Research Article

Application of chitosan and *Satureja khuzestanica* essential oil coating on the shelf life of *Mugil cephalus* L. fillets

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Received: July 2022 **Accepted:** November 2022

Abstract

In this regard, the present study is aimed to assess the effect of chitosan and *Satureja khuzestanica* essential oil (SKEO) on the prolongation of the shelf life and quality of *Mugil cephalus L.* fillet. Edible coating based on biopolymers and phenolic compounds is an effective way to preserve the quality of fish. There is a growing demand for bio-based and active packaging as one of the preferred emerging technologies to improve food quality and extend shelf-life. To this end, the influences of various variables such as storage time (0, 7days+12hours (7.5) and 15 days), storage temperature (-10, -3, and 4 ℃), and essential oil content (0.5, 1 and 1.5) were assessed on the shelf life of *M. cephalus* L fillets through the use of RSM software. A significant rise was observed in the pH of all samples by increasing the storage time ($p<0.05$). Thiobarbituric acid and nitrogen bases (mainly composed of trimethylamine, dimethylamine, and ammonia), as well as peroxid, also increased by prolonging the storage time and reached their highest level at the end of the storage period ($p<0.05$). On days 7.5 and 15, treatment (temperature of -3℃, and 2% essential oil) and treatment (temperature of -10℃ and essential oil of 2%) showed the lowest microbial load (2.39 ± 0.55 and 5.96 ± 0.23 log cfu/g, respectively) while the highest microbial load was detected in the treatment involving 0.5% essential oil and storage temperature of 4℃ ($p<0.05$). Based on sensory tests, no significant difference was observed in the total acceptance of the treatments. The results of the current research indicated that coating with chitosan (2%) and *S. khuzestanica* essential oil (especially 1%) can enhance the storage time of *M. cephalus* fillets in the refrigerator.

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Keywords: Edible coating, Herbal essential oil, *Mugil cephalus*, Shelf life

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Introduction

The growing demand for biological antimicrobial and antioxidant compounds, along with the consumer awareness concerning the use of synthetic chemical preservatives have contributed to the rise of a new trend called "green consumerism" in food industry, which is the basis for the development of alternative approaches for food preservation (Pabast *et al.*, 2018). Chitosan is one of the cationic polysaccharides which can be prepared by chemical and microbiological de-acetylation of chitin from crustacean's skin such as crabs and shrimps. It is the second most abundant natural polymer after cellulose (Peniche *et al.,* 2008). This polysaccharide has functional properties such as antibacterial, antifungal, and antioxidant properties. It is also benefitted from environmental compatibility, biodegradability, non-toxicity, and various physicochemical properties (Keykhosravy *et al.,* 2020). Other functional properties of chitosan include its ability to form a protective film, texturizing agent, proper adhesion, adsorbent and purifying properties, as well as high dietary fiber and applicability as a barrier against gas and moisture in the food and pharmaceutical industries, cosmetics, and textile dyeing. The antimicrobial properties of chitosan can be assigned to positively charged amino groups that react with the negatively charged cell membrane of microorganisms (Antunes *et al.,* 2021). This reaction leads to leakage of protein and other intracellular components of microorganisms. Through chitinase accumulation which inhibits the synthesis of proteinase, chitosan can also reduce the proteinase of the fungal cell wall (Mehdizadeh *et al.,* 2020).

The genus *Satureja* consist of more than 200 species of annual or perennial aromatic plants that is part of family *Lamiacea*e (Dordevic *et al.,* 2022). *S. khuzestanica* belongs to an endemic plant that are widely spread in South-west Iran and known for its clinical uses as analgesic and antiseptic (Pabast *et al*., 2018). *S. khuzestanica* essential oils (EOs) have been introduced to have biological properties, such as antioxidant, antifungal and antibacterial,that have been mostly belonged to their phenolic compounds content, especially carvacrol. (Hadian *et al*., 2011). In addition to the coating, natural essential oils with polyphenolic groups, terpenes, terpenoids, and aliphatic compounds can act as antioxidants and serve as a barrier against free radicals. In addition to their non-toxicity and no side effects (Perdones *et al.,* 2014 and Thielmann *et al.,* 2017), essential oils are also highly acceptable to consumers (Khanzadi *et al*., 2019). Accordingly, SKEO can be exploited as a potential new agent to replace synthetic ones.

