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ORIGINAL ARTICLE

Effect of a chitosan-based nanocomposite containing ZnO and *Zataria multiflora* essential oil on quality properties of Asian sea bass (*Lates calcarifer*) fillet

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Abstract This research aimed to estimate the effects of chitosan (CH) coating in combination with zinc oxide nanoparticles (ZnONPS) and *Zataria multiflora* essential oil (ZEO) on the bacterial and biochemical properties of the Asian sea bass (*Lates calcarifer*) fillets during refrigeration storage (4 ± 1 °C). The fillets were randomly divided into five treatments (CH, CH-ZnONPS, CH-ZEO, CH-ZnONPs-ZEO, and control). Then, the treated fillets were kept at 4 °C and quality analysis was performed on days 0, 4, 8, 12, and 16. The results revealed that the combination of ZnONPs and ZEO with CH coating is an active coating with antimicrobial effects. Also, the coated fillets improved the biochemical properties (such as FFA, TBA, TVBN, pH) as well as color properties during refrigeration storage. The highest rate of FFA (3.59 ± 0.08%oleic acid), TBA (1.43 ± 0.00 mg MDA/kg), TVBN (30.82 ± 0.30 mg/N100g), and pH (7.38 ± 0.03) was recorded in control fillets while the lowest rate of FFA (2.19 ± 0.00%oleic acid), TBA (0.61 ± 0.00 mg MDA/kg), TVBN (19.60 ± 0.20 mg/N100g), and pH (6.99 ± 0.04) was recorded in CH-ZnONPs-ZEO coated fillets ($p < 0.05$) on day 16. The sensory acceptance score was better than that of the control treatment on days 8 and 12 in Sea bass fillet coated with CH-ZnONPs, and CH-ZnONPS/CH-ZEO, respectively, and it was lower the critical score for fishery products. The combination of nanoparticles or essential oils (individually or in combination together) with edible coatings (chitosan) could increase and optimize the storage time of refrigerated seafood.

Keywords *Lates calcarifer* · Chitosan · ZnO nanoparticles · *Zataria multiflora* · Edible coating · Refrigeration storage

Introduction

Nowadays, several novel techniques have been tested by researchers to preserve various fresh seafood products. Biopolymers, from biodegradable sources, constitute a highly desired preservation compound in food processing and packaging to protect foodstuff against bacterial contamination and oxidation responses (Kumar et al. 2020). Chitosan (CH) is a linear polymer of β-(1–4)-D-glucosamine and *N*-acetylDglucosamine. CH is a polysaccharide with natural origin, and is derived from the chitin deacetylation which is obtained extensively from some species, such as shrimp, crab, and fungi (Kumar et al. 2019). CH has different properties such as environmentally-friendly nature, edibility, biodegradability, biocompatibility, visual appearance, protection characteristics, non-toxicity, and as a carrier for food additives (Coma et al. 2002). The mentioned properties of CH have made this natural material a candidate as an active packaging material in seafood processing. Despite all the mentioned advantages, CH has some complications such as low hydrophobic and some mechanical characteristics. For example, chitosan coatings are highly sensitive to moisture and therefore may not be suitable for use when placed in direct contact with food (Rhim et al. 2006). These properties must be optimized to an acceptable level (Wang et al. 2019). To overcome this issue, metal oxide nanoparticles,

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as inorganic compounds, are reported to improve the inherent shortcomings of CH and can add antibacterial plus antioxidant properties (Wang et al. 2019). Metal oxide nanoparticles (e.g. magnesium oxide (MgO), copper oxide (CuO), titanium dioxide (TiO_2), aluminum oxide (Al_2O_3), zinc oxide (ZnO), silica (SiO_2), iron oxide (Fe_2O_3), and cerium oxide (CeO_2) NPs) are frequently used as antimicrobial agents at low concentrations. Also, metal oxide nanoparticles have greater stability at high temperatures during food packaging (Singh et al. 2016). Among nanoparticles, ZnO nanoparticles (ZnONPs) have good antibacterial properties against foodborne pathogenic bacteria. Also, ZnONPs are cost-effective with high accessibility, and they are non-toxic in comparison with other metal oxides (Heydari-Majd et al. 2019). ZnO NPs have been extensively used in products such as foods due to their excellent antimicrobial and photocatalytic activities in recent years. The average particle size of the ZnONPs is between 20 and 30 nm. A mixture of CH and ZnONPs can form nanocomposite coatings. There are few publications on the application of CH-ZnO nanocomposite in the packaging of fish and fishery products (Shahbazi and Shavisi 2018; Amjadi et al. 2019; Kumar et al. 2019, 2020). The application of biopolymers as a potential for seafood product preservation, either alone or in combination with metal oxide nanoparticles, has offered positive effects on the quality of fishery products (Heydari-Majd et al. 2019; Arfat et al. 2015). Meanwhile, CH/ZnONPs have been applied for food preservation of various foods such as chicken and meat due to their higher antibacterial activity (Amjadi et al. 2019). Although there are several reports on the inhibition effects of ZnO-nanoparticles on bacterial contamination of food products, there is spare information on the effects of ZnONPs-coated coatings on the fish quality during refrigeration storage. Further, CH-ZnO nanocomposites with essential oil (EO) may optimize the antimicrobial activities of active coatings. Besides nanocomposite materials, EOs have been tested in food packaging for the increasing demand for natural preservatives (Baptista et al. 2020). The application of plant-derived essential oils may lead to the development of packaging materials to preserve foodstuff (Shadman et al. 2017). EO is a concentrated hydrophobic liquid containing volatile chemical compounds. EOs are natural compounds extracted from plants especially the aromatic plants and can be used as promising sources of potential antioxidant plus antimicrobial activities on foods. They can also be employed as alternative food packaging to synthetic additives. *Zataria multiflora* essential oil (ZEO) has antimicrobial and antioxidant properties (Mojaddar Langroodi et al. 2019). Some researchers have reported the antioxidant and antimicrobial properties of this EO (Shadman et al. 2017; Mojaddar Langroodi et al. 2019). This study addresses the effects of CH-ZnO nanocomposite coatings containing *Zataria multiflora* essential oil on chemical properties, bacterial load, and sensory analyses during the storage period (shelf life) of sea bass fillets during storage in refrigerator (4 ± 1 °C). Also, a key objective of this study was to evaluate the combination of nanoparticles or essential oil (individually or in combination together) with edible coatings (chitosan) to enhance and optimize the storage time of refrigerated seafood.

