Journal of Food Safety

Journal of Food Safety ISSN 1745-4565

EFFECT OF CHITOSAN EDIBLE COATING ENRICHED WITH EUCALYPTUS ESSENTIAL OIL AND α-TOCOPHEROL ON SILVER CARP FILLETS QUALITY DURING REFRIGERATED STORAGE

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Received for Publication November 14, 2015
Accepted for Publication May 3, 2016

doi: 10.1111/jfs.12295

ABSTRACT

The effects of chitosan coating incorporated with eucalyptus essential oil and α-tocopherol on the quality of silver carp fillet during chilled storage (4 + 1°C) were examined over a period of 16 days. The control samples (without any coating), chitosan coating (CH), chitosan coating incorporated with eucalyptus essential oil (CH+EEO) and chitosan coating incorporated with eucalyptus essential oil and α-tocopherol (CH+EEO+α) were analyzed by chemical, microbiological and sensory characteristics. Results showed that, total viable counts, total psychrotrophic counts and total volatile basic nitrogen of CH+EEO and CH+EEO+α were significantly lower than control and CH ($P \leq 0.05$). Addition of α-tocopherol to EEO retarded lipid oxidation. According to the sensory analysis results, CH+EEO+α was acceptable even at the end of the 16-day storage. The results indicated that chitosan coating was improved extending of meat shelf life. Using eucalyptus essential oil and α-tocopherol increased its efficiency significantly.

PRACTICAL APLLICATIONS

Fresh fish are usually more perishable than most other muscle foods. Many attempts have been made to prevent the spoilage of fresh fish. To avoid the use of synthetic food additives, numerous studies are currently focused on using natural products to improve the quality of sea food products. The results of the present study report the efficacy of chitosan edible coating enriched with eucalyptus essential oil and α-tocopherol as a new natural preservative for developing the shelf life of silver carp fillets.

INTRODUCTION

Silver carp (*Hypophthalmicthys molitrix*) is one of the most important economic freshwater fish species cultured in eastern countries due to its fast growth rate, easy cultivation, high feed efficiency ratio and high nutritional value (Fan *et al.* 2009). However, silver carps are highly susceptible to quality deterioration caused by both microbiological and chemical spoilage and lipid oxidation of their highly unsaturated fatty acids, catalyzed by the presence of high concentrations of hematin compounds and metal ions in their muscle (Decker and Hultin 1992).

In order to protect lipids, microorganism and avoid deterioration of appearance, seafood product manufacturers in the past few decades have used several food additives with antioxidative and antimicrobial properties. The increasing consumer awareness and health consciousness, however, results in pressure to avoid the use of synthetic additives, which necessitates the use of natural additives or alternative methods to extend shelf life and improve safety (Georgantelis *et al.* 2007). One such solution could be the use of natural antioxidants and antimicrobial. Many studies have categorized a broad range of plant essential oils that have

antioxidant and antibacterial properties (Sánchez-González *et al.* 2011). These compounds can be used directly to help preserve refrigerated fish. Eucalyptus essential oils (EEO) have been used in traditional medicine. Their antimicrobial activity has been reported and their ability to inhibit the growth of other organisms has resulted in the use of leaves in grain storage and their proposed use as potential natural herbicides (Dellacassa *et al.* 1990). α-tocopherols are effective natural antioxidants for lipid containing foods. α-tocopherol behaves like a chain-breaking electron donor antioxidant by competing with the substrate for the chain-carrying peroxyl radicals. Moreover, α-tocopherol has also been associated with retarding the decomposition of hydroperoxides (Georgantelis *et al.* 2007).

Despite the great potential of essential oils, their use in food preservation remains limited mainly due to their intense aroma and toxicity problems. To minimize the required doses, one interesting option would be the use of edible coatings as vehicles of these natural compounds. Edible coatings have recently gained more interest in the field of food preservation due to the promising results obtained (Sánchez-González *et al.* 2011). Chitosan coatings from polysaccharides, proteins and lipids can extend the shelf life of foods by functioning as solute, gas and vapor barriers. It has been reported to have a number of functional properties that make chitosan useful in nutrition these include its antimicrobial activity and its ability to form protective films or coatings its binding action and antioxidant activity (Ojagh *et al.* 2010).

