droplet size and polydispersity index (PDI) of TCEO-NE were obtained by Dynamic Light Scattering (DLS) method using nano series zetasizer (Nano ZS model, ZEN 3600, Malvern, UK) with a constant dispersion angle of 173°. In this study we used Shahavi et al. (70) method to prepare stable EO nanoemulsions. The measurements were repeated three times at 25 °C. Zetasizer Nano software (version 7.03) was used to collect and analyse the obtained data.

Preparation of treatments: Live golden trouts (*Oncorhynchus aguabonita*) were purchased from a fish farm in Amol, then their head, tail and fins were removed and the contents of the abdomen were emptied and they were immediately transferred to the food hygiene laboratory of the Faculty of Veterinary Medicine in Amol University of Special Modern Technologies, Amol, Iran. Then, under full observance of hygienic principles, the fish was divided into 50 g fillets. The fillets were divided into three groups of control, TCEO-NE 500.00 and TCEO-NE 666.66. The control group was treated with double-distilled water and a mixture of some surfactants (tween 80/ span 80) while, each of TCEO-NE 500.00 and TCEO-NE 666.66 fillets were immersed in 200 ml TCEO-NE with concentrations of 500.00 µl/ml and 666.66 µl/ml for 3 min at room temperature, respectively. They were

then placed in zippered nylon bags sterilized by UV and labeled, then transferred to a refrigerator at 4 °C (19). All the tests were done two times. The TBARS value determination was performed at 0, 2, 4, 6, 8, and 11th day at 4 °C and also at the 30th day under freeze condition (55).

Analysis of the approximate composition of fish fillets: The moisture content (2), ash (3), protein (4) and fat (5) of fish fillets in the control group on day zero were determined according to the method proposed by the Association of Official Agricultural Chemists.

Chemical analyses: The pH values of fish fillets on days 0, 2, 4, 6, 8 and 11 were quantified by immersing the glass electrode of a digital pH meter (Mettler Toledo, Seven Easy, USA) in the homogenized solution of 10 grams of the ground beef with 90 ml of distilled water (75).

Total Volatile Basic Nitrogen (TVB-N) of the samples (mg/100 g fish meat) was determined according to (AOAC) (6). Peroxide value (meq/kg fish meat) was quantified in 1 g of fish meat based on (AOAC) (7). Thiobarbituric Acid Reactive Substances (TBARS) values of the samples were measured using 5 g of fish fillet and absorbance readings at a wavelength of 532 nm (44).

Microbiological analyses: To perform microbial tests, 10 g of each sample was homogenized with 90 ml of 0.1% sterile buffered peptone water (i23029 Ibresco, Iran) for 3 min in sterile bags in a stomacher (Iul Masticator Classic 400 ml, 240 W, Barcelona, Spain). Then, making dilutions was performed with 0.1% peptone water and the dilutions were cultured in culture media (duplicate) and were counted after incubation at the required temperature and time (73). Total counting of aerobic mesophilic bacteria was done by surface culture method in Plate Count Agar (PCA) medium (105463 Merck, Germany) after incubation at 37 °C for 48 h (63). Psychrotrophs were counted by surface culture in PCA medium after incubation at 7 °C for 10 days (23). *Enterobacteriaceae* were counted by pour plate method in Violet Red Bile Glucose agar (VRBG) medium (i23193 Ibresco, Iran) and incubation was performed at 30 °C for 24 h (25). Lactic acid bacteria were counted by surface culture in MRS agar medium (110660, Merck, Germany). Incubation was performed at 30 °C for 48 h under anaerobic conditions (using anaerocult® A gas packs, Merck, Germany) (25). *S. aureus* was counted by surface culture in Baird Parker medium (i23013 Ibresco, Iran) after incubation at 37 °C for 48 h (27). Colony count results were reported as log cfu/g.

Sensory evaluation: Sensory analysis was performed by a trained group of 6 students and staff of Amol University of Special Modern Technologies, unaware of the samples' nature. They were 2 women (21 and 35 years old) and 4 men (26, 27, 30 and 35 years old). To score the color index, the samples were randomly divided among them in an environment that was almost white without the evaluators being aware of the nature of the samples, and they used an 8-point scale for scoring (8 = very bright red, 7 = relatively light red, 6 = light red, 5 = low light red, 4 = bold red, 3 = light dark red, 2 = relatively dark red, 1 = dark red). To score the odor index, the samples were randomly divided among them in a well-ventilated environment without the evaluators being aware of the nature of the samples. Then they used a 9-point scale for scoring. Moreover, to score the taste index, first the fillets were fried in a small amount of frying oil and the oil was removed. Next, without the evaluators being aware of the type of samples, they were randomly divided between them and they used a 9-point scale to rate the fried fillets (0 = very undesirable, 9 = very desirable) (13, 32, 36, 51, 71, 73).

