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Enhancing *Hypophthalmichthys molitrix* Fillet Shelf Life with Innovative Chitosan-Coated *Cuminum Cyminum* Essential Oils

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ABSTRACT

This study investigated the effects of applying a chitosan coating with *Cuminum cyminum* L. essential oil (CCEO) as an antioxidant and antibacterial agent on the quality and duration of storage of silver carp fillets in a refrigerator at 4°C temperature. The chitosan coating was made with varying concentrations of CCEO, specifically 0%, 0.3%, 0.45%, and 0.6%. The antioxidant and antibacterial properties of the packaged fillet sample were assessed and tested at 4 ◦C for 0, 1, 3, 5, 7, 9, 11, 13, and 15 days. After fifteen days, the amounts of TVC (total viable counts), EBC (enterobacteriaceae), and LAB (lactic acid bacteria) were significantly lower when chitosan coating with 0.6% CCEO was used compared to other treatments ($P < 0.05$). The logarithmic colony-forming unit (CFU) counts per gram for total viable count (TVC), enterobacteriaceae count (EBC), and lactic acid bacteria (LAB) were 4.86, 4.96, and 4.02, respectively. The application of chitosan coating in conjunction with 0.6% CCEO effectively suppressed the increase in thiobarbituric acid (TBA), total volatile base nitrogen (TVB-N), peroxide value (PV), and pH levels in the silver carp fillets. The results indicate that applying a chitosan coating with CCEO can extend the shelf life of silver carp fillets when stored in refrigerated conditions for up to 15 days without adversely affecting their taste, aroma, or texture. Our research has shown that applying a chitosan coating with CCEO can significantly extend the freshness of silver carp fillets when kept in the refrigerator.

Keywords: Silver carp, Shelf life, Chitosan, Coating, *Cuminum cyminum*, Essential oil

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INTRODUCTION

The rising popularity of fish and seafood can be attributed to their exquisite taste and numerous health advantages.

The global seafood market has had consistent growth, amounting to USD 159,312 million in 2019, with projections indicating an increase to USD 193,914 million by 2027. Fish is a vital source of macronutrients, including fats, proteins, and carbohydrates, as well as micronutrients, such as minerals and vitamins. Its high nutritional value is fostering an increasing need for economical, convenient, and ready-to-cook fish products, hence stimulating innovation [1].

Over the past few decades, the production and consumption of silver carp (*Hypophthalmicthys molitrix*) have significantly risen in numerous nations. The silver carp plays a crucial role in the polyculture system of Eastern countries [2].

Whole, fresh fish and fillets are the primary types of fish available in the marketplaces [3]. Recently, consumers' attitudes toward food items have experienced notable shifts, primarily due to concerns about the potential toxicity of artificial preservatives. There has been a growing interest in using essential oils (EOs) as organic green preservatives instead of chemical preservatives in food. This is because EOs have high antibacterial, antiviral, antifungal, and antioxidant properties [4, 5].

As a result, disrupting the bacterial enzyme system causes an increase in ion permeability and the leakage of essential cell components [6].

Cuminum cyminum L. (Apiaceae) is a little plant that reaches a height of up to 40 cm. People have grown it for its seeds and essential oils for many centuries. India, Turkey, Iran, Pakistan, and China use cumin in both cuisine and traditional medicine, making it one of the spices with the broadest worldwide distribution [7].

Recently, there has been a growing apprehension regarding the use of plastics and their detrimental impact on the environment.

To address this challenge, various food protection techniques have emerged, including the incorporation of antimicrobial compounds into edible films and coatings to preserve food [8]. Edible coatings are thin layers that are applied to the surfaces of meals. It is a highly effective method for slowing down the growth of microorganisms and preserving the physical and chemical characteristics of food [9].

Conventional techniques for coating food involve submerging the food in the coating mixture and applying the mixture to the surface of the meal via spraying [10].

Chitosan, a natural polysaccharide, is commonly utilized in food manufacturing, particularly in biopolymer packaging, because of its polyelectrolyte nature and unique characteristics [11].

A lot of studies have shown that using chitosan coating helps with the controlled release of bioactive substances that are lipophilic. Essential oils are a mixture of secondary metabolites from plants that are very volatile and lipophilic.

Additionally, chitosan coating has the potential to enhance the effectiveness of plant essential oils (EOs) due to their intrinsic antibacterial properties [12].

The meat's susceptibility to microbial deterioration and oxidation is due to its low oxidative stability and unique makeup [13].