The flathead grey mullet is an important edible fish species in the mullet family *Mugilidae*. It is shown in coastal subtropical and tropical waters around the world. Its length is typically 30 to 75 centimeters. Mullet (*M. cephalus*) is one of the most widely distributed marine food fish species in the world. Commonly, it is found in association with shallow weed beds and empty coastal water beds and estuaries (Bouzagarrou *et al*., 2016). Fish is a valuable marine protein. Fresh fish is extremely perishable because of its natural

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composition. Deterioration of fish muscle caused by changes accompanied by some biological processes such as lipid oxidation, decrease in protein functionality, reactions generated by the activities of the fish's autolytic enzymes, and the metabolic activity of microorganisms (Aref *et al*., 2022). Due to its high level of oxidizable fatty acids, is highly susceptible to oxidation of fats, which in addition to reducing the nutritional value, decline the desirability and acceptance of this valuable food. As microbial growth on the surface of food is the major cause of spoilage, the use of food coatings capable of delaying the growth of bacteria can enhance the safety of food products while prolonging their shelf life. Regarding the functions of chitosan, this substance seems to be a suitable candidate for this purpose (Azizian *et al.,* 2019). Therefore, the use of active packaging could be a proper solution for microbial protection of food compounds, but, there are some topics related to the direct incorporation of essential oils consist of some chemically reactive compounds such as phenolic compounds into complex food systems such as negative effects on the integrity of the food chemistry or physical stability, reduction of the biological activity of bioactive (Pabast *et al*., 2018).

Farzaneh *et al.* (2015) introduced the presence of carvacrol compounds in *S. hortensis*, *S. spicigera*, and *S. khuzistanica* as the cause of antibacterial and antioxidant activity. Pabast *et al.* (2018) studied the effects of chitosan consists of incorporating with free or nanoencapsulated *Satureja* plant essential oil on quality characteristics of fillet and showed that encapsulation decreased the release of SKEO and led to a prolonged antioxidant and antimicrobial activity and also induced sensory characters; their study suggests that chitosan coating containing encapsulated SKEO can be a promising candidate for increasing the shelf-life. Yousefi *et al.* (2020) used plant essential oils against *Listeria monocytogenes* and showed reducing bacteria count with coating. Chambre *et al.* (2020) assigned the high antioxidant capacity of *S. hortensis* to the presence of carvacrol. Rezaei *et al.* (2021) used electrospun chitosan nanofibers and *Artemisia sieberi* extract to control the growth of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Rezaei *et al.,* 2021). Aref *et al.* (2022) investigated improvement of the shelf life of grey mullet (*M. cephalus*) fish steaks using edible coatings containing chitosan, nanochitosan, and clove oil during refrigerated storage and the results showed that a nanochitosan coating with clove oil was the best treatment long-term the shelf life of mullet steaks and efficiently kept the quality attributes to an acceptable level during refrigerated storage for 24 days. According to the studies there are no reports on the fabrication of edible active coatings using SKEO and chitosan. Additionally, no definite data exist on the application of the edible coating containing chitosan and herbal essential oils in fish products so the present study is performed to determine the best level of *S. khuzestanica* essential oil that coating with fixed amount of chitosan to improve and increase the shelf life of *M. cephalus* fillet.

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Materials and methods

Preparation of fish samples

Twenty *M. cephalus* fishes with an average weight of 600 g were freshly purchased from the fish market and immediately transported to the laboratory in ice boxes. The fish samples were washed with drinking water and after descaling and cutting off the fans, viscera were removed to prepare the samples for further experiments.

Extraction of *S. khuzestanica* essential oil

The leaves *S. khuzestanica* were dried in shade for one week. The essential oil was then extracted with water in a glass Cleavenger machine (Farzaneh *et al.*,2015) for 4 hours. The obtained extract was dehydrated by sodium sulfate and stored in vials at 4 °C until further analysis. The compositions of the essential oil were determined by chromatography (Agilent Technologies-7890A) apparatus connected to a mass spectrometer (AgilentTechnologies-5975C) with HP-5MS capillary column (length of 30 m × outer diameter of 0.25 mm × inner diameter of 25 μm).

Preparation of chitosan solution

One% acetic acid solvent was used to prepare the mentioned concentrations of chitosan. For coating, chitosan solutions with a constant concentration of 2% w/v were used (Azizian *et al.*, 2019) which were obtained by dissolving 20 g chitosan powder in 1% v/v acetic acid and their volume to 1000 ml. Then *S. khuzestanica* essential oil (SKEO) was added to chitosan according to the output of RSM software and mixed at 12000 rpm for 1 min. The weighted fillets were poured into 200 cc beakers for 10 min and then removed and hung from sterile mesh plates. They were then exposed to mild airflow on sterile aluminum foils until full drying. The fillets were transferred to the refrigerator and kept at the specified temperatures for 15 days (Khezri *et al*., 2012).

Treatments

The treatments that were subjected included control treatment 1, treatment 2 containing 0.5% SKEO + 2% chitosan, treatment 3 containing 1% SKEO + 2% chitosan, and treatment 4 containing 1.5% SKEO + 2% chitosan.