Statistical analysis: Shapiro-Wilk test was performed to check the normality of the data. The linear procedure model test for Repeated Measures ANOVA and Bonferroni post hoc tests were performed to compare microbial and chemical change trends during each group's study period. For comparison between groups at any time, one-way ANOVA and Tukey post hoc tests were performed. Regarding the variables of color, odor and taste, non-parametric Friedman test was selected to compare the trend of their changes during the study period in each group and also Wilcoxon signed-rank test with Bonferroni correction was used to evaluate the two measured indices between different times. Moreover, Kruskal-Wallis non-parametric test was performed to compare groups at any time and Mann-Whitney U-test with Bonferroni correction was used to compare them in pairs. Results were expressed based on mean and standard deviation. Data analysis was performed using SPSS statistical software version 25 (SPSS Inc., Chicago, IL, USA). In all analyses, a significance level of less than 5% was considered.

Results

The results regarding the identification of the components of *T. copticum* EO by GC/MS are shown in Table 1 and Figure 1. Sixteen different components of *T. copticum* EO were identified, accounting for 97.9% of the EO. Thymol (53.17%), limonene (21.12%) and alpha-terpinene (19.46%) were the three major compounds determined ($P<0.05$).

Droplet size and PDI of TCEO-NE using the DLS method were 127.6 nm and 0.21 (Figure 2). The average moisture, ash, protein and fat in *O. aguabonita* fillets on day zero were 73.66%, 1.30%, 17.50% and 1.36%, respectively.

Table 1. The amount of compounds identified in *T. copticum* EO by GC/MS.

No.	Constituent	Quantity (%)	Retention Time (min)
1	α-thujene	0.31	10.86
2	α-pinene	0.17	11.95
3	Limonene	21.12	13.12
4	Myrcene	0.74	14.02
5	α-terpinene	19.46	15.16
6	p-cymene	0.21	16.25
7	D-limonene	0.63	17.48
8	Cineol	0.54	18.33
9	γ-terpinene	0.48	19.55
10	Terpinolene	0.17	20.47
11	Thymol	53.17	22.19
12	α-terpineol	0.21	25.88
13	Carveol Acetate	0.36	27.63
14	Carvone	0.17	29.46
15	Carvacrol	0.12	31.89
16	Bergaptene	0.04	34.19
	Total	**97.9**	

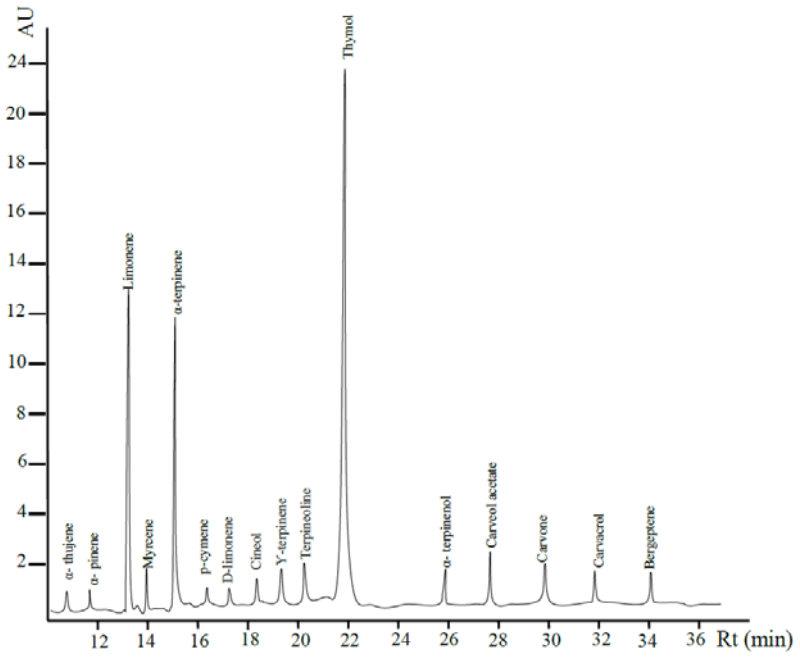

Figure 1. GC-MS chromatogram of *T. copticum* EO.

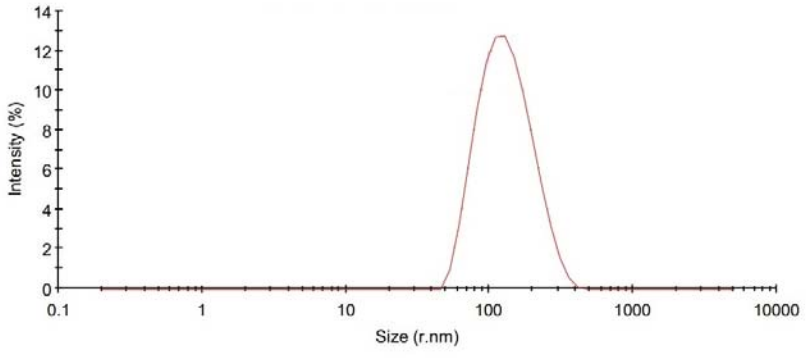

Figure 2. Particle size distribution of *T. copticum* essential oil nanoemulsion

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Table 2. Chemical properties of *O. aguabonita* fillet coated with *T. copticum* EO nanoemulsion stored at 4 °C.