The objective of this study is to enhance the development of packaging systems that have biological activity by using chitosan to CCEO. Additionally, the study seeks to assess the antibacterial and antioxidant effects of these packaging systems on the shelf life of silver carp fillet.

MATERIALS AND METHODS

We used chitosan powder sourced from Sigma-Aldrich in Germany. The chitosan powder had a medium molecular weight ranging from 190 to 310 kilodaltons (kDa) and a degree of deacetylation between 75% and 85%.

The plasticizer glycerol was obtained from Merck Company (Darmstadt, Germany). Several reputable suppliers, including Merck in Darmstadt, Germany, Sigma in the USA, and Quelab in Canada, provided the analytical-grade chemicals and reagents used in this study.

CCEO Extraction

We crushed 150 grams of air-dried *Cuminum cyminum* L. and then combined it with 1.5 liters of distilled water. The essential oil (EO) was extracted using the process of hydrodistillation utilizing a Clevenger device for 3 h. The EO was dried using sodium sulfate and then stored in opaque glass vials in the refrigerator for further study. The assessment of the retrieved electro-optical (EO) components was carried out in our previous study [14].

Chemical Characterization of CCEO

The CCEO was analyzed using a gas chromatograph model 7890B (Agilent Technology, USA) equipped with a split-splitless injector port and a mass selective detector (MSD) model 5975C (Agilent Technology, USA) and was fitted with an HP-5MS capillary column. The helium gas was used as the carrier with a flow rate of 1 mL/min. The temperature of the injector was held at 210 °C at a split ratio of 5: 1. The temperature of the GC–MS interface was held at 300 ºC. MSD was operated in the electron ionization mode (70 eV). A full scan (40–450 m/z) was selected for identification of the compounds in the sample. The initial column temperature was 40 °C, which was then increased to 290 °C at a rate of 5 °C min^{-1}, where it was finally held for 1 min. The detector was a quadruple mass spectrometer. Chemstation software was used for the instrumentation control and data acquisition. The identification of the primary components of the essential oil (EO) was conducted by comparing their retention indices, standard mass spectral fragmentation patterns, and the National Institute of Standards and Technology (NIST). Subsequently, the proportion of them was determined by calculating the percentage based on the areas of GC peaks [15].

Preparation of the chitosan coating solution

The chitosan solution with a medium molecular weight was made as follows: A solution was prepared by dissolving 2 g of chitosan powder in 100 cubic centimeters of 1% acetic acid (volume/volume) and stirring it for three hours at room temperature. A plasticizer, glycerol, was introduced to the solution at a rate of 0.75 mL/g of chitosan weight. The mixture was then agitated for 10 min. To exclude solid particles that had not dissolved, the chitosan coating solution was passed through Whatman No. 3 filter paper. The chitosan solution was supplemented with Tween 80 at a concentration of 0.25% v/v, along with different concentrations of CCEO (0%, 0.3%, 0.45%, and 0.6% v/v). The solution was uniformly mixed under sterile conditions at a speed of 21600 revolutions per minute for 1 minute [16].

Preparation and Processing of the Fish Samples

A fish farm in Tehran Province caught silver carp (*Hypophthalmichthys molitrix*), which had an average weight of 1000 grams and an average length of 350 millimeters. The fish were transported to the meat science laboratory of the Food Hygiene Department at Tehran University at a temperature of 4 ºC in proximity to ice and subsequently divided into square pieces weighing approximately 60 g each. The fat and protein content percentages of three fillets were analyzed using the Association of Analytical Communities method [3].

The fillets were immersed for 30 s in 500 mL of different coating solutions, including A (control samples, distilled water), B (chitosan), C (chitosan enriched with 0.3% CCEO), D (chitosan enriched with 0.45% CCEO), and E (chitosan enriched with 0.6% CCEO). The fish fillets were extracted and left to drain for 30 minutes at a temperature of 25 °C in a microbiological hood to create the coatings. Subsequently, they were stored at a temperature of 4 ± 1 °C [16].

The shelf life of the fish was determined by conducting microbiological, chemical, and sensory investigations at eight specific time intervals (0, 1, 3, 5, 7, 9, 11, 13, and 15 days).

Microbial Analysis

The bacterial characteristics of each treatment were assessed on sampling days by homogenizing 10 g of the respective samples with 90 mL of 0.1% peptone water using a stomacher for 2 min. The enumeration of various types of bacteria, including total viable count (TVC), enterobacteriaceae (EBC), and lactic acid bacteria (LAB), was conducted by culturing appropriate decimal dilutions on selective media [17].