Thus, the present study was aimed to investigate the effect of chitosan coating incorporated with eucalyptus (*Eucalyptus globulus* Labill) essential oil and α-tocopherol on the quality of silver carp fillet during storage at refrigeration condition (4 ± 1°C).

MATERIALS AND METHODS

Materials

Food grade EEO extracted by the hydrodistillation method, was obtained from Barij Essence Pharmaceutical Co., Kashan, Iran, and stored in a dark container at 4°C until use. Medium molecular weight chitosan (450 KDa) was purchased from Sigma Aldrich Chemical Co. Polyethylene glycol and Tween 80 was acquired from Merck (Frankfurt, Germany). All other chemicals were analytical grade.

Gas Chromatography-Mass Spectrometry (GC-MS) Analysis of EEO

EEO was analyzed using a Varian 3400 GC-MS(Agilent) system equipped with a DB-5 fused silica column (30 m × 0.25 mm, film thickness 0.25 μm, J and W Scientific Corporation); the oven temperature was 50–260°C at a rate of 4°C min^{-1}. The transfer line temperature was 270°C, carrier gas, helium at a linear velocity of 31.5 cm/s, split ratio 1:60, ionization energy 70 eV, scan time 1 s, mass range 40–300 amu (Ariaii *et al.* 2015).

Coating Preparation

Chitosan solution was prepared with 2% (w/v) chitosan in 1% v/v acetic acid. To achieve complete dispersion of chitosan, the solution was stirred at room temperature (25 ± 2°C) for 3 h. The solution in beakers was placed on a hotplate/magnetic stirrer, and glycerol was added to chitosan at 0.75 mL/g concentration as a plasticizer and stirred for 10 min. The resultant chitosan coating solution was filtrated through a Whatman No. 3 filter paper to remove any undissolved particles. Then the eucalyptus oil and α-tocopherols, mixed with Tween 80 (Aldrich Chemical Co., Steinheim, Germany), to help distribute and completely incorporate the eucalyptus oil and α-tocopherols, was added to the chitosan solution. The final coating forming solution consisted of 2% chitosan, 1% acetic acid, 0.75% glycerol, 0.2% Tween 80, 1% eucalyptus oil and 0.04% α-tocopherols (α). The final coating forming solution was homogenised under aseptic conditions at 21,600 rpm for 1 min (Model D500, Wiggenhauser Machinenbau, 10965 Berlin, Germany). The control solution was prepared without addition of eucalyptus oil and α-tocopherols (Ojagh *et al.* 2010). The solution was kept overnight at 4°C in order to remove all bubbles.

Fish Sample Preparation and Storage Condition

Fresh silver carp, varying from 900 to 1,100 g in weight were purchased at a public market alive and transferred to the laboratory at the Islamic Azad University of Amol (Ayatollah Amoli branch) in Iran, in sealed foamed polystyrene boxes containing flaked ice. Then, the fishes were killed by slurry ice, skinned, filleted and washed by tap water in a laboratory. Fillet samples (100 ± 10 g fish in each group) were randomly assigned into four treatment lots consisting of one control lot (uncoated) and three lots treated with the coating solutions: chitosan (CH), CH+ 1% EEO, CH+ 1% EEO+ 0.4% α. For each treatment, approximately 15 fillets were immersed for 30 s in 500 mL of the coating solution. Then they stood for 2 min, followed by a second immersion in the coating solution for 30 s. Next, the fish fillets were removed and allowed to drain for 5 h at 4°C on a presterilized metal net under a biological containment hood in order to form the edible (Ojagh *et al.* 2010), then stored at 4 ± 1°C for 16 days. Chemical, microbiological and sensorial analyses were performed at 4 day intervals to determine the overall quality of fish.

Proximate Composition Analyses

The moisture content and crude ash were determined in an oven at 103 and 550°C, respectively, until the weight became constant. The total crude protein was determined by Kjeldahl's method (AOAC 2005) and the lipid content was analyzed according to the procedure of Bligh and Dyer (1959).

Chemical Analyses

The pH was determined by homogenizing 5 g of the fish sample in 45cc of distilled water with Ultra-Turrax (IKA T25-Digital Ultra-Turrax, Staufen, Germany) at 3,000 rpm for 30 s. Then, the pH was measured with a digital pH meter.