Parameter	Day / Group	0	2	4	6	8	11
pH	Control	7.00±0.06^{a,A}	7.18±0.03^{a,AB}	7.30±0.04^{a,AB}	7.39±0.07^{a,AB}	7.53±0.04^{a,AB}	7.78±0.03^{a,B}
	TCEO-NE 500.00	7.00±0.06^{a,A}	7.10±0.01^{b,AB}	7.21±0.02^{a,AB}	7.30±0.05^{ab,AB}	7.43±0.02^{a,AB}	7.51±0.03^{b,B}
	TCEO-NE 666.66	7.00±0.06^{a,A}	7.08±0.01^{b,AB}	7.12±0.03^{b,AB}	7.21±0.08^{b,AB}	7.26±0.06^{b,AB}	7.39±0.01^{c,B}
TVB-N (mg/100 g)	Control	14.64±0.07^{a,A}	19.83±0.03^{a,B}	26.35±0.47^{a,C}	36.39±0.06^{a,D}	38.53±0.18^{a,E}	40.24±0.18^{a,E}
	TCEO-NE 500.00	14.64±0.07^{a,A}	17.58±0.35^{b,B}	21.07±0.03^{b,C}	28.73±0.03^{b,D}	36.74±0.06^{b,E}	38.84±0.11^{b,F}
	TCEO-NE 666.66	14.64±0.17^{a,A}	16.81±0.12^{c,B}	18.37±0.06^{c,C}	27.80±0.02^{c,D}	35.91±0.14^{c,E}	37.71±0.23^{c,F}
Peroxide value (meq/kg)	Control	2.10±0.15^{a,A}	5.09±0.52^{a,A}	7.78±0.61^{a,A}	10.71±0.32^{a,B}	12.69±0.17^{a,C}	16.52±0.35^{a,D}
	TCEO-NE 500.00	2.10±0.15^{a,A}	3.54±0.09^{b,A}	5.55±0.30^{b,A}	7.64±0.35^{b,B}	11.61±0.24^{b,C}	14.23±0.07^{b,D}
	TCEO-NE 666.66	2.10±0.15^{a,A}	3.11±0.08^{b,A}	4.48±0.28^{b,A}	6.28±0.43^{c,A}	10.12±0.07^{c,B}	13.60±0.18^{c,C}

*Different lowercase letters show a significant difference at any time point between the three groups (P<0.05).
** Different uppercase letters show a significant difference in each group between six time point (P<0.05).

The results of pH in *O. aguabonita* fillets coated with TCEO-NE are shown in Table 2. The pH was 7.00 on day zero, which reached 7.78 (P<0.05) during an increasing trend on day 11 in the control group, while this increasing trend was less steep in the other two groups and in TCEO-NE 500.00 and TCEO-NE 666.66 reached 7.51 and 7.39, respectively. On all days except day zero, there was a significant difference between the pH of the control group and the pH of TCEO-NE 666.66 (P<0.05), while only on days 2 and 11, a significant difference was observed between the control group and TCEO-NE 500.00.

The results of TVB-N in *O. aguabonita* fillets coated with TCEO-NE are demonstrated in Table 2. TVB-N of fish fillets was 14.64 mg/100 g on day zero, which reached 40.24, 38.84 and 37.71 in the control group, TCEO-NE 500.00 and TCEO-NE 666.66, respectively on day 11. Regarding TVB-N content, no difference was observed between the groups on day zero (P>0.05). At other time points, there were significant differences among all three groups (P<0.05).

The results of peroxide value in *O. aguabonita* fillets coated with TCEO-NE are shown in Table 2. The highest and the lowest amount of peroxide at the end of the storage period were 16.52 and 13.60 meq/kg belonging to the control group and TCEO-NE 666.66, respectively.

Figure 3 shows the amount of TBARS in *O. aguabonita* fillets coated with TCEO-NE and stored at 4 °C. TBARS value in fish fillets of the control group on day zero was 0.57 mg MDA/kg of fish and after 11 days of storage at 4 °C reached 4.76 mg MDA/kg fish meat and 2.90 mg MDA/kg fish meat in the control group and TCEO-NE 666.66, respectively. These values were 5.88, 4.65 and 3.48 mg MDA/kg fish meat on day 30 of the storage period (freezing conditions) in the control group, TCEO-NE 500.00 and TCEO-NE 666.66, respectively.