Measurement of peroxide value

Peroxide number was measured according to the method stated by AOAC (2005). Typically, 50 g of the sample was added to a 500 ml Erlenmeyer flask and heat to 60 ℃ in a water bath for 3 min to melt the fat. Then, 30 ml of acetic acid and chloroform solution (3 V / V) was added followed by 3 minutes of stirring to dissolve the fat. The sample was passed through a Whatman filter paper under vacuum to separate the fish particles. To the filtered solution was added 0.5 ml of potassium iodide saturated solution. It was then poured into a titrator burette and titrated with 25 ml of standard sodium thiosulfate solution. The peroxide number can be calculated using the following equation:

$$\text{POV (meq } O_2\text{/kg Fat)} = \frac{S \times N}{W}$$

S = titration volume (ml), N= normality of sodium thiosulfate solution =0.01, W= sample weight (kg)

Measurement of thiobarbituric acid (TBARS)

Ten g of the sample was mixed with 50 ml of distilled water. The resulting mixture was

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transferred to distillation Erlenmeyer along with 47.5 ml of distilled water. HCl (4 normal, 2.5 ml), along with anti-foam and anti-boiling agents, was added to the mixture and the flask was connected to the distillation device. The mixture was heated and 50 ml of the distilled substance was collected from the mixture after boiling. 5 ml of the distilled material and 5 ml of the TBA reagent were transferred to the capped tubes. After shaking, they were placed in boiling water for exactly 35 min. The same time steps were simultaneously repeated for the control samples. The samples were cooled for 10 min and their optical density was read vs. the control at a wavelength of 538 nm (Egan *et al.*, 1981).

TBA=Optical density × 7.8 (mg malondialdehyde/kg Fat)

pH measurement

The sample (5 g) was transferred to a sterilized bag followed by adding 45 ml distilled water. The bag content was then mixed and homogenized in ablender for 15 s. The digital pH-meter (Metrohm pH-meter, model 827 pH-lab, Switzerland) was first calibrated and placed in the sample until the emergence of a fixed number (Parvaneh, 1998).

Measurement of total volatile nitrogen (TVN)

Volatile nitrogenous species in the samples are formed upon the decomposition of proteins and can be measured by the macro-Kjeldahl method. The sample (10 g) together with 2 g of magnesium oxide catalyst, 300 ml distilled water, and several glass pearls were transferred into a Kjeldahl distillation flask (capacity of 500-700 ml). Boric acid (2%, 25 ml) and a few drops of 0.1% alcoholic methyl red reagent were added to the receiver Erlenmeyer underneath the refrigerant (such that the end of the refrigerant was in solution), which decolored the solution. Then the digestion balloon was heated whose contents boiled within min minutes. The distillation process was continued for 25 min from the time of boiling. Therefore, volatile bases of the fish meat were distilled and absorbed by the contents of the receiver Erlenmeyer, the color turned blue (as the environment got alkaline). The heat was switched off and the distilled solution was diluted with 0.1 N sulfuric acid until the emergence of pale purple color. As each milliliter of 0.1 N sulfuric acid corresponds to 0.0014 g or 1.4 mg of nitrogen, the volatile bases can be determined through the following equation (Parvaneh 1998):

TVN = 0.1% Acid consumption for sample × 1.4× 100

Total viable count (TVC)

Fish fillets (5 g) and 45 ml of distilled water were transferred to a sterile bag and homogenized. The sample was then diluted to 10^5 ml. Each dilution (1 ml) was cultured on count agar medium plates. All plates were incubated upside down for 48 hours at 37 ℃ (Iranian standard No. 2325, 1380).

Psychrotrophic count (PTC)

At sterile conditions and under the laboratory hood, the containers containing the sample were opened and 5 g of shrimp meat was separated by pliers and sterile scissors and placed in sterile plastic bags. Then 45 ml of sterile distilled water was added and the bag was transferred to the Inter-science 400 OstoMicro blender for homogenization in 1 minute. The homogenized sample was

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sequentially diluted and cultured on nutrient agar plates and surface cultured. The psychrotrophic bacteria were counted by placing the plates at 10 ^{o}C for 7-10 days (Iranian National Standard No. 5272, year).

Sensory tests

A five-factor Hedonic questionnaire was employed for this test (Table 1). Fish samples were fried and their texture, color, taste, smell, and total acceptance were evaluated (Hedayatifar *et al.,* 2011).

Data analysis

The obtained data were analyzed by State-Ease Design Expert V7 software based on face-centered composite design technique and using SPSS software (ver. 16), One-way ANOVA and Tukey's HSD post-hoc test were used to investigate the difference in treatments. Data are presented as mean ± standard deviation (SD) and pvalues <0.05 were considered significant.