Materials and methods

Materials preparation

The main materials used for this study are as follows (1) Chitosan (≥ 92.0% deacetylation; Tahoorkaran Bahador Pars Company, Shiraz, Iran, CAS no.: 9012-76-4, EC no.: 618-480-0). (2) Glycerol (Code no.: 6279, Sigma-Aldrich Company, Germany, CAS no.: 56-81-5). (3) ZnONPs powder (mean diameter: 30 nm; Iranian Nanomaterials Pioneers Company, Mashhad, Iran, Stock #: US3590). (4) ZEO (Code no.: 1411569, Barij Essence Pharmaceutical Company, Tehran, Iran).

Preparation of coated fillets

Fresh sea bass (*Lates calcarifer*) (mean weight: 300 ± 20 g) were transferred from a local farm (Choebdeh, Khuzestan, Iran) to the fisheries laboratory. The fillets were manually prepared.

CH solution was prepared by stirring 1% (w/v) CH in 1% (v/v) of acetic acid at 25 °C in distilled water (Caner and Cansiz 2007). ZnONPs (5% of dry CH) were dissolved in distilled water for 15 min. To prepare chitosan containing ZnONPs, CH solution and ZnONPs solution were stirred at room temperature (25 °C) for 30 min. Then, glycerol was added as a plasticizer (0.75 ml/g) and stirred for 10 min. In order for all treatments to have the same conditions, an equal amount of glycerol was added to the distilled water (as control treatment) so that the possible effects observed would not be due to the effect of glycerol.

The fillet samples were randomly divided into five groups and in triplicates (in 15 packages). The fillet samples were immersed in drinking water (first group), CH (second group), CH-ZnONPs (third group), CH-ZEO (fourth group), and CH-ZnONPs-ZEO (fifth group), respectively. After 15 min, all of the samples were drained, packaged into polyethylene bags (75 μm in thickness), and stored at 4 ± 1 °C for 16 days. The biochemical and bacteriological properties, color, and sensory properties were analyzed at 4-day intervals to estimate the storage status of Asian sea bass (Shadman et al. 2017). All analyses

were performed as triplicates per treatment per sampling day.

Bacteriological analyses

Total mesophilic bacteria (TMB) and total psychrophilic bacteria (TPC) were counted on plate count agar (PCA) using pour plate method for 48 h, at 30 °C, and 7 days at 7 °C, respectively. The pour plate method was used for lactic acid bacteria (LAB) counted on MRS agar, at 30 °C for 72 h. The results of the bacterial counts were stated as log CFU/g fillet (Khater and Farag 2016).

Biochemical analyses

Total volatile basic nitrogen (TVB-N) contents were measured via the distillation method (Goulas and Kontominas 2005). The pH content of fish fillets was measured using a digital pH meter (913 pH meter, Metrohm, Herisaw, Switzerland) (Suvanich et al. 2000). Thiobarbituric acid reactive substances (TBARS) were measured by the method described by Lemon (1975) with modifications according to Cyprian et al. (2015). Free fatty acid (FFA) values were measured according to Woyewoda et al. (1986) and Bernardez et al. (2005) by extracting lipid from 10 g of fillet sample using chloroform/methanol, and titration of free carboxylic groups with sodium hydroxide.

Color

The sample color was analyzed by a color meter (Shanghai Precision & Scientific instrument Co., Ltd., Shanghai, China) which would report the results in the CIE system. Three measurements were run per sea bass fillet sample. The colorimeter was calibrated to the white standard. In this system, L* indicates color lightness on a scale of 0–100 points from black to white. a* denotes the position between red (+) and green (−), and b * represents the position between yellow (+) and blue (−) (Zhang et al. 2013).