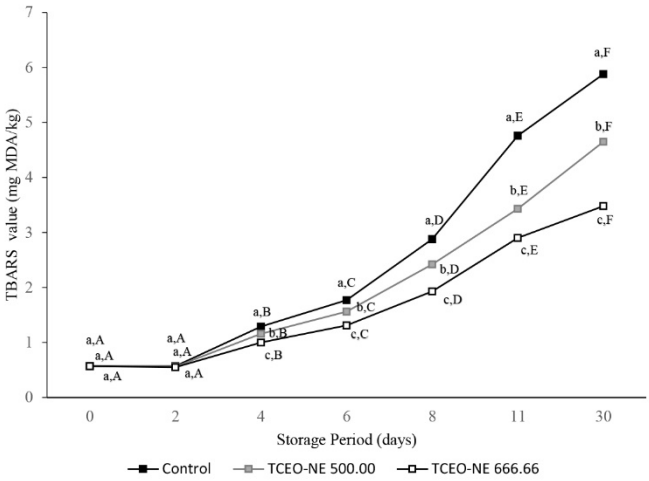

Figure 3. TBARS value of *O. aguabonita* fillet coated with *T. copticum* EO nanoemulsion stored stored at 4 °C and freezing condition.
* Different lowercase letters show a significant difference at any time point between the three groups (P<0.05).
** Different uppercase letters show a significant difference in each group between seven time points (P<0.05).

Figure 4 shows the total aerobic mesophilic count in *O. aguabonita* fillets coated with TCEO-NE and kept at 4 °C. This count increased in all groups during the storage period, with this trend being faster in the control group than in the other two groups. There was a significant difference between the control group and TCEO-NE

666.66 on all days except day zero. As can be seen in Figure 3, there was a significant difference among all groups on days 4, 6 and 8 (P<0.05).

Figure 4 shows the count of psychrotrophic bacteria in *O. aguabonita* fillet coated with TCEO-NE stored at 4 °C. Psychrotrophic bacteria count in fish fillets was 2.39 log cfu/g which reached 12.45 log cfu/g in the control group after 11 days of storage at 4 °C, while in TCEO-NE 666.66, this count reached 9.98 log cfu/g after 11 days of storage at 4 °C. There was a significant difference between the control group and the other two groups on all days except day zero (P<0.05), but TCEO-NE 500.00 and TCEO-NE 666.66 were not significantly different from each other.

Enterobacteriaceae count in *O. aguabonita* fillets coated with TCEO-NE, stored at 4 °C is shown in Figure 4. During the storage period, an increasing trend in the number of Enterobacteriaceae was observed in all groups. *Enterobacteriaceae* count in the control group reached 11.61 log cfu/g, while in TCEO-NE 500.00 and TCEO-NE 666.66 reached 10.58 log cfu/g and 8.41 log cfu/g at the end of the storage period, respectively. There was a significant difference among all groups regarding *Enterobacteriaceae* count on all days except day zero.

Changes in the number of lactic acid bacteria in *O. aguabonita* fillet coated with TCEO-NE and stored at 4 °C are shown in Figure 4. The difference of the number of lactic acid bacteria in the control group between day zero and day 11 was approximately 9 log cfu/g, but in TCEO-NE 666.66, it was approximately 5.5 log cfu/g. Significant differences were observed among the groups from day 6, so that on days 6, 8 and 11, all three groups had significant differences from each other (P<0.05).

Figure 4 shows the changes of *S. aureus* in the *O. aguabonita* fillet coated with TCEO-NE and stored at 4 °C. *S. aureus* count on day zero was 2.19 log cfu/g and in the control group, TCEO-NE 500.00 and TCEO-NE 666.66 reached 10.54 log cfu/g, 8.66 log cfu/g and 8.35 log cfu/g respectively, after 11 days of storage at 4 °C. *S. aureus* count in the control group was significantly different from the other two groups on all days except day zero, but no significant difference was observed between TCEO-NE 500.00 and TCEO-NE 666.66 (P<0.05).