Table 1. Sensory parameters of the treated *Mugil cephalus* fillets

Featured	Description	Grade
Color	Completely pale, yellow and mucous…without color change, shining, bright color	1…5
Odor	Completely undesirable, strong ammonia… desirable, fresh, sea weedy	1…5
Taste	Completely unpleasant, sour, sulphide…sweet, pleasant	1…5
Texture	Pasty, very soft… fresh fish cohesion, firm, springy	1…5
Total acceptance	Completely unacceptance… desirable	1…5

Results

pH variations in the fillest coated with chitosan and *S. khuzestanica* essential oil

According to the model (Fig. 1), pH = 0.98 + 0.13A + 0.44B-0.11C-1.89AB-2.09AC-5.11BC risk parameters of temperature, time and concentration of the extract, as well as the interaction of time and temperature, time and essential oil concentration, and essential oil concentration and time were among the effective parameters on pH variations (*p <0.01*). Regarding the F-Value of essential oil concentration (921.42) compared to the F-Value of time (51.21) and temperature (77.59), the essential oil concentration was the most effective variable on pH. The pH increased significantly with raising the temperature and time; while increment of the concentration declined this parameter in the fillets.

Thiobarbiotic acid and peroxide levels of *M. cephalus* fillet covered with chitosan and *S. khuzestanica* essential oil

According to the results of the analysis of variance, the test model significantly justified the production of PV and TBA in *M. cephalus* fillet (*p <0.01*). The linear effect of temperature, essential oil concentration, and time was effective on PV and TBA production at a 99% confidence level. The linear parameter of temperature showed a lower slope compared to time و so that the decrease in fillet storage temperature caused a significant decrease in the amount of thiobarbiotic acid and peroxide (*p*

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<0.01). According to the p-value values, the interaction of temperature and time was not effective on PV production at 95% level, thus it was removed in the proposed model (*p <0.01*). Figs. 2 and 3 significant exhibit a decline in PV and TBA values by decreasing the temperature and storage time and increasing the level of essential oil concentration (*p <0.01*).

Volatile basic nitrogen of *M. cephalus L.* fillets coated with chitosan and *S. khuzestanica* essential oil

According to the F-values, the concentration of essential oil was more effective on TVB-N compared to temperature and time (*p <0.01*). As suggested in Fig. 4, the lowest TVB-N was recorded at low temperatures and the highest extract concentration. Regarding the F-Value, the extract concentration had the highest impact on the TVB-N parameter at a significant level of 5%, so, it had a greater effect than the variables of temperature and storage time (Fig. 6).

Variations of bacterial load of *M. cephalus* L. fillet covered with chitosan and *S. khuzestanica* essential oil

The results of the analysis of variance of the proposed model for the total viable count and the psychrotrophic count of *M. cephalusL*. fillets are shown in Figs. 4 and 5. Linear parameters of temperature, essential oil concentration, and time had a significant influence on the TVC of *M. cephalus L.* fillet (*p <0.01*). The concentration of essential oil regarding the variables of temperature and storage time had the greatest effect on the bacterial load at a significant level of 5% (with an F-Value of 253.77). According to Fig. 5, the slope of the temperature factor was greater than the time variable in terms of their impact on bacterial load and show that the decrease in temperature causes a significant decrease in bacterial load (*p <0.01*). Regarding the psychrotrophic bacteria, temperature, essential oil, and time were effective (*p <0.01*). According to the F-Value, increasing the concentration level of essential oil caused a significant decrease in the amount of bacteria (*p <0.01*). The synergistic effect of temperature and concentration of essential oil on bacterial load was seen. Based on three-dimensional figures, an increase in temperature and storage time increased significantly in psychrotrophicbacterial load (*p <0.01*).

Optimization

Predicted and real R2 values of the independent variables and their dependent responses showed high desirability (Table 2) this means that the predicted values are closer to the real values with no significant differences (*p<0.05*).

Sensory analysis

The results showed that increasing the percentage of chitosan solution and the percentage of S. khuzestanica essential oil caused a significant increase in the score of sensory features such as color, different tempratures, taste, texture, odor, and total acceptance (*p<0.05*). Only in treatment 4, no significance was observed with other treatments (at -10 ℃, -3 ℃ and 4 ℃). Treatment 3 and -10 ℃ was the best in terms of color, odor, taste, texture, and total acceptance. In other words, 2% chitosan solution and 1% *S. khuzestanica* essential oil could be the best treatment for *M. cephalus* fillet. According to Fig. 7, the worst sensory features were recorded in treatment 1, where only *M. cephalus* fillet was used.