Chemical Analysis

The levels of total volatile base nitrogen (TVB-N), peroxide value (PV), and thiobarbituric acid reactive compounds (TBA) in silver carp fillet samples were assessed using the techniques developed by Goulas and Kontominas [18-20].

Sensory Evaluation

A sensory analysis was performed on samples of fried fish fillets to evaluate the sensory impact they produced. An assessment was carried out using a panel test to assess the fish fillets from each treatment of chitosan supplemented with different concentrations of CCEO (0, 0.3, 0.45, and 0.6% v/v) and a control group. The panel had 50 judges who lacked formal training but were affiliated with the Faculty of Veterinary Medicine at the University of Tehran. The panelists evaluated the samples by assigning ratings on a 9-point scale, where 9 represents a significant level of liking, 8 represents a high level of liking, 7 represents a moderate level of liking, 6 represents a mild level of liking, 5 represents a neutral stance, 4 represents a mild level of disliking, 3 represents a moderate level of disliking, 2 represents a high level of disliking, and 1 represents an intense level of disliking. The evaluations were assigned based on various aspects, including appearance, color, odor, and flavor [21].

Statistical Analysis

The statistical analysis was conducted using SPSS 16. The data underwent analysis using one-way ANOVA, followed by Turkey's multiple comparison test. The values are presented as the mean and corresponding standard deviation for all outcomes. Statistical significance was determined at a p-value of less than 0.05 [21].

RESULTS AND DISCUSSION

Chemical Makeup of Essential Oil

According to the GC-MS studies (Table 1), cumin aldehyde (41.6%), γ-Terpinene (18.2%), and o-Cymene (14.6%) were the main parts of CCEO.

Table 1 Composition of *Cuminum cyminum* essential oil by GC–MS

Number	Components	Retention time	Retention index
1	Heptanal	9.215	1.158
2	β-Myrcene	9.632	1.163
3	α-Phellandrene	10.164	1.170
4	o-Cymene	10.240	1.161
5	β-Phellandrene	10.315	1.162
6	1,8-Cineole	10.412	1.163
7	γ-Terpinene	10.550	1.164
8	α-Terpinolene	10.583	1.165
9	4-Terpineole	10.635	1.165
10	Myrtenol	10.750	1.167
11	Cumin aldehyde	11.325	1.173
12	Carvacrol	11.964	1.180
13	Myrtenol	11.963	1.180
14	o-Cumenol	12.012	1.180
15	p-Cymen	12.312	1.183
16	Curcumene	12.452	1.185
17	Thymol	12.845	1.189
18	Carvacrol	12.923	1.698
19	Carotol	12.945	1.699
20	Trans-Caryophyllene	12.986	1.699

Other researchers have documented these findings.

According to Bisht *et al* [22]. The main chemicals found in the GC-MS analysis of CCEO were γ-Terpin-7-al (22.9%), γ-Terpinene (22.6%), β-Pinene (22.2%), and Cumin aldehyde (13.1%). Researchers found that cumin aldehyde (49.4%), p-Cymene (17.4%), β-Pinene (6.3%), α-terpinen-7-al (6.8%), γ-Terpinene (6.1%), p-Cymen-7-ol (4.6%), and Thymol (2.8%) are the main parts of cumin seed essential oil [23]. Typically, based on extensive research, the primary constituents of cumin essential oil (CCEO) are cumin aldehyde, α-Terpinene, β-Pinene, γ-Terpinene, and o-Cymene [24] The composition of herbal essential oils can vary based on factors such as geographic region, climate, harvest season, variety and genetic condition, age of the plant, extraction method, and type of cultivation [25].

Proximate Analysis

Using the Soxhlet technique, the fat content in the fillets was determined to be 5.12 ± 0.48%, according to our evaluation. The moisture content of the samples was measured to be 70.62% using a hot air oven. Furthermore, the protein concentration of 13.97 ± 0.59% was determined using the Kjeldahl method.

Bacteriological Changes

Microbiological examination, in conjunction with chemical markers, commonly assesses the quality and shelf life of meat. This study analyzed the microbiological parameters TCV, EBC, and LBC over 15 days to see any changes.

Figures 1–3 show the changes in the bacterial composition of silver carp fillets treated with various concentrations of CCEO (0, 0.3, 0.45, and 0.6% v/v) over 15 days of refrigerated storage.