Sensory analyses

The fish samples were steamed for 15 min at 85 °C. The sensory analysis was performed in a standard test room with standard light and air conditioning. The parameters that were assessed in the sensory analysis component of the study included color, texture, odor, appearance, and total acceptance. A descriptive five-point hedonic scale (1 = dislike extremely as completely rejected, and 5 = like extremely as very accepted) was used in this study. Unpleasant odor, dull color, and general unacceptability of fish fillet with a sensory score under 3 were considered unacceptable for human consumption. The sensory evaluation of the control and coated fillets was performed by 32 panelists (aged between 18 and 32 years old) (Meilgaard et al. 2016).

Statistical analysis

All data were analyzed by one-way and two-way ANOVA with factors coating and/or time of storage using SPSS software version 16 (SPSS Inc., Chicago, Illinois, USA). Moreover, Duncan's post-test was used to compare the means. A level of confidence of 95% was considered as significant.

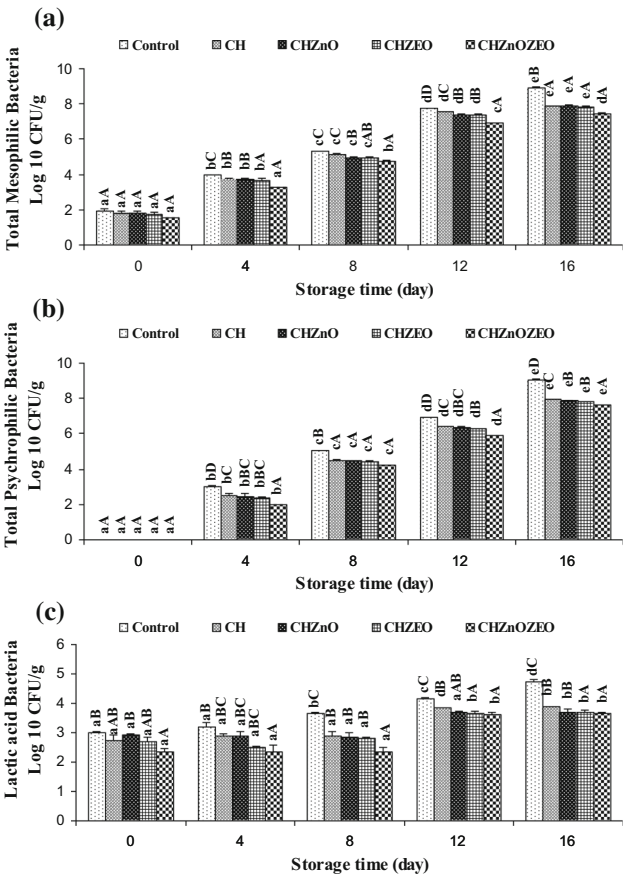

Fig. 1 The effect of ZnONPs and ZEO nanocomposite on mesophilic bacteria (**a**), psychrophilic bacteria (**b**), and lactic acid bacteria (**c**) of Asian sea bass during storage at the refrigerator. Mean values and standard errors from the three replicates are presented. Different uppercase letters indicate significant differences between treatments ($P < 0.05$). Different lowercase letters show significant differences in storage time in each treatment ($P < 0.05$)

Results and discussion

The bacterial analyses

The TMB and TPC values of sea bass fillets, during storage at 4 °C, for 16 days, are presented in Fig. 1. The initial TMB and TPC values in the sea bass fillets in control group were 1.95 ± 0.13 log10 CFU/g and 0.00 log10 CFU/g, respectively. This indicates that the sea bass fillets were in high quality regarding bacterial contamination (Sikorski et al. 1990; Kumar et al. 2020).

The initial LAB count for all fillets ranged from 1.23 to 1.68 log10 CFU/g (Fig. 1). LAB counts for the control and coated fillets were lower than the other bacterial counts analyzed in the current study during the storage period. The LAB count increased gradually in all samples during the storage. The addition of ZnONPs and EO into the CH solution did not change the LAB of treated fillets. There was no significant difference between control and coated fillets at the end of the refrigeration storage.

According to Kostaki et al. (2009), the LAB has more resistance to EOs due to the bacterial ability to withstand osmotic stress. However, TMB and TPC of sea bass samples in the control samples increased rapidly during the refrigeration storage and were generally higher than in other samples ($P < 0.05$). Jeon et al. (2002) and Pereda et al. (2011) reported that CH can be used for the quality preservation various types of food packaging. The mechanism of antibacterial effects of CH involves interacting with the positive charge of the NH_3^+ groups of glucosamine monomers in CH molecules with the negative charge of macromolecules on the microbial cell surface (Ojagh et al. 2010). Also, CH acts as a barrier for oxygen transfer (Jeon et al. 2002). Comparatively, the highest and lowest abundance of TMB was observed in the fillets coated with CH, and CH-ZnONPs-ZEO coatings, respectively. While CH had little effect on reducing the bacterial growth, both ZnO nanoparticles and ZEO might enhance the antibacterial activity. This antibacterial activity would reduce the bacterial growth when ZnO and ZEO were combined with the CH solution. This result coincided with the antibacterial activity of the prepared nanocomposite coatings (CH-ZnONPs nanocomposite) and their potential to improve the storage time of sea bass fillets. ZnONPs act as antimicrobial agents as formation of reactive oxygen species (ROS) (de Azeredo 2013) interferes with the permeability of bacterial cells and causes cytolysis.