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Table 2. Fitted models for the measured parameters of the treated *Mugil cephalus* fillets

Variable	R^2	R^2-adj
TVC	0.91	0.89
FFA	0.91	0.9
TVB-N	0.96	0.94
TBA	0.90	0.86
PV	0.90	0.82
Psychrotrophic bacteria	0.90	0.83
pH	0.90	0.89

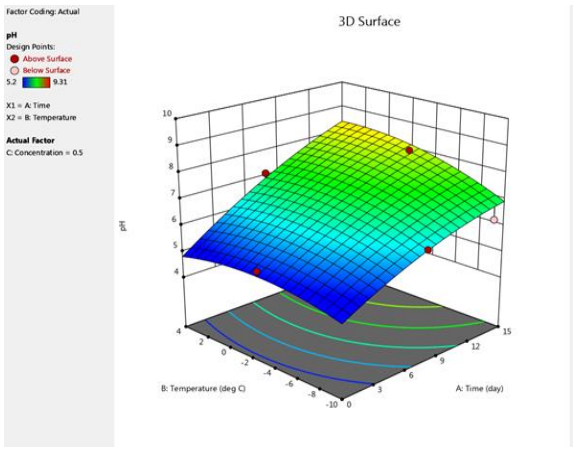

Figure 1. Three-dimensional effect of the input variables on pH variations of *Mugilcephalus L* fillets.

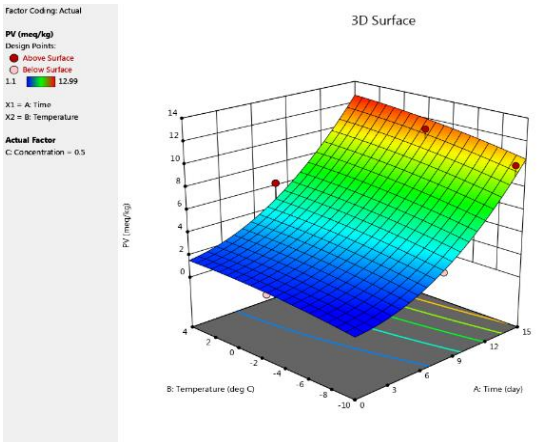

Figure 3. 3D effect of input variables on PV of *Mugil cephalus* fillet

PV=125+0.17A+0.44B-0.19C-0.231AC

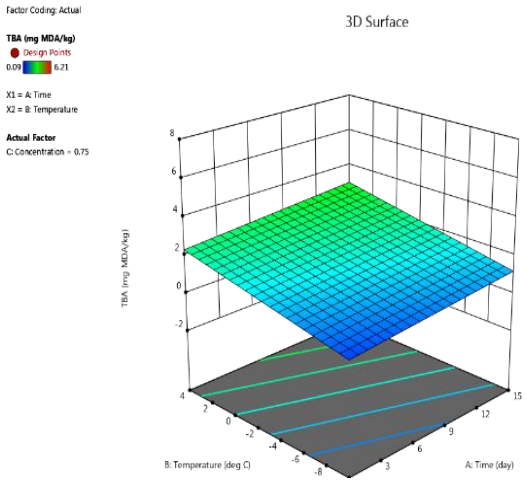

Figure 2. 3D effect of input variables on TBA of *Mugil cephalus* fillet

TBA =0.91+0.78A+0.38B-2.39C-4.11AC-0.19BC

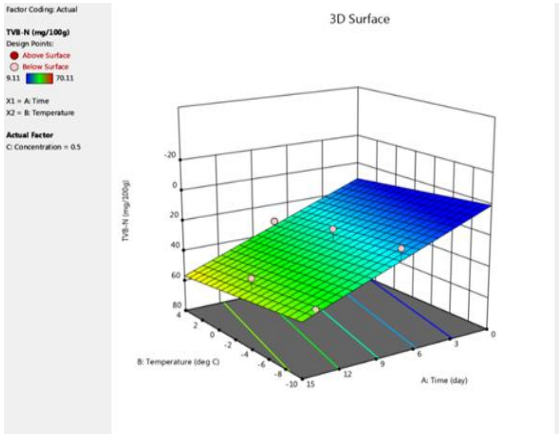

Figure 4. 3D effect of input variables on TVB-N of *Mugil cephalus* fillet

TVB-N=62+2.11A-0.09B-0.2C

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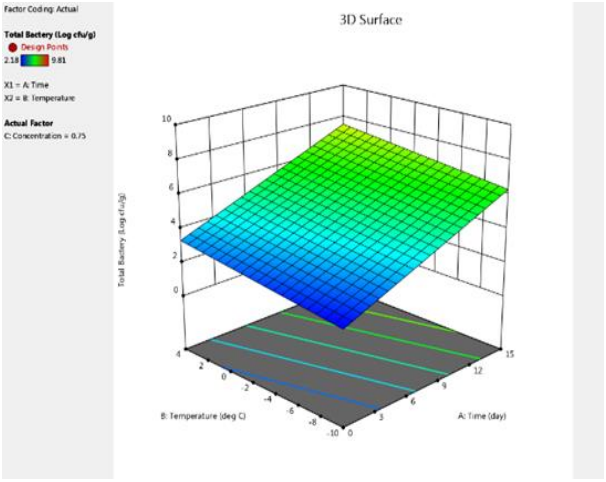