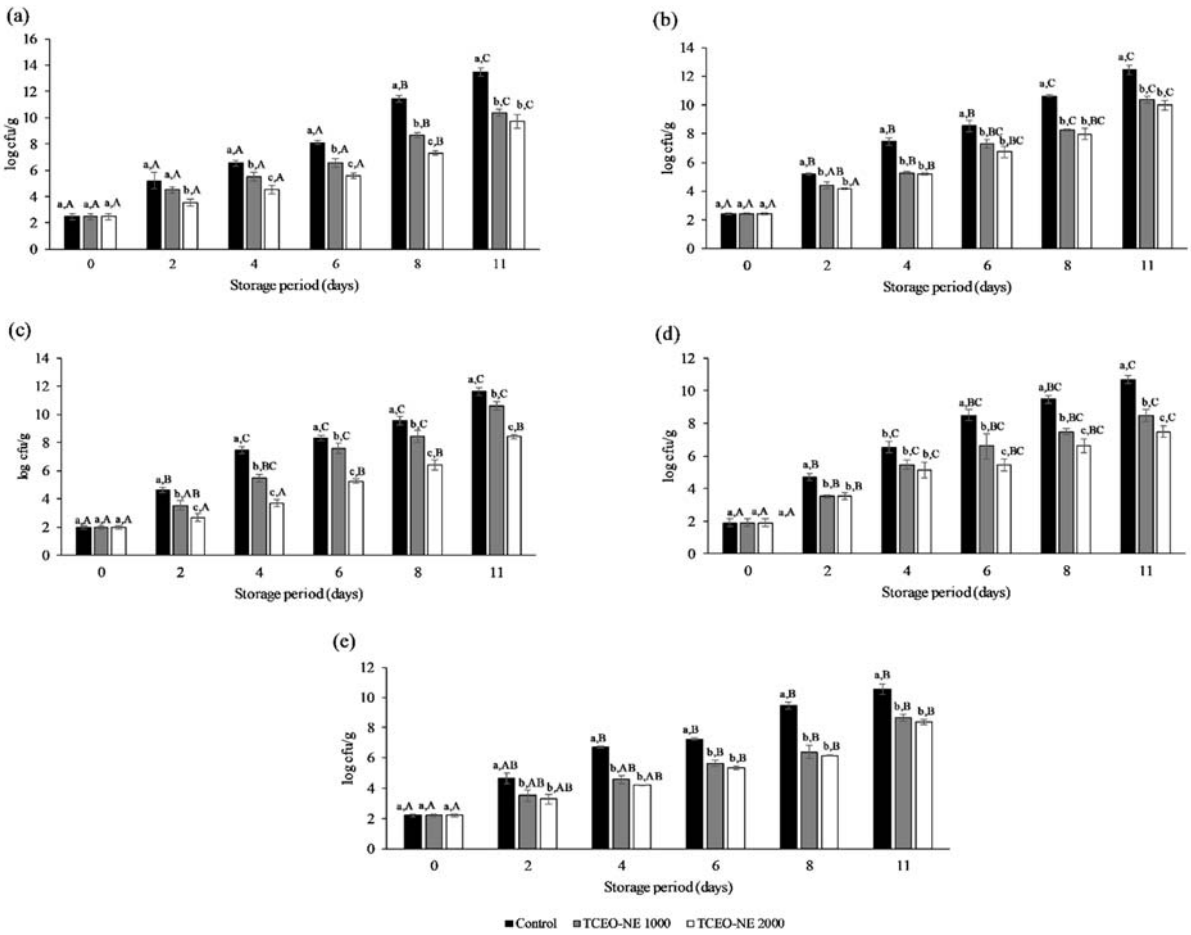

Figure 4. Microbial changes of *O. aguabonita* fillet coated with *T. copticum* EO nanoemulsion stored at 4 °C. (a): Total aerobic mesophilic count, (b): Psychrotrophs, (c): *Enterobacteriaceae*, (d): Lactic acid bacteria, (e): *S. aureus*
* Different lowercase letters show a significant difference at any time point between the three groups (P<0.05).
** Different uppercase letters show a significant difference in each group between seven time points (P<0.05).

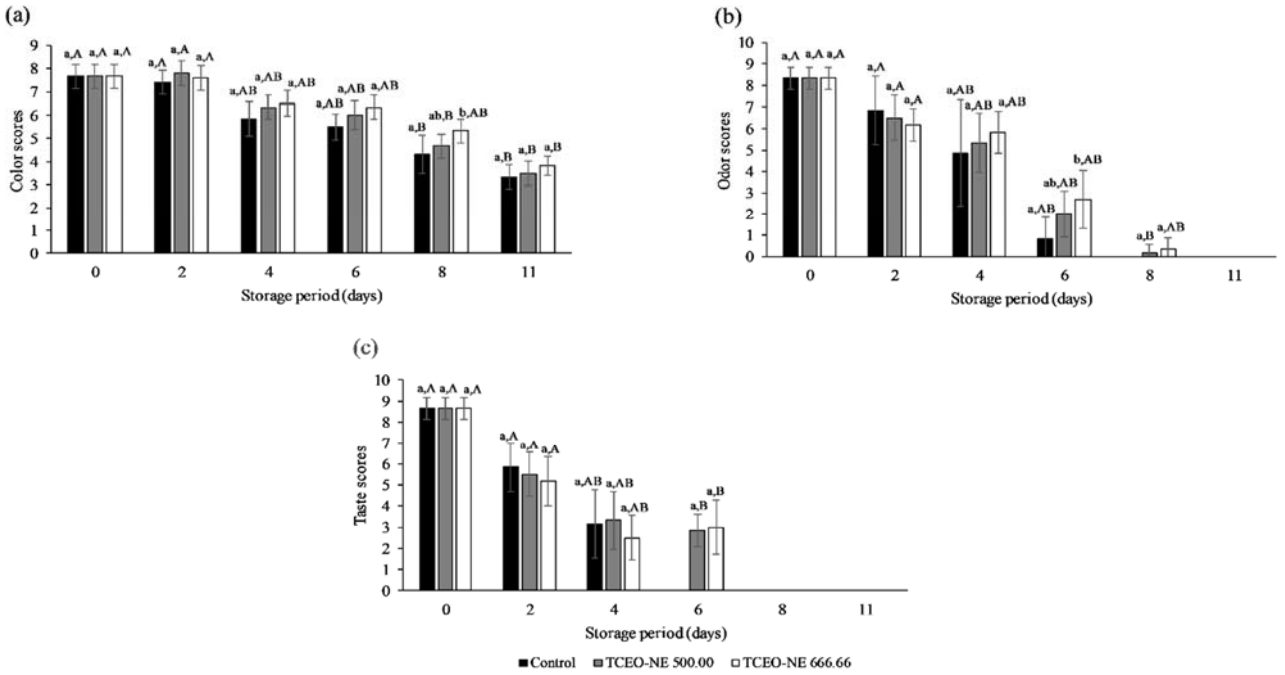

Figure 5. Sensory changes of *O. aguabonita* fillet coated with *T. copticum* EO nanoemulsion stored at 4 °C. (a): Color, (b): Odor, (c): Taste