Stan et al. (2016) found that ZnONPs had antibacterial activity against some foodstuff bacteria. Further, previous studies have demonstrated that different EOs can be used for limiting of TMB growth in seafood (Huang et al. 2018). To improve the inhibitory effect of CH coating, the addition of EOs to CH solution enhanced the synergistic antibacterial effect in sea bass fillet. EOs components hydrolyze the peptidoglycan layer of the bacterial cell membrane, and thus EOs would reduce the bacterial growth (Gómez-Estaca et al. 2010). The mechanism of plant EOs depends on the presence of bioactive compounds such as phenolic acids and terpenoids (Baptista et al. 2020).

Raeisi et al. (2014) showed that *Z. multiflora* EO is rich in thymol and carvacrol which have suitable antibacterial and antioxidant properties. Among the phenolic compounds, carvacrol has been shown to have the highest antimicrobial activity due to its hydrophobic nature and the presence of a free hydroxyl group which is essential for its activity on cell membranes (Raeisi et al. 2014; Ali et al. 2000). According to the results of TMB and TPC counts, CH coating combination with ZnONPs and ZEO presented the highest antibacterial activity in comparison with other coated fillets. Similar results were reported by Heydari-Majd et al. (2019) and Arfat et al. (2015). They reported that use of ZnONPs alone or in combination with EO into the biopolymer solution significantly inhibited TMC on *Otolithes ruber* fillets, and sea bass slice, respectively. It was found that successful interaction of ZnONPs and ZEO had the highest antibacterial activity for sea bass fillet, reducing the bacterial load in the sea bass samples. The acceptable level for TMB in raw seafood is 7 log CFU/g (ICMSF 1986).

Microbiologically, the samples coated with CH, CH-ZnONPs, CH-ZEO, and CH-ZnONPs-ZEO coatings were acceptable up to 12-day storage. TMB was higher in uncoated sea bass fillets than the recommended limit in fresh fish after 8 days of refrigeration storage, suggesting that the best time storage in terms of bacterial contamination is around 8 days in control fillets.

Changes in pH values

All changes in biochemical parameters are shown in Fig. 2. According to pH values, significant differences were detected among the treatments. The initial pH value of samples was 6.01–6.04, which was within the normal range of pH in fishery products (Arfat et al. 2015). The pH value of the control and coated fillets increased dramatically during the storage period. The main reason for the increased pH levels in coated and control fillet is probably protein decomposition through bacterial load and production of alkaline metabolites such as ammonia and trimethylamine (Nirmal and Benjakul 2011). On day 16 of refrigeration storage, pH values of control fillets, CH, CH-ZnONPs, CH-ZEO, and CH-ZnONPs-ZEO were 7.38 ± 0.03, 7.18 ± 0.00, 7.20 ± 0.01, 7.07 ± 0.02, and 6.99 ± 0.04, respectively. The pH values were significantly lower in coated samples than in the uncoated fillets ($P < 0.05$). pH is a critical factor affecting microbial