Figure 1 illustrates the changes in the TVC of silver carp fillets for each treatment. The initial TVC of fresh silver carp fillets was 3.15 ± 0.04 log cfu/g. All treatments showed an increase in TVC when held at a temperature of 4ºC.

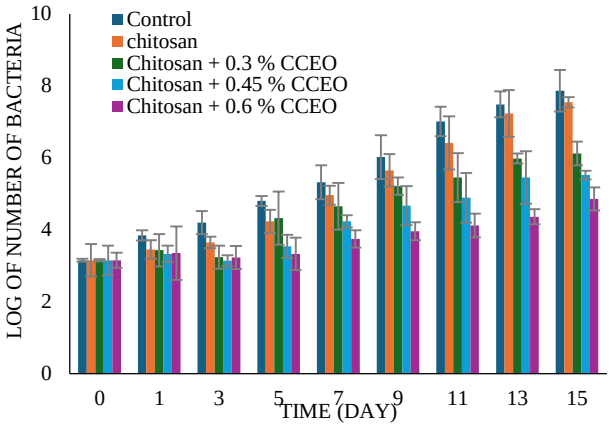

Fig. 1 Changes in total viable counts (TVC) of silver carp during chilled storage; variations in total viable count (Mean ± SD) in silver carp fillet during refrigerated storage (4 ± 1 ˚C). The counts for the control group; chitosan; chitosan + 0.3% CCEO; chitosan + 0.45% CCEO; chitosan + 0.6% CCEO are represented in various colors.

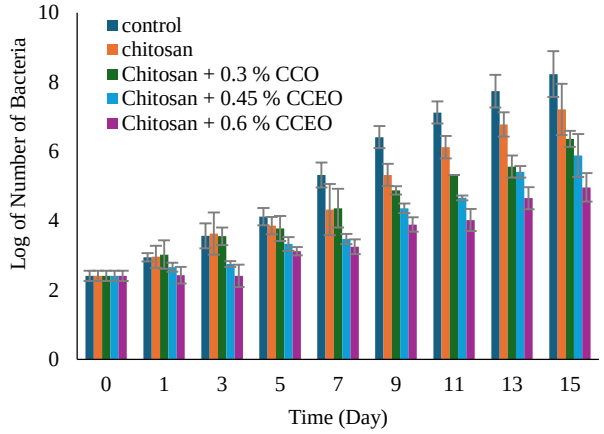

Fig. 2 Changes in enterobacteriaceae count (EBC) of silver carp during chilled storage; Variations in enterobacteriaceae (Mean ± SD) in silver carp fillet during refrigerated storage (4 ± 1 ˚C). The counts for the control group; chitosan; chitosan + 0.3% CCEO; chitosan + 0.45% CCEO; chitosan + 0.6%CCEOare represented in various colors.

The number of TVC in the treated samples was significantly reduced ($P < 0.05$) compared to the control group. Throughout days 13 to 15, the TVC in the fresh fish control sample reached a maximum limit of 7 log cfu/g at 11 days, as established by Salam [26]. Applying chitosan supplemented with CCEO at several concentrations (0, 0.3, 0.45, and 0.6% v/v) as a coating on silver carp fillets resulted in an extended shelf-life. According to Figure 2, the initial quantity of EBC was 2.41 ± 0.15 log cfu/g. The treated samples exhibited significantly reduced EBC compared to the control sample, with a p-value of less than 0.5. On the first day of research, the initial population of LAB in the silver carp fillets was 2.63 ± 0.38 log cfu/g (Fig. 3).

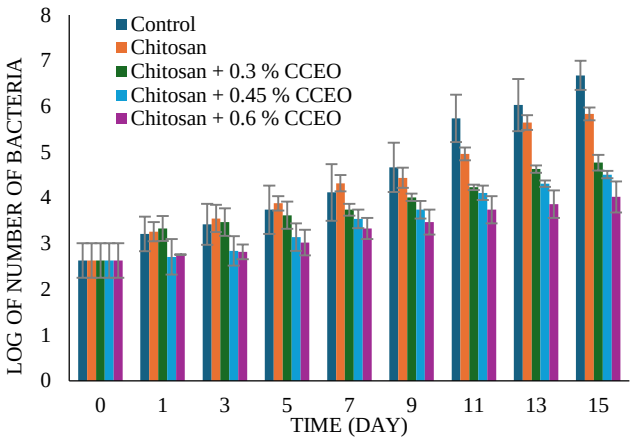

Fig. 3 Changes in lactic acid bacteria count (LAB) of silver carp during chilled storage; Variations in lactic acid bacteria (Mean ± SD) in silver carp fillet during refrigerated storage (4 ± 1 ˚C). The counts for the control group; chitosan; chitosan + 0.3% CCEO; chitosan + 0.45% CCEO; chitosan + 0.6% CCEO are represented in various colors.