* Different lowercase letters show a significant difference at any time point between the three groups (P<0.05).

** Different uppercase letters show a significant difference in each group between six time point (P<0.05).

Figure 5 shows the color changes of *O. aguabonita* fillet coated with TCEO-NE and stored at 4 °C. Color index had a decreasing trend during the study period which was more pronounced in TCEO-NE groups than in the control group (P>0.05). But the only significant difference was observed between the color of the control group and TCEO-NE 666.66 just on day 8 (P<0.05).

Figure 5 shows the odor changes in *O. aguabonita* fillet coated with TCEO-NE and stored at 4 °C. The odor index decreased during the storage period and this trend was more intense in the control group, so the control group did not receive any points on days 8 and 11 and TCEO-NE 500.00 and TCEO-NE 666.66 on day 11 (P<0.05).

Figure 5 shows the taste changes in *O. aguabonita* fillet coated with TCEO-NE and stored at 4 °C. In evaluating the taste of fish fillets, the groups containing TCEO-NE scored higher than the control group, so that the control group received no points on days 6, 8 and 11 and the other two groups on days 8 and 11. The taste index showed a significant difference between days 0 and 2 with days 6, 8 and 11 in all three groups (P<0.05).

Discussion and Conclusion

In this study, the major constituent of *T. copticum* EO was thymol (53.17%). In the study of Jebelli Javan et al. (39) and Rabiei et al. (58), the major constituents of *T. copticum* EO were thymol, p-cymene and gamma-terpinene. Goudarzi et al. (30) also showed that the main constituent of *T. copticum* EO was thymol (36.7%). Differences between the constituents of *T. copticum* EO in different studies can be due to the genetic differences, geographical area of growth, plant age, part of the plant and the method used for essential oil extraction, and the type of solvent used in essential oil (11, 58).

According to our study, the droplet size of TCEO-NE was 127.6 nm, and PDI was 0.21. In other works, researchers focused on evaluating critical parameters for preparing stable essential oil nanoemulsions (69, 70). Their optimized formula was used for this study. Generally, oil in water nanoemulsions are very stable and can only be separated into two phases quickly using unique methods such as electric fields (34, 35, 69, 70). Naeim et al. (50) investigated the antifungal effect of *T. copticum* EO and its nanoemulsion against *Aspergillus niger* and its antioxidant properties in hamburgers and reported that the droplet size of the nanoemulsion was 146 nm. Sahraneshin Samani et al. (65) evaluated the effect of thyme and *T. copticum* EOs nanoemulsions in preventing the growth of *Byssochlamys fulva* in apple juice and showed that the droplet size of the nanoemulsion by ultrasound method was 15.13 nm and PDI was 0.253. Ozogul et al. (52) studied the effects of rosemary, laurel, thyme and sage EOs nanoemulsions on the sensory, chemical and microbial quality of rainbow trout fillets and reported the droplet size of the nanoemulsions prepared by the ultrasonic method as 112.82, 66.02, 63.02 and 59.48

nm, respectively. It seems that these differences in droplet size are due to the type of EOs, surfactants and methods of nanoemulsion preparation (19). The droplet size of nanoemulsions is as a criterion for determining their stability. Thus, the larger the droplet size of the nanoemulsion, the less stable the nanoemulsion (48).

We found that the moisture content of fillets was 73.66% on day zero. Askary Sary et al. (9) and Gokoglu et al. (29) reported the moisture content of rainbow trout carcasses as 77.9% and 73.38%, respectively. According to our study, the Ash content in fillets was 1.30% on day zero. Ash content in rainbow trout fillets was reported to be 1.57% and 1.35% (9, 29). In this study, the amount of protein in fillets was 17.50% on day zero. Askary Sary et al. (9) and Gokoglu et al. (29) reported the amount of protein in rainbow trout fillets as 19.46% and 19.80%, respectively. We found that the fat content of fillets was 1.36% on day zero. Askary Sary et al. (9) and Gokoglu et al. (29) reported the average fat content in rainbow trout fillets as 0.83% and 3.44%, respectively. Moisture, ash, protein and fat can vary based on fish species, size, diet, fishing time, spawning cycle, habitat and other environmental conditions (51).