The peroxide value (PV) was determined in the total lipid extracts according to the method of Pearson (Egan *et al.* 1997). Results were expressed in meqO_2/kg.

The thiobarbituric acid (TBA) value was determined colorimetrically by the method as described by Kirk and Sawyer (1991) and expressed as mg malondialdehyde/kg sample.

Free fatty acid (FFA) was estimated by the procedure explained by AOAC (2005) and its content was expressed as percentage of oleic acid.

The total volatile basic nitrogen (TVB-N) of the fish samples was determined by the micro-diffusion method as described by Goulas and Kontominas (2005). Results were expressed in mg N/100 g of fish.

Microbiological Analysis

Bacteriological counts were determined by placing a 10 g sample in 90 mL of 0.85% NaCl solution, and homogenizing it with a stomacher. Total viable count (TVC) and total psychrotrophic count (TPC) determined by the pour plate method, using plate count agar (PCA, Merk, Darmstadt, Germany). The inoculated plates were incubated at 37°C for 2 days for TVC and at 10°C for 7 days for TPC. All counts were expressed as log colony-forming units (cfu) g^{-1} and performed in triplicate (Ibrahim-Sallam 2007).

Sensory Evaluation

The sensory quality of the silver carp fillets during 16 days of preservation was based on a 5-point scale. A six member trained panel were asked to judge the texture (5, firm; 1, very soft); color discoloration (5, no discoloration; 1, extreme discoloration); odor (5, extremely desirable; 1, extremely unacceptable/off-odors); and overall acceptability (5, extremely desirable; 1, extremely unacceptable) of the samples. The fish samples were defined as unacceptable when the sensory attributes declined below 4.0 (Ojagh *et al.* 2010).

TABLE 1. PERCENTAGE OF CHEMICAL COMPOUNDS IDENTIFIED IN EUCALYPTUS ESSENTIAL OILS (EEO)

Peak	Compound	Gc area (%)
1	1,8-Cineol	66.28
2	α-Pinene	12.31
3	Limonene	9.24
4	α-Phellanderene	1.89
5	β-Pinene	0.93
6	Camphor	0.84
7	Sabinene	0.72
8	Linalool	0.62
9	α-Terpineol	0.47
10	Borneol	0.35
11	4-Terpineol	0.09
12	Fenchol	0.07

Statistical Analysis

One-way ANOVA was used and mean comparison was performed by Duncans' new multiple range test. Statistical analysis was prepared using the SPSS statistical software, (release 18.0) for Windows (SPSS Inc., Chicago, IL). All data are presented as mean ± SD. Significant differences were considered at the 95% confidence level ($P \leq 0.05$).

RESULTS AND DISCUSSION

Chemical Composition of EEO

Twelve components were identified representing 93.81% of EEO. The qualitative and quantitative of EEO compositions are in Table 1. The major constituents of EEO were 1,8-Cineol (66.28%), α-Pinene (12.31%) and Limonene (9.24%). The results are in agreement with the results of Boukhatem *et al.* (2014) and Barra *et al.* (2010) that they all distinguish Eucalyptus essential oil is rich with 1,8-Cineol.

Proximate Composition

The composition of fish can affect the sensory characteristics that strongly influence the acceptability of fish as food. It may also affect microbial growth (Ibrahim-Sallam 2007). The proximate composition of fresh silver carp samples at day 0 showed 17.67 ± 0.58% crude protein, 76.62 ± 0.36% moisture, 3.22 ± 0.45% crude fat and 1.47 ± 0.03% ash. The proximate composition of the silver carp reported in different studies (Zhang *et al.* 2009; Ariaii *et al.* 2015) showed some differences especially for the lipid content. Such variations in the chemical composition of fish is strongly related to the nutrition, fish size, sex, age, environment and catching season (spawning cycles) (Ibrahim-Sallam 2007).