Figure 5: 3D effect of input variables on TVC of Mugilcephalus L. fillet

Total bacterial

4.83+2.1A+0.607B-0.781C-3.72AC

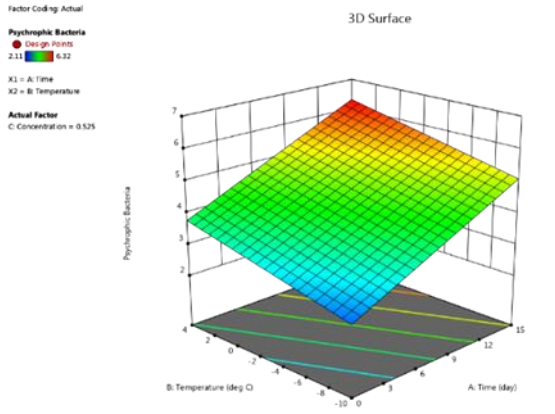
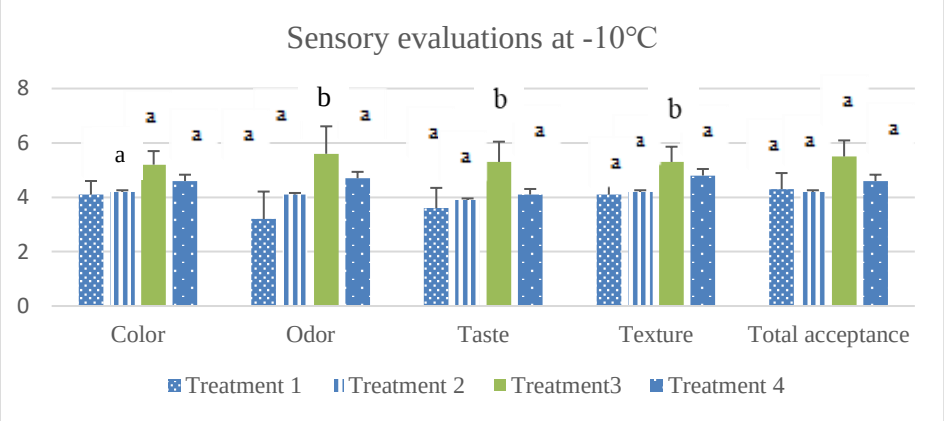
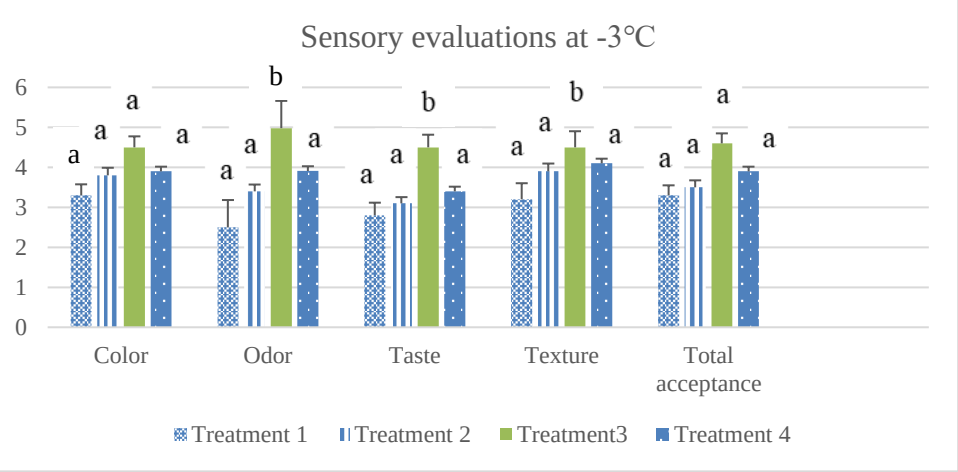


Figure 6. 3D effect of input variables on PTC of *Mugil cephalus* fillet

Psychrotrophic bacterial

60.36+6.96A+5.12B-0.98C-84.11AC





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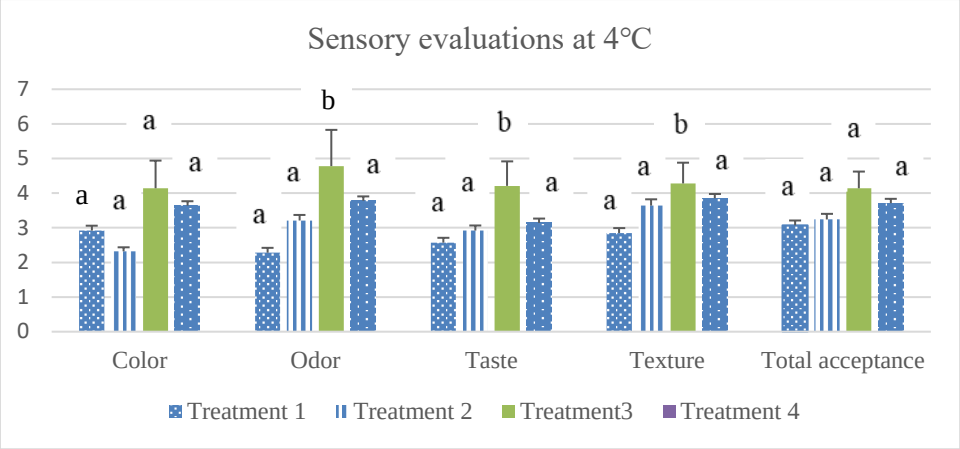