Using different amounts of essential oils in conjunction with chitosan led to a notable decrease in LABs ($P < 0.05$). Our research aligns with the findings of Fernández-Pan et al. [27] regarding the impact of using WPI and EOs in food preservation. After 13 days of storage, they observed a reduction of 2.01 log CFU/g and 1.16 log CFU/g in the APC of chicken fillets coated with WPI-containing clove and oregano EOs, compared to the control group [28]. Alirezalu, Moazami-Goodarzi, Roufegarinejad, Yaghoubi, and Lorenzo (2022) made a similar observation. They discovered that when chicken breast was coated with calcium-alginate containing Artemisia fragrance EO, it had a significant inhibitory effect on APC [29]. In a study conducted by Zheng et al. (2023), it was found that the application of chitosan with different concentrations of oregano EO (0.25%, 0.5%, 1.0%, and 2.0%) to chicken breast led to decreases of 1.02, 1.40, 2.61, and 3.74 log10 CFU/g, respectively [30].

The present investigation's findings regarding the combined effect of EOs and chitosan are consistent with the results reported by Alparslan and Baygar (2017). They observed that the growth of deep-water pink shrimp was inhibited when the samples were wrapped in chitosan film infused with orange peel EO [31].

The LAB, or lactic acid bacteria, are a group of bacteria that can survive with or without oxygen and are commonly found in the microbial community of fish meat [32] As shown in Figure 2, the initial number of LAB in the control group was 2,63 log CFU/g and increased to 6,68 log CFU/g during storage ($P < 0.05$). The treated group exhibited a decreased bacterial count compared to the control group. Applying a chitosan coating containing 6 % CCEO resulted in a significant reduction in the LAB count in silver carp fillets. Specifically, the LAB count was around 2.66 log CFU/g lower than the control group at the end of the study period ($P < 0.05$).

During the investigation, it was observed that the bacterial growth rate dropped significantly ($P < 0.05$) in the chitosan + 6 % CCEO and chitosan + 4.5 % CCEO groups, compared to the samples without EO. Chen et al. (2021) examined the effects of chitosan coating in combination with cinnamon or oregano essential oils (EOs) on the microbiological populations of roast duck in modified environment packaging, explicitly focusing on the storability of the meat at cold temperatures [33]. Chen et al. (2021) found that the LAB count in the chitosan and chitosan-CEO groups was lower than that in the control samples after 21 days of storage, which aligns with our findings. Dehnad, Mirzaei, Emam-Djomeh, Jafari, and Dadashi (2014) conducted a study to assess the impact of bacterial nanocellulose on meat packaging. They specifically examined the antibacterial properties of nanocellulose-chitosan films to extend the shelf-life of meat. According to Dehnad et al. (2014), the LAB count decreased by up to 3.1 log cycles after 6 days of storage at 3 ◦C, compared to the control sample [34]. The study conducted by Bazargani-Gilani et al. (2015) showed that immersing chicken flesh in pomegranate juice and covering it with chitosan laced with *Zataria multiflora* Boiss EO led to a notable reduction in LAB counts compared to control samples during refrigerated storage [35].