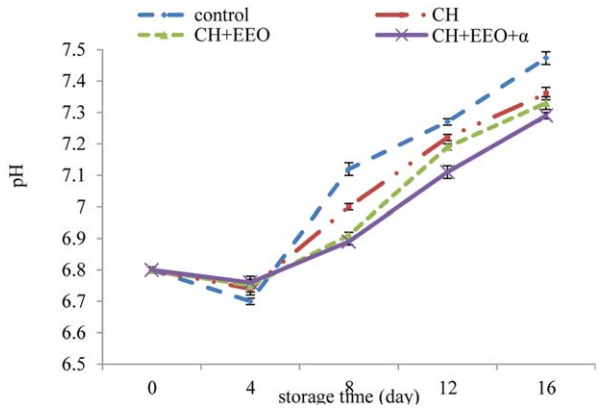

FIG. 1. CHANGES IN PH OF COATED FILLETS DURING STORAGE (C: CONTROL, CH: COATED WITH CHITOSAN, CH+EEO: COATED WITH CHITOSAN AND EUCALYPTUS ESSENTIAL OIL, CH+EEO+α: COATED WITH CHITOSAN, EUCALYPTUS ESSENTIAL OIL AND α-TOCOPHEROL)

Assessment of Biochemical Spoilage

pH. pH values for control, CH, CH+ EEO and CH+ EEO+α for silver carp fillet are shown in Fig. 1. The initial pH of silver carp samples was 6.8. According to Abdollahi *et al.* (2014), the initial pH of fresh silver carp fillets was 6.79 while Ariaii *et al.* (2015) and Fan *et al.* (2013) reported initial pH of silver carp fillet equal to 6.2 and 6.3, respectively. In all fish samples, the values of pH initially decreased and then increased. The initial pH decrease may be attributed to the dissolution of CO_2 in the fish sample, while the increase of pH was postulated to be due to an increase in volatile bases produced, e.g., ammonia and trimethylamine, by either endogenous or microbial enzymes (Manat *et al.* 2005). The pH values of coated samples significantly ($P < 0.05$) lower than the control samples during storage time. The lowest pH value at the end of storage period was observed in CH+ 1% EEO and CH+ 1% EEO+α. The observed significant difference may be related to the antibacterial properties of EEO which prevent production of alkaline compounds and contributes to the extending of the preservation of fish samples by prohibiting the activity of the endogenous proteases (Fan *et al.* 2009).

Lipid Oxidation of Fish Fillets. The PV provides a measure of the hydroperoxides which are the primary products of auto-oxidation and they are odorless. However, their decay leads to the formation of a wide range of carbonyl compounds, hydrocarbons, furans and other products that contribute to the rancid taste of decaying food (Yanishlieva and Marinova 2001). The PV values of treatments during the refrigerated storage are shown in Fig. 2a. PV of fresh fish was approximately 0.89 meqO_2/kg sample. The PV values of the control and coated samples increased significantly