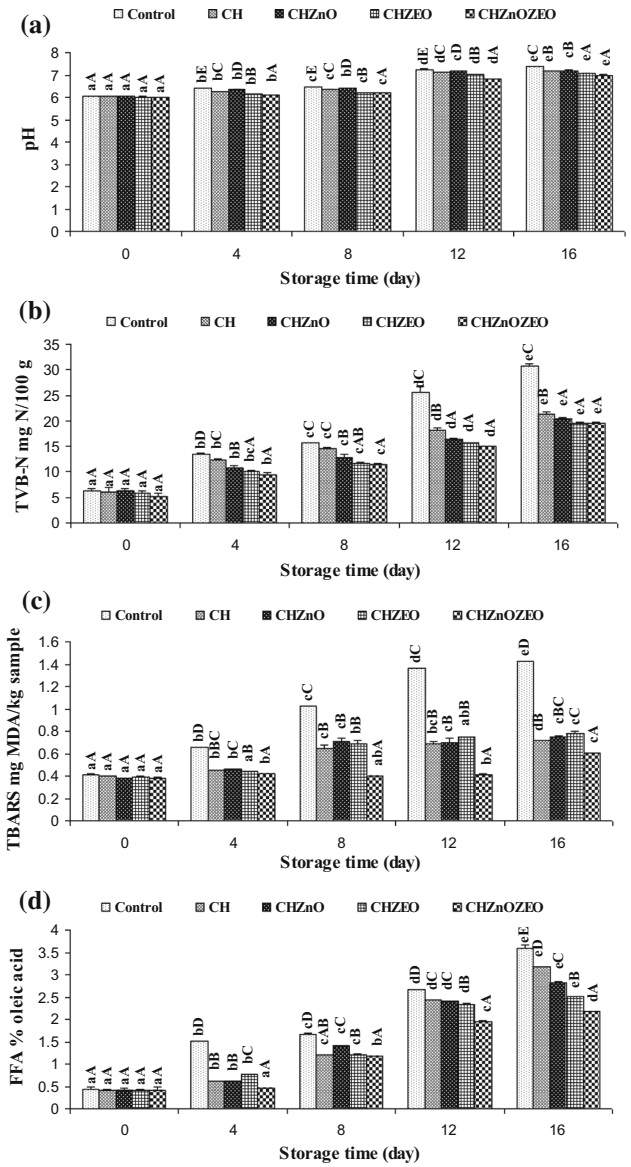

Fig. 2 The effect of ZnONPs and ZEO nanocomposite on the biochemical analyses (pH (**a**), TVB-N (**b**), TBA (**c**), FFA (**d**)) of the Asian sea bass during storage at the refrigerator. Mean values and standard errors from the three replicates are presented. Different uppercase letters indicate significant differences between treatments ($P < 0.05$). Different lowercase letters show significant differences in storage time in each treatment ($P < 0.05$)

growth and spoilage of foods (Khater and Farag 2016). Based on the report by Suo et al. (2017), the coating containing a mixture of sodium carboxymethyl cellulose dissolved in distilled water and ZnONPs presented an inhibitory effect on the bacterial flora of pork meat. When the pH is larger than seven, fish will not be acceptable (Khater and Farag 2016). By decreasing bacterial growth and breakdown of nitrogenous compounds, the pH value of fillets coated with CH-ZnONPs-ZEO was below seven throughout the entire storage and at the end of the storage period. Also, the pH value of CH-ZnONPs-ZEO coated fillets was lower than that of other treatments throughout the storage period. One of the most important reasons for increasing pH of fish fillets is bacterial contamination during the storage (Raeisi et al. 2014). In this respect, lactic acid bacteria play the main role. Increasing pH during the storage period may be correlated to the accumulation of basic compounds released by either endogenous or microbial enzymes activities. Thus, the ability to maintain the pH of fish fillets at lower value indicates better preservation ability. Lower pH of the coated samples can enhance microbial inhibition and contribute to extending the preservation of fish samples by inhibiting the activity of the endogenous proteases (Li et al. 2018). Also, as mentioned above, the highest antibacterial activity was observed in the combination of ZnONPs with ZEO into CH coatings. Thus, it seems that the alteration in pH values is related to changes of TMB and TVB-N values.

TVB-N values alteration

The initial TVB-N values in the fillets ranged between 5.27 ± 0.55 mg N/100 g and 6.35 ± 0.32 mg N/100 g of tissue. These values reflected the freshness of raw fish (Idakwo et al. 2016). TVB-N content of fresh seafood fillets increased gradually during the storage period ($P < 0.05$) in the control and coated fillet. This alteration in TVB-N content was moderate in treated fillets than in untreated fillets. The increase of TVB-N of samples could emanate from the endogenous enzyme activities, and the microorganism's metabolic activity producing ammonia and biogenic amines (Kakaei and Shahbazi 2016). During the storage period, TVB-N values of sea bass fillets treated with ZnONPs had no significant difference with the ZEO treated fillets, suggesting that ZnONPs and ZEO have a similar effect on decreasing the degradation of nitrogenous compounds in sea bass fillets. TVB-N values of the control and fillets coated with pure chitosan increased to 15.60 ± 0.20 mg N/100 g and 14.67 ± 0.20 mg N/100 g on day 8, respectively, while the values of TVB-N in chitosan-coated with ZnONPs or ZEO and in combination together was lower than the control fillets ($P < 0.05$), and it was less than 12 mg N/100 g on day 8. The TVB-N values increased on day 16 and ranged from 30.82 ± 0.30, 21.33 ± 0.33, 20.37 ± 0.31, 19.60 ± 0.20 and 19.42 ± 0.29 mg N/100 g for control fillets and all coated fillets. According to the acceptable levels of TVB-N in seafood (20–30 mg N/100 g) (Idakwo et al. 2016), the best storage period for sea bass control fillets group was 8 days, while CH-coated fillets or CH fillets coated with ZnONPs, ZEO, or their combination reached this limit on day 12 of the storage period, indicating delayed chemical spoilage. Similarly, Qiu et al. (2014) reported that TVB-N values in

Japanese sea bass treated with CH coating alone or chitosan incorporated with plant extract dropped to 60.5 and 48 mg N/ 100 g, respectively, during storage period at 4 °C for 12 days. The low initial TVB-N value of treated fillets was relatively in agreement with the low initial TMB count (1.87 log10 CFU/g), again indicating the good quality of the sea bass fillets. Similar results have been reported for sea bass fillets wrapped/or coated with plant extract/ or essential oil incorporated biopolymers film/or coating (Arfat et al. 2015; Qiu et al. 2014). They showed that use of these materials can improve the efficiency of a coating procedure to protect sea bass fillets from spoiling.