In this study, the initial pH of the fillets was 7.00 on day zero and reached 7.78, 7.51, and 7.39 in the control group, TCEO-NE 500.00 and TCEO-NE 666.66, respectively on day 11. The increasing trend of pH value during the storage period is explained by the accumulation of nitrogen and alkali compounds such as ammonium and trimethylamines resulting from an increase in the number of spoilage bacteria and enzymatic activity (15). Durmus et al. (21) studied the effects of nanoemulsions of several edible oils (hazelnut, corn, canola, soybean, olive and sunflower) on chemical, sensory and microbial changes of vacuum-packed and refrigerated sea bass fillets. The lowest and the highest pH values at the end of the storage period (day 18) belonged to the group containing nanoemulsions of olive oil (6.88) and the control group (7.22), respectively. These differences were attributed to the inhibitory effect of the nanoemulsion on microbial growth. Ozogul et al. (51) reported that the initial pH of the control fish fillets was 6.89 on day zero and reached 7.21 on the final day of storage period (day 24). The lowest pH was reported in the group containing laurel nanoemulsion (7.07).

In the present study, TVB-N has reached the maximum acceptable level on day 4 in the control group and on day 6 in TCEO-NE 500.00 and TCEO-NE 666.66. TVB-N is an important indicator of fish quality and spoilage, which increases due to microbial spoilage and the activity of fish enzymes (61). The maximum acceptable level of TVB-N was 25 mg/100 g fish (28). Raeisi et al. (60) reported that rainbow trout flesh coated with 1.5 and 3% *T. copticum* extract, contained less than the permissible amount of TVB-N for up to days 9 and 15 of storage, respectively. This may be due to a rapid decrease in bacterial population or a decrease in the bacterial capacity for oxidative deamination of non-protein nitrogen compounds or a combination of these two mechanisms.

The maximum permissible level of peroxide in fish fillets for human consumption has been reported to be 10 meq/kg fish meat (57). In this study, the control group exceeded the acceptable limit on day 6 and TCEO-NE 500.00 and TCEO-NE 666.66 on day 8. Peroxide content is an indicator of fat oxidation that is used to measure hydroperoxides (24). Hydroperoxides are of the primary products of oxidation and tasteless compounds that consumers do not recognize. Still, secondary compounds such as aldehydes and ketones cause unpleasant taste and odor in food products (53). Raeisi et al. (60) investigated the antioxidant and antimicrobial effects of shallot (*Allium ascalonicum L.*) and ajwain (*T. ammi* (*L.*) Sprague) seed extract on semi-fried rainbow trout fillets. They found that the amount of peroxide in all treatments increased during the storage period, exceeding the allowable limit in the control group on day 6 and in the groups containing 1.5 and 3% of *T. copticum* seed extract on days 12 and 15, respectively.

TBARS index indicates the amount of secondary metabolites of fat oxidation, especially aldehydes (43). The maximum acceptable amount of TBARS in fish meat is 1-2 mg MDA/kg fish meat (52). We found that among the fillets stored in the refrigerator (4 °C), TCEO-NE 666.66 fillets exceeded the permissible limit on day 11, while the index in the other two groups exceeded the acceptable limit on day 8. Durmus (19) showed that none of the rainbow trout fillets containing citrus EO at the end of the storage period (day 16) exceeded the maximum TBARS level, but the control group exceeded this level on day 12 and reached 2.77 mg MDA/kg fish meat. The amount of TBARS in mandarin and grapefruit EOs reached 1.56 and 1.69 mg MDA/kg fish meat, respectively, which were the lowest amount of TBARS among all groups during the storage period.

In this study, the total aerobic mesophilic count on day zero was 2.47 log cfu/g indicating the fillets' good quality (66). The maximum allowable number of aerobic mesophilic count in fish is 7 log cfu/g (1). In the current study, aerobic mesophilic count in the control group exceeded the allowable level on day 6 and in TCEO-NE 500.00 and TCEO-NE 666.66, on day 8, increasing the shelf life by two days. A lower count of aerobic mesophilic in groups containing TCEO-NE may imply its antibacterial properties and its effective role in reducing the total microbial count. Similarly, Durmus (19) showed that aerobic mesophilic count in rainbow trout fillets was 2.43 log cfu/g on day zero. The lowest level of aerobic

microorganisms at the end of the storage period was observed in the groups containing mandarin (7.43 lof cfu/g) and grapefruit nanoemulsions (7.63 log cfu/g). This amount exceeded the allowable level in the control group on the 10th day. Thus, the use of these nanoemulsions extended the shelf life from 4 to 6 days. Using virgin olive oil nanoemulsion extended the shelf life of lamb loins for 4 days (38). Shadman et al. (68) reported that aerobic mesophilic count in rainbow trout fillets in the control group was 2.31 log cfu/g which is similar to the present study.