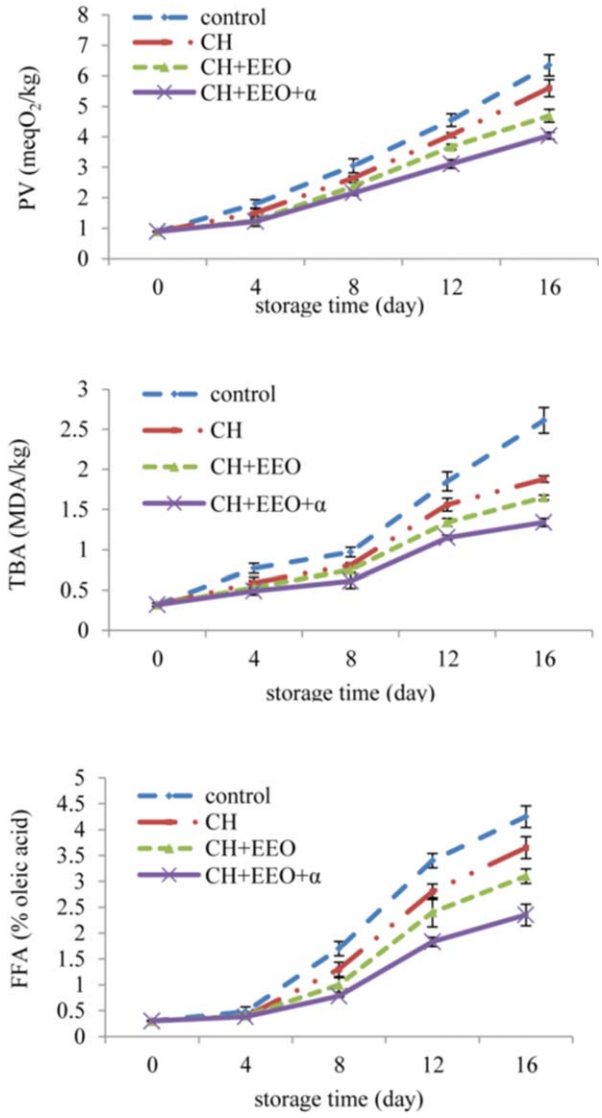

FIG. 2. CHANGES IN PEROXIDE VALUE (PV) (a), THIOBARBITURIC ACID (TBA) (b) AND FREE FATTY ACID (FFA) (c) OF COATED FILLETS DURING STORAGE (C: CONTROL, CH: COATED WITH CHITOSAN, CH+EEO: COATED WITH CHITOSAN AND EUCALYPTUS ESSENTIAL OIL, CH+EEO+α: COATED WITH CHITOSAN, EUCALYPTUS ESSENTIAL OIL AND α-TOCOPHEROL)

($P < 0.05$) with storage time; by the end of the storage time (day 16), PV in coated samples significantly lower than in the control. Chitosan coatings have low oxygen permeability, which produce an oxygen-resistant layer on the surface of fish fillets and decrease lipid oxidation and PV (Mohan *et al.* 2012; Abdollahi *et al.* 2014). Samples treated with CH+EEO showed lower (maximum 5.6 meqO_2/kg) PV in comparison with chitosan-coated samples (maximum 4.69 meqO_2/kg). It may be attributed to antioxidant ability of eucalyptus which is related to its phenolic compounds such as 1,8-cineole, α-Pinene, etc. are considered to have antioxidant ability

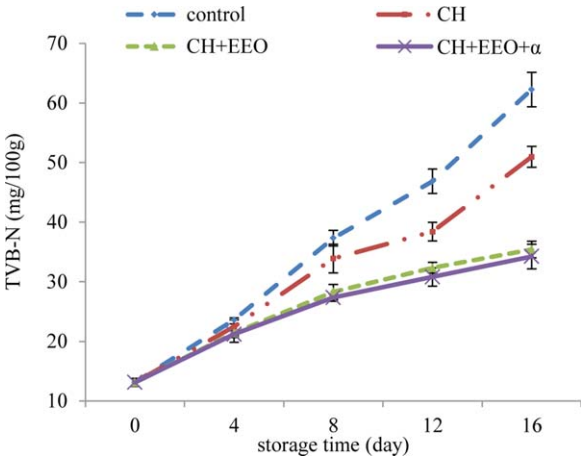

FIG. 3. CHANGES IN TOTAL VOLATILE BASIC NITROGEN (TVB-N) OF COATED FILLETS DURING STORAGE (C: CONTROL, CH: COATED WITH CHITOSAN, CH+EEO: COATED WITH CHITOSAN AND EUCALYPTUS ESSENTIAL OIL, CH+EEO+α: COATED WITH CHITOSAN, EUCALYPTUS ESSENTIAL OIL AND α-TOCOPHEROL)

(Barra *et al.* 2010). However, the lowest PV during the storage period was observed in the samples coated with CH+EEO+α ($P < 0.05$), consequently α-tocopherol had more efficient inhibitor effect on primary oxidation. All the tocopherols contain a hydroxyl-bearing aromatic ring structure, which enables them to donate hydrogen to free radicals and thus act as biological antioxidants (Kamal-Eldin and Appelqvist 1996). In this way, chain reactions initiated by hydroxy radicals and could be broken by the formation of a stable radical as a result of interaction with α-tocopherol (Tolouie *et al.* 2013). Similar results were observed by others (Tolouie *et al.* 2013; Abdollahi *et al.* 2014; Ariaii *et al.* 2015).