Changes in TBARS values

Thiobarbituric acid reactive substances (TBARS) are valuable indicators to measure the lipid oxidation of seafood (Alsaggaf et al. 2017). On day 0, the TBARS value of all fillets was between 0.38 and 0.41 mg MDA/ kg fillet. Our findings of the initial TBARS of fresh sea bass fillets concurred with the results reported by Qiu et al. (2014) in Japanese sea bass (*Lateolabrax japonicas*) fillets. The TBARS values in sea bass fillets changed during the storage period and were affected by coating substances ($P < 0.05$). The TBARS of the control and coated fillets increased significantly ($P < 0.05$). Its maximum levels were recorded on day 16 of the storage period. At the end of the storage period, TBARS values for CH, CH-ZnONPs, CH-ZEO, CH-ZnONPs-ZEO, and control were 0.72, 0.75, 0.78, 0.61, and 1.43 mg MDA/kg fillet, respectively. The TBARS value of the control fillets was significantly higher than that of the coated fillets ($P < 0.05$). According to the result of TBARS values, CH coating had little antioxidant activity compared to the other treatments. Several studies have shown that the combination of different nanoparticles or EOs with chitosan coatings enhances the inhibitory effects of oxidation (Heydari-Majd et al. 2019). In the current study, the combination of ZnONPs or ZEO with CH solution significantly decreased lipid oxidation. Moradi et al. (2012) observed the incorporation of ZEO into CH solution boosted the antioxidant activity of CH. Both oxygen barrier properties and antioxidant activity of ZnONPs and ZEO may contribute to the reduction of lipid oxidation in fillets (Arfat et al. 2015), suggesting chitosan has a good synergistic effect on reducing lipid oxidation when combined with ZnONPs and ZEO. Based on previous studies on *Zataria multiflora* essential oil, the polyphenol compounds in ZEO were introduced as antioxidant compounds. The polyphenol compounds in ZEO exhibit oxidation of free radicals, so the ZnONPs using ZEO may exhibit enhanced antioxidant activity (Stan et al. 2016). The mechanism of the antioxidant activity of ZnONPs may be due to the electrostatic attraction bioactive compounds (COO-, O-) of essential oil with nanoparticles with a positive charge (ZnO = Zn2 + + O2-) (Stan et al. 2016). Further, ZnONPs can protect fish fillets from oxidation by forming a barrier to oxygen permeability in treated fillets during refrigeration storage (Arfat et al. 2015) using their positive electric charge. TBARS values of 1 to 8 mg MDA/ kg are acceptable sensory limits (Connell 1990; Alsaggaf et al. 2017). In the current study, TBARS values of all fillet samples were less than 2 mg MDA/kg fillet. This showed the acceptable quality of sea bass fillets during the refrigeration storage.

Alteration of FFA values

The initial FFA value of sea bass fillet ranged from 0.4 to 0.43% of oleic acid. On day 16 after the refrigeration period, FFA values of control, CH, CH-ZnONPs, CH-ZEO, and CH-ZnONPs-ZEO were 3.59 ± 0.08, 3.18 ± 0.01, 2.83 ± 0.02, 2.51 ± 0.00, and 2.19 ± 0.00% of oleic acid, respectively. The FFA values of all fillets increased during the refrigeration period. The FFA values of coated fillets were kept relatively low. These might have resulted from the antioxidant properties of the ZnONPs and ZEO to prevent hydrolysis of unsaturated fatty acids in the fish fillet. The progress of lipid hydrolysis were studied by measuring free fatty acids (FFA) which are triacylglycerols products formed either via chemical or enzyme mediated hydrolysis (Barthet et al. 2008). The overall increase displays hydrolytic oxidation in the fillets caused by internal or bacterial enzymes, while the decrease may be related to the interaction of triacylglycerol products with proteins (Pereira De Abreu et al. 2011). The lower content of FFA in the samples treated with CH-ZnONPs-ZEO may be due to the influence of the essential oil on meat enzymes and their activity (Silva and Ammerman 1993). In general, plant essential oils do not absorb oxygen due to the presence of phenolic compounds, but they prevent the formation of free radicals of fatty acids, so the process of lipid or oil autooxidation occurs later.