The Enterobacteriaceae is a family of gram-negative bacteria that often resides in the gastrointestinal tract of humans and livestock. It constitutes a fraction of the microbial flora found in chicken meat. Enterobacteriaceae found in food, particularly meat, might indicate the level of food hygiene and the possibility of fecal contamination [6, 36]. The levels of EBC ranged from 2.41 to 8.23 logs CFU/g over, for 15 days in control groups. The control group did not exhibit any statistically significant difference compared to the other groups until day 5 ($P > 0.05$). The results (Fig. 3) showed that the proliferation of EBC decreased as the concentration of CCEO increased in all treatments. Specifically, the count of EBC in Chitosan + 6 % samples was considerably lower than in the other samples after day 4 ($P < 0.05$). The control group exhibited the highest levels of EBC. The study conducted by Abbasi et al. (2021) examined the microbiological quality of chicken meat. The researchers investigated the effects of corn starch coating mixed with *Zataria multiflora* EO and cinnamon essential oil on the meat's quality when stored at refrigerated temperature. During the 20-day storage period, the control samples showed a bacterial count ranging from 4.30 log CFU/g to 10.94 log CFU/g. Furthermore, the findings of their study demonstrated that incorporating the cinnamon essential oil into the coating solution resulted in a reduction of around 2.61 log CFU/g of Enterobacteriaceae on the last day of storage, compared to the control samples [6]. In addition, the findings of Sani et al. (2017) demonstrated a notable reduction in the number of EBC on the lamb that was wrapped in nanocomposite films made of cellulose nanofibers and WPI, along with titanium dioxide and rosemary EO. This reduction was observed when compared to the control group, which aligns with our results [28].

Chemical Analysis

Organic amines, which are produced by the digestion of proteins and other nitrogen-containing compounds by enzymes within the human body as well as bacterial growth, can be measured as indicators of spoilage referred to as total volatile basic nitrogen(TVB-N)[6, 37-39].

Figure 4 shows that the TVB-N value for the control group increased significantly from 7.21 to 40.08 mg N/100 g as the storage duration was prolonged. By day 9, the levels of TVB-N in both the control and chitosan-coated samples exceeded the permissible limit of 25 mg/100 g. In contrast to the chitosan + 0.3 % CCEO and chitosan + 0.45% CCEO group, which did not see spoiling until day 13. In the chitosan + 06 % CEO group, TVB-N remained within the acceptable range even on the final day of the research. Additionally, from day 3 until the end of storage, there was a significant difference in the TVB-N levels between the samples from the chitosan + 0.6 % CCEO group and the other groups ($P < 0.05$).

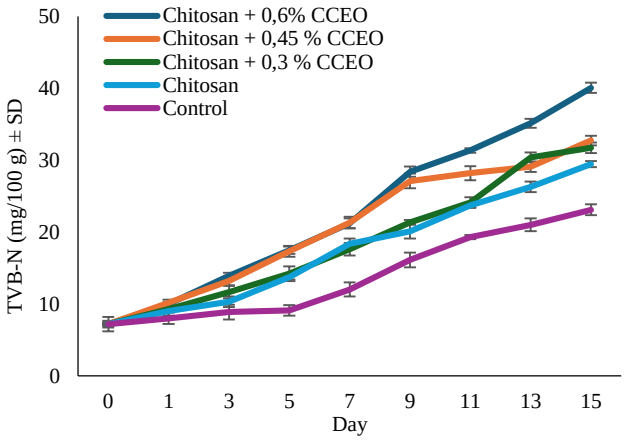

Fig. 4 Total volatile basic nitrogen (TVB-N) in various treatments of silver carp samples stored at refrigerated temperature (4 ± 1 °C). TVB-N evolution of control group; chitosan; chitosan + 0.3% CCEO; chitosan + 0.45% CCEO; chitosan + 0.6% CCEO samples during storage. Data are presented as mean ± standard deviation (SD).

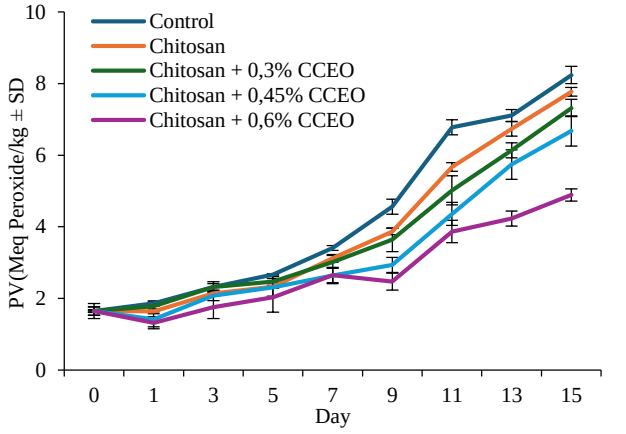

Fig. 5 Peroxide value (PV) of various treatments of silver carp samples at chilled temperature (4 ± 1 °C). Evolution of lipid oxidation in control control group; chitosan; chitosan + 0.3% CCEO; chitosan + 0.45% CCEO; chitosan + 0.6% CCEO samples during storage. Data are presented as mean ± standard deviation (SD).