TBA value has been widely used to reckon the degree of lipid oxidation and the presence of TBA reactive substances is on account of the second stage auto-oxidation during which peroxides are oxidized to aldehyde and ketone (Li *et al.* 2012). In the present study, changes in TBA values of different treatment groups were shown in Fig. 2b. The TBA values of all treatment increased during storage. The increase in TBA value during the chilled storage may be attributed to the partial dehydration of fish and to the increased oxidation of unsaturated fatty acids (Mendes *et al.* 2008). According to Connell (1990), a TBA value of 2 mg MDA/kg is regarded as the acceptability limit. The initial TBA value of the fish samples was 0.332 mg MDA/kg and this value increased to 2.61, 1.88, 1.65 and 1.34 mg MDA/kg at the end of storage for the Control, CH, CH+EEO and CH+EEO+α, so only control samples exceeded the maximal permissible limit. Chitosan has been well-known for its good film-forming property and excellent compatibility with other substances (Duan *et al.* 2010). In this study, incorporation of EEO and α-tocopherol into chitosan enhanced the antioxidant properties of coating and improved inhibitory effects of coating on TBA formation. The use of chitosan films or coatings to incorporate antimicrobials, antioxidants and nutrients in food systems has been reported in several previous studies (Duan *et al.* 2010; Ojagh *et al.* 2010; Li *et al.*, 2012; Tolouie *et al.* 2013; Abdollahi *et al.* 2014).

The presence of FFAs is caused by the hydrolysis of lipids. Therefore, quantifying the FFAs percentage can be profitably used as an index of the degree of lipolysis, which in turn is an indicator of the fish freshness (Andevari and Rezaei 2011).FFA values of treatments during the refrigerated storage are shown in Fig. 2c. FFA of fresh fish was approximately 0.31% oleic acid sample, until it reached a maximum (Control = 4.25, CH = 3.65, CH+EEO = 3.01 and CH+EEO+α = 2.25) on the day 16. A gradual increase in FFA content was observed in all treatments during storage period. The overall increase displays hydrolytic oxidation in the fillets caused by internal or bacterial enzymes and the decrease may be related to the interaction of triacylglyceride products with proteins (Pereira de Abreu *et al.* 2011). The lower content of FFA in the samples treated with the essential oil may be due to the influence of eucalyptus on meat enzymes and their activity (Silva and Ammerman 1993), the activity of it was improved when it combined with α-tochophorol. The results coincide with results of Irfan Aksu (2007) for sliced, vacuum packaged kavurma which observed lower content of FFA in samples treated with α-tochophorol.

All in all, the result of lipid oxidation of silver carp fillets were in agreement with those reported by Georgantelis *et al.* (2007) about the effect of rosemary extract, chitosan and a-tocopherol on lipid oxidation of beef burgers during frozen storage. They reported that, combined use of chitosan with both rosemary and α-tochophorol had positive effects on lipid oxidation.

Total Volatile Basic Nitrogen. TVB-N, which is mainly composed of ammonia and primary, secondary and tertiary amines, is widely used as an indicator of fish deterioration. Its increase is related to the activity of spoilage bacteria and endogenous enzymes (Fan *et al.* 2009). TVB content of treatments during the refrigerated storage are shown in Fig. 3. TVB content of all samples at day 0 was approximately 13.11 mg N/100 g, indicating the good quality of the fresh samples. The concentration of TVB-N in freshly caught fish is typically between 5 and 20 mg N/100 g, whereas levels of 30–35 mg N/100 g fish are generally regarded as the limit of acceptability for fish (Connell 1995). The TVB-N content increased in all treatments during storage. It exceeded the limit by day 4 for control and by day 8 for CH coated samples. This fact was indicative of either a faster reduction of bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds (or both) due to the effect of chitosan in the fish samples