Changes in color

The color properties of fillets are significant indicators in accepting the food materials by the consumers. Table 1 reports the color in terms of lightness (L*), redness (a*), and yellowness (b*) of uncoated and coated sea bass fillets during refrigerated storage for 16 days. The color properties of fish muscle showed an interaction between packaging material and storage time. Generally, the L*, a*, and b* values of uncoated samples significantly ($P < 0.05$) increased during the refrigerated storage. The L*, a*, and b* values of fillet samples coated with chitosan with or without ZnONPs/ZEO showed no significant differences

Table 1 Effects of ZnONPs and ZEO nanocomposite on color changes of Asian sea bass during storage in refrigerator

Color index	treatments	Time of Storage (day)				
		0	4	8	12	16
L*	Control	68.17 ± 2.20^{Aa}	53.55 ± 1.71^{Cc}	61.15 ± 1.89^{Ab}	54.48 ± 1.82^{Bc}	52.21 ± 1.85^{Bc}
	CH	68.17 ± 2.20^{Aa}	61.87 ± 0.01^{ABb}	62.31 ± 2.18^{Ab}	59.17 ± 1.71^{ABbc}	54.38 ± 1.70^{ABc}
	CH-ZnONPs	68.17 ± 2.20^{Aa}	59.13 ± 1.97^{Ba}	61.24 ± 4.92^{Aa}	59.01 ± 4.23^{ABa}	60.15 ± 4.14^{Aa}
	CH-ZEO	68.17 ± 2.20^{Aa}	62.61 ± 0.61^{ABab}	61.76 ± 1.41^{Abc}	56.44 ± 2.35^{ABc}	59.60 ± 1.88^{ABbc}
	CH-ZnONPs-ZEO	68.17 ± 2.20^{Aa}	65.72 ± 1.26^{Aab}	64.24 ± 1.91^{Aab}	63.66 ± 2.08^{Aab}	60.36 ± 0.18^{Ab}
b*	Control	11.81 ± 0.55^{Ab}	13.52 ± 0.59^{ABb}	12.24 ± 0.90^{ABb}	12.15 ± 0.80^{ABb}	20.89 ± 1.22^{Aa}
	CH	11.81 ± 0.55^{Aa}	14.04 ± 0.22^{Aa}	13.47 ± 0.61^{Aa}	11.54 ± 0.61^{ABa}	11.61 ± 1.42^{Ba}
	CH-ZnONPs	11.81 ± 0.55^{Aa}	16.33 ± 0.47^{Ab}	11.12 ± 0.36^{Bbc}	13.34 ± 1.16^{Ab}	10.05 ± 1.34^{Bc}
	CH-ZEO	11.81 ± 0.55^{Aa}	13.36 ± 0.07^{ABa}	11.36 ± 0.36^{Ba}	4.91 ± 0.32^{Cc}	8.83 ± 1.57^{Bb}
	CH-ZnONPs-ZEO	11.81 ± 0.55^{Aa}	10.66 ± 1.93^{Ba}	10.30 ± 0.60^{Ba}	10.75 ± 0.08^{Ba}	9.30 ± 0.19^{Ba}
a*	Control	5.31 ± 0.34^{Ab}	7.15 ± 0.56^{Ab}	4.01 ± 0.66^{Ab}	6.57 ± 1.10^{Ab}	12.76 ± 1.54^{Aa}
	CH	5.31 ± 0.34^{Aa}	3.70 ± 1.12^{BCa}	4.49 ± 1.01^{Aa}	6.07 ± 0.30^{Aa}	3.90 ± 0.72^{Ba}
	CH-ZnONPs	5.31 ± 0.34^{Aab}	5.02 ± 0.63^{BCab}	4.12 ± 1.53^{Aab}	6.28 ± 0.25^{Aa}	3.09 ± 0.02^{Bb}
	CH-ZEO	5.31 ± 0.34^{Aa}	3.27 ± 0.14^{Ca}	4.76 ± 1.08^{Aa}	4.25 ± 1.61^{Aa}	4.55 ± 0.23^{Ba}
	CH-ZnONPs-ZEO	5.31 ± 0.34^{Aab}	5.75 ± 0.10^{ABa}	4.74 ± 0.56^{Aab}	5.16 ± 0.99^{Aab}	3.85 ± 0.28^{Bb}

*Mean values and standard errors from the three replicates are presented. The different uppercase letters in the same columns within the same storage time indicate the significant differences ($P < 0.05$). The different lowercase letters in the same rows within the same treatment indicate the significant differences ($P < 0.05$)

($P > 0.05$) during the entire storage time. Uncoated sea bass fillets were brighter (higher L* value), more reddish (greater a* value), and more yellowish (higher b* value) in comparison with coated fillets samples, on day 16 of the storage period ($P < 0.05$). The b* values of uncoated fillets indicated a significant ($P< 0.05$) increase during the storage period, though the b* values of the fillets coated with CH did not show any significant difference with the fillet coated with CH-ZnONPs and CH-ZnONPs-ZEO during the storage period ($P > 0.05$). According to Zhang et al. (2013), the aldehyde formed by auto-oxidation of lipids may have an interaction with the amino groups of proteins. This reaction leads to improvements of yellowish color (b* value). Further, the a* value of the fillets increased significantly over time ($P < 0.05$). Lipid oxidation is one of the main factors in oxidizing pigments which change color of fillets (Giteru et al. 2017). Use of CH in the coating, even without ZnONPs/or ZEO, was effective in reducing the oxidation of heme pigments and reduce the reduction–oxidation potential of lipids, thereby reducing the reddish color (a* value). These results concur with the results reported by Amjadi et al. (2019), who stated that the use of CH nanofiber and ZnONPs in the production of gelatin-based nanocomposite was effective in reducing a* value in chicken fillets stored at 4 °C. They stated that one of the main factors affecting the fillets color is lipid oxidation. Also, they found that use of coatings can reduce lipid oxidation, thus a* value was reduced. Some parameters such as the structure of muscle, hematin pigment, and the extent of unbound water, with changes in the surface muscle light scattering, could lead to higher L* values in control fillets at the end of the storage period, indicating an opaque color (Erikson et al. 2018). The combination of CH coatings with additives could protect the color quality of sea bass fillet. These results suggested that chitosan individually or in combination with ZnONPs and ZEO induced an increase in the L* values of sea bass fillets and made the fillets darker. This result was in line with the results reported by Vital et al. (2018) who did not observe any significant differences in the L* values of chitosan-coated *Oreochromis niloticus* fillet with or without additives during the storage period.