Figure 7. Sensory evaluations for *Mugil cephalus* fillets treated with chitosan and Satureja khuzestanica essential oil for a storage time of 15 days at different temperature (-10℃, −3℃, 4℃)
Different characters in a row imply a significant difference.
Treatment 1: control; Treatment2: 2% chitosan solution and 0.5% essential oil; Treatment 3: 2% chitsoan solution and 1% essential oil; Treatment 4: 2% chitosan solution and 1.5% essential oil.

Discussion

This study was aimed to use the response surface method for simultaneous investigation of the three variables of temperature, time, and concentration of essential oil on the shelf life of *M. cephalus L.* fillets coated with chitosan and *S. khuzestanica* essential oil. The findings showed that the concentration of essential oil has a significantly higher effect than the temperature and time of fillet storage against antibacterial and antioxidant activities, Ozyurt *et al.* (2015) confirm this results that cold storage and freezing can increase the shelf life, but they do not completely improve the quality of fish fillet (Alboghobeish, and Khodanazary, 2018).

Peroxide value (PV) in the early stages of oxidation is due to the binding of oxygen to the double bond of unsaturated fatty acids (Yanar 2007) while TBA can be assigned to the reaction of malondialdehyde with other oxidizing compounds such as amines, nucleotides, fatty acids, proteins, and phospholipids in the secondary stages of oxidation (Connell 1975). The output of the response surface software revealed that the three variables of temperature, storage time, and extract content were effective on the oxidation parameters of *M. cephalus L.* fillet. Among them, significantly, the the concentration of essential oil (treatment 3, 4) and then low temperature had significant effect on fillet oxidation (TBA = 0.91 + 0.78A + 0.38 B-2.39C-4.11AC-0.19BC and = 125 + 0.17A + 0.44B-0.19C-0.231AC PV). Numerous studies such as Chambre *et al.* (2020) and Raji *et al.* (2019) have reported the presence of antioxidants as well as antibacterial compounds such as phenolics, carvacrol, and thymol in *S. khuzestanica*(Chambre *et al.*, 2020) which is consistent with the results of the present study. By reacting with active oxygen compounds such as superoxide, hydroxyl radicals, and hydrogen peroxide, the mentioned compounds increase oxidation-related parameters (TBA, PV, FFA); they also prolong the shelf life of *M.*

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cephalus L. fillets while reducing the oxidation rate (Raeisi *et al.,* 2015) so the findings of these studies are consistent with the results of the present study. *S. khuzestanica*-containing chitosan coating, in addition to having antioxidant compounds, also prevents oxygen molecules from reaching the surface of the fillet (Dinis *et al.,* 1994). The results of Li *et al.* (2012) and Fan *et al.* (2009) also confirm the results of this study which indicate the positive influence of chitosan coating in combination with plant extracts in increasing antioxidant properties and reducing bacterial enzymatic reactions associated with lipid oxidation(Fan *et al.,* 2009). Chitosan is a natural food additive due to its non-toxic nature, biocompatibility, biodegradability, antimicrobial and antioxidant effects, and film-forming ability (Vieira *et al.,* 2019). Phenolic compounds of savory extract decelerate the primary and secondary oxidation compounds and reduce the oxidation in the fillet by trapping free radicals such as proxy radicals, which are a type of reactant in the intermediate parts of oxidation (Fernando *et al.,* 2016), this result (reduce the oxidation) was obtained in the present research.