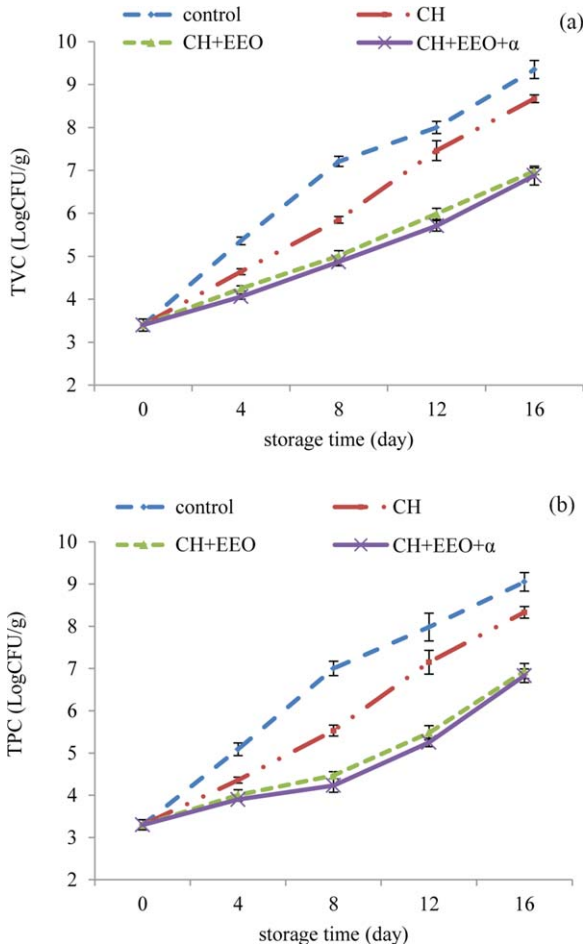

FIG. 4. CHANGES IN (a) TOTAL VIABLE COUNT (TVC) AND (b) TOTAL PSYCHROTROPHIC COUNT (TPC) OF COATED FILLETS DURING STORAGE (C: CONTROL, CH: COATED WITH CHITOSAN, CH+EEO: COATED WITH CHITOSAN AND EUCALYPTUS ESSENTIAL OIL, CH+EEO+α: COATED WITH CHITOSAN, EUCALYPTUS ESSENTIAL OIL AND α-TOCOPHEROL)

(Fan *et al.* 2009). At the end of storage period, samples coated CH+EEO and CH+EEO+α reached a significantly ($P < 0.05$) lower TVB-N value of 35.41 and 34.23 in comparison with the others. Its value remained lower than acceptable limit for these samples until 16 of storage, respectively. The lower value of TVB-N observed in samples coated CH+EEO can be related to the antibacterial activity of the essential oil which would more rapidly reduce bacterial population and this could be due to the decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds (Banks *et al.* 1980).

Microbial Analysis

It has been estimated that about one-third of the world's food production is lost annually on account of microbial spoilage (Abdollahi *et al.* 2014). Variation in TVC and TPC of the samples treated with different treatments during the refrigerated storage are shown in Fig. 4a,b. TVC and TPC of silver carp samples were initially low (3.43 and 3.31 log10 cfu/g, respectively), indicating the high quality of fish fillets used in this study (ICMSF 1986). Both TVC and TPC of all samples increased with storage time and the value of control increased faster and exceeded the maximum 10^7 $\log_{10}$c cfu/g after 8 day. This acceptability limit of 10^7 cfu/g has been recommended for fresh fish (ICMSF 1986). As can be seen, all coated samples significantly inhibited the growth of mesophilic bacteria compared with the control during the storage period. At the end of storage period, samples coated CH+EEO and CH+EEO+α reached a significantly ($P < 0.05$) lower TVC value (6.98 and 6.88 cfu/g, respectively) in comparison with the others. It also had a similar trend about TPC of fillets stored at 4 ± 1°C. Some components of eucalyptus like components such as 1,8-cineole, limonene, linalool have shown antibacterial properties (Nezhad *et al.* 2009). However, addition of EEO into the chitosan caused to it be released to the surface of coated fillets and can keep its inhibitory effect for a longer period.

In this study, α-tocopherol doesn't have antibacterial activity; similar results were observed by the other researches (Cheah and Gan 2000; Tolouie *et al.* 2013).

Sensory Evaluation

Table 2 shows sensory score for the texture, odor, color and overall acceptability of the silver carp fillets during the storage period. Initially, all samples had a bright and acceptable appearance. Tang was not reported by any of the panelists. Therefore, the application of EEO and α in combination with CH does not negatively affect sensory properties of fish products. The sensory scores of all treatment samples decreased with storage time. The fish samples were considered to be acceptable for human consumption until the sensory score reached 4 (Ojagh *et al.* 2010). According to the results of the sensory analysis acceptability of control samples were given "unacceptable" scores by the 4th day, 8th day for samples coated with CH, 12th day for samples coated with CH+EEO and 16th day for samples coated with CH+EEO+α. In general, application of EEO and α in combination with CH could improve sensory properties of the silver carp fillets, it can be at first related to the barrier properties of the coating and then the antibacterial and antioxidant properties of the essential oil and α-tocopherol which has prevented lipid oxidation and microbial deterioration in the samples (Abdollahi *et al.* 2014).