Sensory analysis

The total scores of acceptance in the control and treated sea bass fillets are shown in Fig. 3. The tot scores of acceptance ranged from 1 to 5. On the first day of storage, all sea bass fillets revealed the best quality, obtaining the highest sensory scores (5). The total score of acceptance in the control and coated sea bass fish fillets decreased during storage (1.08 ± 0.08 for control fillets and 2.58 ± 0.83 for CHZnZEO treated fillets on day 16). Regarding the appearance scores of chilled sea bass fillets, there was no significant difference between CH-ZnONPs and CH-ZEO coated fillets, though the CH-ZnONPs coated fillets

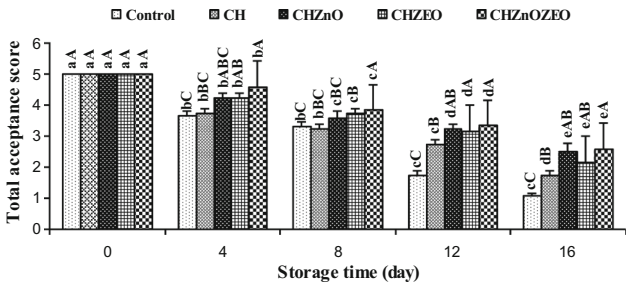

Fig. 3 The effect of ZnONPs and ZEO nanocomposite on changes in total acceptance score of Asian sea bass during storage at the refrigerator, Mean values, and standard errors from the three replicates are presented. Different uppercase letters indicate significant differences between treatments ($P < 0.05$). Different lowercase letters show significant differences in storage time in each treatment ($P < 0.05$)

showed a higher score during the 16 days of storage. The sensory properties of control fillets were unacceptable on day 8. On day 16, the CH-coated fillets were not acceptable (1.75 ± 0.14). The unpleasant appearance and adhesive surface of the fillets are the probable reasons for this result. On the other hand, the combination of ZnONPs or ZEO in CH increased the acceptance score. Thus, the sensory scores of nanocomposite + ZEO coated fillets showed the best fillets quality and they were more pleasant for the panelists (as consumers). This score arose from lower bacterial load of the treated fillets and lower lipid oxidation in comparison to the control fillets (untreated fillets). These results concurred with the results of Arfat et al. (2015) for sea bass treated with gelatin-ZnO nanocomposite with basil leaf EO stored at 4 °C. Amjadi et al. (2019) also observed that the incorporation of ZnO NPs into a gelatin solution extended the quality of chicken fillets.

Conclusion

Based on the results obtained from this study, bacterial growth was inhibited in coated sea bass fillets during the storage period. The decreased bacterial load led to reduced TVB-N and pH values. The combination of EO and ZnO had the highest antibacterial effect. However, coating the fillets did not lead to LAB growth inhibition. Further, ZnONPs and ZEO combination with CH coating significantly decreased the values of oxidation of lipids thus increasing the antioxidant effect. Overall, the synergistic effect of ZnONPs and ZEO might be a valuable method to expand the antioxidant and antibacterial effect of nanocomposites.

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Authors' contributions SZM: Investigation, methodology, Writing the primary manuscript, SMM: Conceptualization, Supervision, Review & Editing, Project administration, Resources, AK: Supervision, Data Curation, Validition, Methodology, Review & Editing, SMH: Methodology.

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Availability of data and material The data are not shared.

Declaration

Conflict of interest The authors declare that they have no conflict of interest.

Consent to participate All authors of the manuscript are aware from submission of this manuscript.

Consent for publication I hereby accept liability for the scientific integrity of the manuscript contents. I hereby declared that this manuscript is an original research article which has not been submitted or published in other journals. Also, my study does not involve human subjects. This manuscript is resulted from Research Project under financial support of Khorramshahr University of Marine Science and Technology, Khorramshahr, Iran.

Ethical approval The authors of the manuscript declared that the experimental conditions were standard and ethical statements about experimental animals were observed during the experimental. The minimum samples were collected and the animals were anesthetized before any sampling.

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