One of the main causes of spoilage in fresh fish stored in the refrigerator is psychrotrophic bacteria, which produce compounds such as aldehydes and ketones that cause undesirable changes in taste, texture and odor, thereby reducing fish quality (20). The maximum allowable number of psychrotrophic bacteria is reported to be 7 log cfu/g (20, 66). We found that the number of psychrotrophic bacteria in the control group, TCEO-NE 500.00 and TCEO-NE 666.66 groups exceeded the allowable limit on days 4, 6 and 8, respectively, which may be due to the presence of compounds such as thymol, limonene, alpha-terpinene and other antibacterial compounds in *T. copticum* EO.

Enterobacteriaceae are a health indicator for fish meat and are part of the natural microbial flora of fresh trout (1). In the present study, the initial number of *Enterobacteriaceae* in fillets was 1.97 log cfu/g on day zero and reached 11.61, 10.58, and 8.41 in the control group, TCEO-NE 500.00 and TCEO-NE 666.66, respectively on day 11. Durmus (19) reported the initial number of *Enterobacteriaceae* in rainbow trout fillet to be between 1 to 1.35 log cfu/g and at the end of the storage period (day 16), *Enterobacteriaceae* count in the control group reached 9.67 log cfu/g.

Lactic acid bacteria, as part of the natural microbial flora of fish, are important causes of spoilage in meat products (1, 59). In this study, the number of lactic acid bacteria in the control group at the end of the storage period was approximately 9 log cfu/g, but in TCEO-NE 666.66, this number was approximately 5.5 log cfu/g. Pasbani and Amiri (56) showed that the number of lactic acid bacteria in beef in the control group at the end of the storage period increased by approximately 3 log cfu/g, while in the group containing thymol seed lipid nano-particles and aloe vera coating, this increase was approximately 1.4 log cfu/g.

We found that the initial number of *S. aureus* in fillets was 2.19 log cfu/g on day zero and reached 10.54, 8.66, and 8.35 in the control group, TCEO-NE 500.00 and TCEO-NE 666.66, respectively on day 11. Jebelli Javan et al. (39) concluded that *T. ammi* EO and ethanolic extract of propolis, especially when used in combination, reduce the growth rate of some foodborne pathogens such as *S. aureus* which can be caused by compounds such as thymol and carvacrol.

The color of fish is a remarkable criterion for consumers and can indicate freshness or spoilage. Changes in the color of fish tissue are largely due to reactions such as oxidation and Maillard, which are preventable by the presence of a strong antioxidant hindering discoloration by making oxygen unavailable (41). In the present study, the most desirable group during the storage period in terms of color was TCEO-NE 666.66. Khoshbouy Lahidjani et al. (41) reported that the color index of rainbow trout scored by panelists decreased over time, so that fish color in the control group reached an undesirable level after 10 days, but the groups containing the curcumin nanoemulsion coating had an acceptable color even after 15 days.

The odor index decreases with increasing TVB-N and the production of products such as dimethylamine, trimethylamine, ammonia and other volatile nitrogen compounds. Moreover, the increasing psychrotrophs count has a negative effect on the odor index (31). In this study, from the 4th day of storage, the control group received lower scores in the odor test as TVB-N increased and the number of psychrotrophs exceeded their allowable limit. Similarly, Khoshbouy Lahidjani et al. (41) reported that the rainbow trout fillets in the control group had an unpleasant odor from day 5 onwards, which was in line with increasing TVB-N and exceeding the allowable limit on day 5. Furthermore, the presence of nanoemulsion coating of curcumin during the storage period caused a significant difference with the control group so that the presence of curcumin at the level of 5% was able to prevent the formation of unpleasant odors during the storage period.

The results of the present study showed that the groups containing nanoemulsions received higher taste scores from evaluators than the control group, and the most desirable group during the maintenance period was TCEO-NE 666.66. Durmus (19) reported that the use of nanoemulsions had a positive effect on fish taste. The groups containing nanoemulsions received higher scores from the evaluators during the storage period than the control group. Among nanoemulsions, those of mandarin and grapefruit EOs received the highest scores.

Our results showed that the treatment groups and the control group exceeded TVB-N limit on day 6 and on day 4, respectively and the treatment groups and the control group exceeded peroxide limit on day 8 and on day 6 respectively. TBARS index in TCEO-NE 666.66 exceeded the allowable limit on day 11, while in the other two groups, it exceeded the acceptable level on day 8. Based on total aerobic mesophilic count, the use of TCEO-NE coating has extended the shelf life of fish fillets by two days. These effects can be attributed to thymol, limonene

and alpha-terpinene in *T. copticum* EO. The color, odor and taste index had a negatively decreasing trend during the study period and was higher in the groups coated with TCEO-NE at all times than in the control group. The results showed that due to the properties of TCEO-NE and its positive effects on chemical, microbial and sensory changes of *O. aguabonita* fillets, this compound could be used as a natural food additive to increase shelf life.

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Conflict of Interest

The authors declared that there is no conflict of interest.

Author Contributions

RP designed the study and wrote the manuscript. MH and MHS carried out the experiments. All authors provided critical feedback and helped shape the research, analysis and manuscript.

Data Availability Statement

The data supporting this study's findings are available from the corresponding author upon reasonable request.

Ethical Statement

This study does not present any ethical concerns.

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