CONCLUSIONS

In the present work, the effect of combination eucalyptus essential oil and α-tocopherol with chitosan on the quality of

TABLE 2. CHANGES IN THE SENSORY ATTRIBUTES OF COATED FILLETS DURING STORAGE

		Storage period (days)				
Sensory attributes	Treatment	0	4	8	12	16
Texture	C	5.00 ± 0.00a	4.10 ± 0.30b	3.50 ± 0.64 c	2.10 ± 0.26d	1.03 ± 0.33d
	CH	5.00 ± 0.00a	4.60 ± 0.27a	4.20 ± 0.59b	3.90 ± 0.64c	2.23 ± 0.39c
	CH+EEO	5.00 ± 0.00a	4.70 ± 0.45a	4.70 ± 0.32a	4.20 ± 0.62b	3.75 ± 0.20b
	CH+EEO+α	5.00 ± 0.00a	4.70 ± 0.42a	4.50 ± 0.28a	4.30 ± 0.27a	4.00 ± 0.11a
Odor	C	5.00 ± 0.00a	4.40 ± 0.49b	3.20 ± 0.12c	2.63 ± 0.06d	1.24 ± 0.55d
	CH	4.90 ± 0.10a	4.68 ± 0.23a	3.90 ± 0.82b	2.90 ± 0.64c	2.20 ± 0.43c
	CH+EEO	4.80 ± 0.12a	4.70 ± 0.26a	4.40 ± 0.66a	4.00 ± 0.37b	3.65± 0.12b
	CH+EEO+α	4.75 ± 0.20a	4.70 ± 0.00a	4.60 ± 0.38a	4.40 ± 0.28a	4.00 ± 0.19a
Color	C	5.00 ± 0.00a	4.20 ± 0.20b	2.80 ± 0.37c	2.06 ± 0.03d	1.00 ± 0.12d
	CH	5.00 ± 0.00a	4.80 ± 0.40a	3.90 ± 0.75b	2.60 ± 0.74c	2.16 ± 0.08c
	CH+EEO	5.00 ± 0.00a	4.70 ± 0.47a	4.60 ± 0.49a	4.00 ± 0.54b	3.96 ± 0.08b
	CH+EEO+α	5.00 ± 0.00a	4.90 ± 0.10a	4.90 ± 0.28a	4.20 ± 0.51a	4.10 ± 0.10a
Overall	C	5.00 ± 0.00a	4.23 ± 0.25b	3.06 ± 0.48c	3.00 ± 0.51c	1.52 ± 0.43d
	CH	4.90 ± 0.14a	4.65 ± 0.25a	4.00± 0.12b	3.53 ± 0.64b	2.60 ± 0.53c
	CH+EEO	4.85 ± 0.21a	4.80 ± 0.26a	4.35 ± 0.66a	4.10 ± 0.27a	3.75 ± 0.22b
	CH+EEO+α	4.80 ± 0.14a	4.70 ± 0.25a	4.50 ± 0.38a	4.40 ± 0.28a	4.10 ± 0.19a

a–c With different small letters in the same row, represents significant difference ($P \leq 0.05$). (C, control; CH, coated with chitosan; CH+EEO, coated with chitosan and eucalyptus essential oil; CH+EEO+α, coated with chitosan, eucalyptus essential oil and α-tocopherol).

silver carp fillet was studied. CH+EEO and CH+EEO+α had lower bacterial count, TVB-N in comparison to samples coated with chitosan and control samples. CH+EEO+α treatment could maintain silver fillet shelf life till 16 days without any significant loss of texture, odor, color or overall acceptability and it had lower lipid oxidation rate in comparison to the others. Eucalyptus essential oil has shown good antibacterial activity and the antioxidant activity of it was improved when it combined with α-tochophrol. Therefore, chitosan coating enriched with eucalyptus essential oil and α-tocopherol provides an active coating that improves food preservation and shelf-life extension.

ACKNOWLEDGMENT

The authors are thankful to Barij Essence Co. Ltd., Iran for participation in providing and analyzing the EEO.

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