This is likely related to the effect of CCEO combined with coating components and the solid antibacterial properties of the CCEO [40, 41]. The current results are consistent with those of Mehdizadeh and Langroodi (2019), who found that, when kept in the fridge, chicken meat coated with chitosan alone or with a combination of 0.5% and 1% Zataria multiflora EO had much lower TVB-N values than the control group [42]. Similarly, Farsanipour, Khodanazary, and Hosseini (2020) found that at the end of storage, the TVB-N values of control samples were considerably higher than those of fish fillets coated with chitosan + EO. According to Farsanipour et al. (2020), the antibacterial properties of essential oils in the treated samples may be responsible for the occurrence. The TVB-N level increased in both groups throughout storage. Still, the one with orange peel EO had a gentler slope [43], according to Alparslan and Baygar (2017), who investigated the effect of a chitosan coating with orange peel EO on the pink shrimp's shelf life. Furthermore, the control group's TVB-N graph exhibited a substantial increase in slope throughout the trial [31]. Findings from this study corroborate our own.

The unpleasant odor that is caused by fat oxidation in meat products reduces its consumer appeal. The generation of primary oxidation products determines the amount of lipid oxidation [44]. The amount of peroxide and hydroperoxide components generated during preservation is indicated by the Peroxide value (PV) of the samples that were examined. Throughout the storage period, the PV values of the Control samples varied between 1.65 and 8.24 meq peroxide/kg, as shown in Figure 5. Compared to the coated samples containing CEO, the control samples had considerably higher PV values in the present study ($P < 0.05$).

The study showed that the Chitosan + 0.6% CCEO group had a lower peroxide value (PV) than the control group, there was a statistically significant decrease in PV compared to other groups in the coated groups that had 0.3% and 0.4% CCEO ($P < 0.05$). Coatings enhanced with EO may have a more significant impact in preventing peroxide production. EOs can reduce lipid peroxidation by suppressing lipid peroxy radicals and chelating iron ions in lipoxygenase enzymes [45]. The results show that herbal compounds and their derivatives, especially CCEO, can significantly delay lipid oxidation [16, 46]. Also, research has shown that chitosan can extend the shelf life of meat products by acting as an antioxidant [5, 16]. The present study's results also corroborated those of Bazargani-Gilani et al. (2015), who hypothesized that coating chicken fillets with EOs and storing them in the fridge would effectively delay the oxidation of their lipids[35].

The levels of thiobarbituric acid reactive substances (TBA) are assessed as a means of quantifying the quantity of malondialdehyde, the primary secondary product of lipid oxidation in meat and meat products[43]. Figure 6 demonstrates the effect of the combination of chitosan coating and CCEO on the TBA level of silver carp fillets during storage. Across all samples, the concentration of TBA exhibited a consistent increase for the storage period. Initially, the TBA levels in all groups were determined to be 0.18 mg malondialdehyde (MDA)/kg silver carp fillet. The results of the current investigation indicate that there is not a significant difference between the treated group and the control group ($P > 0.05$). Subsequently, from day 1 to day 5, there was a notable rise observed in the samples from the control group ($P < 0.05$), with the value reaching around 0.41 mg MDA/kg silver carp fillet. The intensity of this increase in the control group became more pronounced after day 5. By the 15th day, the value reached 0.75 mg MDA/kg in the control group. In contrast, the TBA level in the chitosan + 06% CCEO groups remained below 0.5 mg MDA/kg of silver carp fillet on the same day and was significantly lower than the control group ($P < 0.05$). The increase in CCEO concentration in our sample can be attributed to the drop in TBA levels, justifying this result. Similarly, numerous research has been carried out to investigate the effects of different coatings and films on the preservation of meat products. These studies found that using either chitosan or EO, or both, resulted in significant reductions in TBA levels compared to the control groups. The findings of these investigations align with the data collected in the current study [16, 47-49]. The interaction between malondialdehyde and primary amino groups of chitosan may enhance the antioxidant activity of chitosan [50, 51]. In a correlated investigation, Langroodi, Nematollahi, & Sayadi (2021) examined the influence of applying chitosan and herbal active components to turkey meat and observed that as the storage time rose over 20 days, all treatments resulted in elevated levels of TBA.