Volatile basic nitrogens are a set of compounds including trimethylamine, dimethylamine, ammonia, and nitrogen bases produced by spoilage bacteria, which cause unpleasant odor (Ojagh *et al.,* 2010). A rise in TVC and PTC will increment the TVB-N significanly; both indicators have a similar trend. In all three variables psychrotrophic bacteria= 60.36 + 6.96A + 5.12B-0.98C-84.11AC, TVC = 4.83 + 2.1A + 0.607B-0.781C-3.72AC, and TVB-N = 62 + 2.11A-0.09B -0.2C, the concentration of *S. khuzestanica* essential oil was more effective than time and temperature. TVB-N values of treatment 3 and 4 showed rapid increase than the others and a significant difference was observed between treatment 3, 4 and control at the end of the cold and frozen storage. At the end, it can be concluded that the coating treatment was effective in controlling TVB-N values of fillet. The presence of carvacroland phenolic compounds led to the release of cellular components and the destruction of the cellular enzyme system by counteracting the production of internal proteases and disrupting the phospholipid bilayer membrane of the cell (Syed *et al.,* 2015), especially carvacrol had propitious antimicrobial and antioxidant activity due to its lipophilic character and phenolic hydroxyl group (Pabast *et al.,* 2018). These features are combined with antibacterial activities by chitosan due to its positively charged amino groups which interact with negatively charged cell walls (Shahidi *et al.,* 1999) and justify significant reduction in bacterial growth followed by a decrease in TVB-N. Genskowsky *et al.,* (2015) examined the effect of chitosan film with Maqui berry extract and showed higher antioxidant activity of the extract-containing film at all concentrations(Genskowsky *et al.,* 2015). The extent of antioxidant activity was dependent on the dose of essential oil, which was in line with the results of the present study. Barrera-Ruiz *et al.,* (2020) considered the composition of plant essential oils of *Schinusmolle*, *Thymus vulgaris*,*Cinnamomum zeylanicum*, and chitosan effective in increasing the antibacterial

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power of chitosan due to phenolic and flavonoid compounds of plant essential oils (Barrera-Ruiz *et al.* 2020). In a study by Sharafati et al. (2015) on the effect of chitosan containing eucalyptus and cumin essential oils was assessed on the TVC of trout fillets, they stated that the use of essential oils reduced the TVC, PTC, and Enterobacteriaceae in trout (Sharafati *et al.*, 2015).An increment in the concentrations of essential oils enhanced their antimicrobial effects, hence, declining the microbial load of fish which can be attributed to the antibacterial effects of essential oils due to the presence of phenolic compounds in essential oils. Dordevic *et al.*, (2022) in similar study, concluded that the chitosan coating *S. montana* L. essential oil itself had good antimicrobial activity, decreasing the number of psychrophilic bacteria, which confirms the result of this research.

In the present study, the pH of fresh fish was close to 7 (Arashisar *et al*. 2004) which rose significantly during the storage period due to increased activity of autolytic enzymes and fish-degrading proteolytic bacteria (Kilincceker *et al.*, 2009). In the present study essential oil coating (treatment 2-4), due to its relatively higher antimicrobial activity than control, was more effective significantly in inhibiting microbial growth and fish fillet spoilage., so such a trend was expected for pH by increasing the amount of volatile basic nitrogen, Wang et al. 2021 in their study showed that the increase of pH value was related to the proliferation of spoilage bacteria that could degrade proteins and produce volatile bases which was consistent with the results. Aşik and Candoğan (2014) reported that shrimp chitosan coating with garlic essential oil reduced the pH of the samples compared to the untreated samples, which can be due to the low pH of the chitosan and essential oil solution as a result of the presence of acetic acid content of essential oil (Aşik and Candoğan 2014).

Increasing the levels of *S. khuzestanica* essential oil significantly raised the sensory properties of *M. cephalus L*. samples was in all treatments except for Treatment 4. All sensory attributes of samples were significantly influenced by the storage time ($p<0.05$). All treatments fish fillet samples assumed unacceptable color, odor, taste and texture after day 10, Of course, lowering the storage temperature caused a significant increase in the score of sensory indices, similar results showed by Raeisi *et al.* 2018 and Vieira *et al.* 2019. Significantly, the best treatment was the one involving 2% chitosan and 1% *S. khuzestanica* essential oil. In a study by Alparslan and Baygar (2017) on pink shrimp (*Parapenaeus longirostris*) packed with essential oil-containing chitosan with essential oil and in the work of Fazlara *et al.* (2017) on the use of chitosan and rosemary, the applied treatments maintained the sensory factors (odor, taste, color, and texture) at an acceptable level over the storage time, that confirmed the results of present studies.

In the present study, *S. khuzestanica*-containing chitosan coatings were prepared and used on *M. cephalus* L. fillets during its storage under refrigerated and frozen conditions to enhance the quality and provide a safer product. The TVC and PTC results showed a lower

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bacterial load in the coated samples. The greatest reduction was observed in treatment 1. The results TVB-N measurements indicated the higher effectiveness of the coating in reducing the bacterial TVB-N than the wrapper. These coatings and wrappers exhibited their antioxidant properties with lower indices of thiobarbituric acid, free fatty acids, pH, and trimethylamine in coated and wrapped samples. Sensory tests also showed the effectiveness of *S. khuzestanica*- containing chitosan wrapping and coating on the sensory properties of the studied fillets. These results suggest that chitosan coatings containing SKEOs can be employed as an active packaging in fish meat processing industry.

Conflict of interest statement

The authors have no patents, whether planned, pending or issued, broadly relevant to the work. There is no aspect of the work covered in this manuscript that has involved animals has been conducted with the ethical approval of all relevant bodies or not. There are no relevant conflicts of interest for all authors.

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