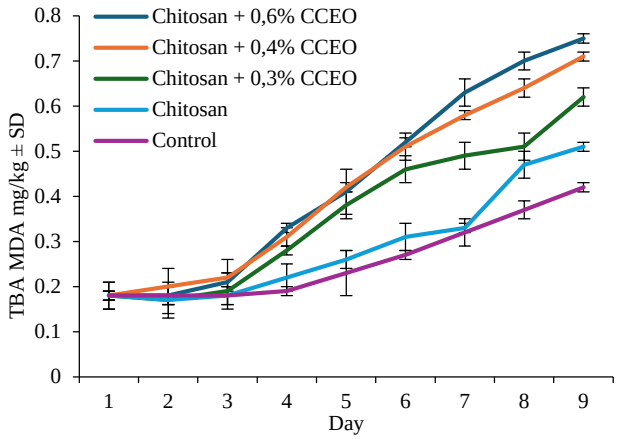

Fig. 6 Thiobarbituric acid reactive substances (TBARS) of various treatments of silver carp samples stored at chilled temperature (4 ± 1 °C). Malondialdehyde (MDA) levels in control samples; chitosan; chitosan + 0.3% CCEO; chitosan + 0.45% CCEO; chitosan + 0.6% CCEO samples during storage. Data are presented as mean ± standard deviation (SD).

After the trial, the TBA level varied between 0.71 and 5.89 mg MDA/kg beef. The samples exhibiting the lowest levels of TBA were those containing the highest quantity of grape seed extract, in addition to being coated with chitosan and treated with *Origanum vulgare* EO. The control and pure chitosan samples had the highest levels of MDA/kg meat, measuring 5.89 and 1.56 mg, respectively[52]. At the same time, there were variations in the quantity of TBA, the general pattern of this investigation aligned with our findings.

Sensory Evaluation

Table 2 presents the median scores for the odor, taste, and acceptability of the control and treated fish fillet samples. The fillet that was treated with chitosan mixed with 0.3% EO and stored for 15 days was the most preferred sample. The experiment revealed that applying a chitosan coating with 0.3% CCEO achieved the desired sensory characteristics. The results of the present investigation were consistent with those of Zheng et al. (2023). A study discovered that the application of a chitosan coating with either 1% or 2% oregano EO significantly enhanced the sensory qualities and prolonged the shelf life of chicken meat by 9 days compared to the control group [53]. The study conducted by Yıldız and Yangılar (2017) demonstrated that rainbow trout fillets coated with whey WPI containing ginger and chamomile EOs had the highest preference in terms of their sensory characteristics [54].

Table 2 Median rating for sensory properties of fish fillets as affected by different concentrations of CCEO

Combination	Odor (mean ± SD)	Taste (mean ± SD)	Overall acceptability (mean ± SD)
Control	6.87 ab ± 1.2	6.87 ab ± 0.4	6.87 ab ± 0.8
Chitosan	7.12 ab ± 0.5	7.88 ab ± 0.6	6.93 ab ± 0.6
Chitosan + 0.3 % CCEO	7.84 a ± 1.1	8.02 ab ± 0.7	8.23 a ± 1.4
Chitosan + 0.45 % CCEO	6.73 ab ± 1.3	5.23 b ± 1.6	5.14 b ± 0.2
Chitosan + 0.6 % CCEO	4.69 b ± 0.8	3.22 ± 0.3	3.98 ± 0.3

Means followed by the same letters are not significantly ($P < 0.05$) different.
SD: standard deviation.

CONCLUSION

The findings of this study indicate that using chitosan and CCEO enhances the longevity of refrigerated silver carp fillets. In addition, the silver carp fillet samples were found to have satisfactory sensory qualities. The findings of this study indicate that the combined use of chitosan and CCEO had a more substantial inhibitory effect on lipid oxidation and microbiological spoilage bacteria compared to utilizing coating alone. The antibacterial and antioxidant properties of the coated silver carp fillets resulted in a reduction of TVB-N, TBA level, and PV values during the 15-day storage period, compared to the uncoated samples. Based on the findings that demonstrate the beneficial combined effect of the materials utilized in this study, along with the growing consumer preference for natural compounds that possess various properties (such as antioxidant, antimicrobial, and shelf-life extension), it is advisable to implement these types of coatings in food products, particularly fish meat.

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Abbreviations

CCEO; *Cuminum cyminum* essential oil
CFU; Colony forming unit
EBC; Enterobacteriaceae
EO; Essential oil
LAB; Lactic acid bacteria
MDA; Malondialdehyde
PV; Peroxide value
TVN; Total viable count
TVB-N; Total volatile bases-nitrogen
TBA; Thiobarbituric acid reactive substances

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