Original Article

Effects of Carboxymethyl Cellulose and Tragacanth Gum Biocomposite Enriched With the Alcoholic Extract of *Dunaliella salina* Algae on the Shelf Life of Sea Bass Fillet

Amirhossien Ebrahimi[1], Zhaleh Khoshkhoo[1, 2*], Afshin Akhondzadeh Basti[1], Marjaneh Sedaghati[1]

1. Department of Food Science and Technology, NT.C., Islamic Azad University, Tehran, Iran.
2. Department of Food Science and Quality Conrtol, University of Tehran, Tehran, Iran.

How to Cite This Article Ebrahimi, A., Khoshkhoo, Z., Akhondzadeh Basti, A., & Sedaghati, M. (2026). Effects of Carboxymethyl Cellulose and Tragacanth Gum Biocomposite Enriched With the Alcoholic Extract of *Dunaliella salina* Algae on the Shelf Life of Sea Bass Fillet. *Iranian Journal of Veterinary Medicine, 20*(3), 537-552. http://dx.doi.org/10.32598/ijvm.20.3.1005858

http://dx.doi.org/10.32598/ijvm.20.3.1005858

ABSTRACT

Background: Food safety has become a global concern due to rising foodborne illnesses, highlighting the need for affordable, eco-friendly methods to detect and prevent spoilage. Smart packaging offers an innovative solution by actively monitoring and preserving food quality.

Objectives: This research aimed to evaluate the effect of a carboxymethyl cellulose (CMC) edible film, combined with the gum of the Tragacanth plant and containing 2% and 4% alcoholic extract of *Dunaliella salina* algae, on the shelf life of sea bass fish fillets.

Methods: Initially, hydroalcoholic extracts of algae were prepared, and edible films were subsequently formulated using a composite base of CMC and carrageenan. The films were divided into three treatment groups: control (CMC/tragacanth), CMC/tragacanth with 2% algal extract, and CMC/tragacanth with 4% algal extract. The antioxidant activity, moisture content, turbidity, and thickness of the films were measured. Thereafter, the films were applied as coatings for sea bass fillets. The physicochemical properties of the fish fillets (pH, total volatile base nitrogen [TVB-N], and thiobarbituric acid [TBA]), microbial parameters (mesophilic and psychrophilic bacteria, MIC), and organoleptic characteristics were evaluated over a 9-day storage period in comparison with the control samples.

Results: The edible films containing hydroalcoholic algal extract showed enhanced antioxidant activity, especially at 4% concentration. Incorporation of the extract slightly increased film thickness and turbidity, while moisture content decreased. Fillets coated with the 4% extract film showed the lowest pH increase, TVB-N, and TBA values over the 9-day period. Microbial analysis revealed that mesophilic and psychrophilic bacteria were significantly lower in treated samples compared to the controls. Additionally, MIC results confirmed the antimicrobial potency of the extract against *Escherichia* and *Salmonella typhi*. Sensory evaluation supported these findings, as treated fillets maintained acceptable texture, odor, and overall appearance, while control samples showed noticeable spoilage signs by day 9.

Conclusion: The edible film containing *D. salina* extract effectively extended the shelf life of sea bass fillets. This approach may open new horizons in the future of food safety and hygiene.

Keywords: Carboxymethyl cellulose (CMC), *Dunaliella salina* algae, Sea bass fish, Tragacanth gum

Article info:
Received: 05 Nov 2025
Accepted: 28 Dec 2025
Publish: 01 May 2026

*** Corresponding Author:**
Zhaleh Khoshkhoo, Associate Professor.
Address: *Department of Food Science and Technology, NT.C., Islamic Azad University, Tehran, Iran.*
Phone: *+98 (21) 77009801*
E-mail: *zh_khoshkhoo@iau.ac.ir*

Introduction

Aquatic animals, particularly fish, are widely acknowledged as nutritionally superior to terrestrial sources due to their easily digestible proteins, high concentrations of polyunsaturated fatty acids (PUFAs), rich macro- and micromineral content, and low caloric density (Ameur et al., 2022; Zhao et al., 2022). Among the PUFAs found in fish, docosahexaenoic acid (DHA; 22:6 n–3) and eicosapentaenoic acid (EPA; 20:5 n–3) are especially recognized for their significant health-promoting properties and are widely recommended for human consumption (Tocher et al., 2019; Lahreche et al., 2022). However, the high unsaturation of these lipids makes them highly susceptible to oxidative degradation during storage, leading to spoilage, sensory deterioration, and reduced shelf life (Lanzarin et al., 2016; Lahreche et al., 2022). In addition, quality degradation, such as protein degradation, fat oxidation, color changes, development of off-flavors, and texture softening can easily occur during the spoilage process of fish (Ameur et al., 2022; Zhao et al., 2023). In response to growing concerns over food safety and the rise of foodborne illnesses (Sobhan et al., 2022), vacuum packaging combined with refrigeration has become the most common method for preserving fat-rich fish products. This technique reduces oxygen levels inside the packaging, thereby limiting lipid oxidation and aerobic microbial growth (Genç et al., 2013; Lahreche et al., 2022). Nevertheless, even under these conditions, fish products often exhibit limited shelf life and reduced consumer acceptance (Zhao et al., 2023; dos Santos et al., 2022). Moreover, researchers have utilized various preservation techniques to delay the quality degradation of fish after death, including irradiation, modified atmosphere packaging, chemical preservatives, and biological preservatives. These methods can also effectively increase the shelf life of fish (Zhao et al., 2023).

Nevertheless, considering the environmental impacts of conventional packaging, over the past few decades, we have witnessed a proliferation of biodegradable and organic materials aimed at reducing human impact on the environment (Hosseini & Gómez-Guillén, 2018). Therefore, there is an urgent need to develop effective, low-cost, and eco-friendly preservation strategies for seafood products to minimize quality deterioration during transport and storage (Zhao et al., 2023). In this regard, the development of techniques to extend the shelf life of fresh seafood and other skinless protein products (such as chunks or fillets) has been an active area of research in recent decades. Edible films, also known as active coatings, have been widely recognized as a promising strategy, and various films have been successfully proposed to enhance the preservation of fresh meat (Maciel et al., 2020). In general, edible films are created using film-forming polymers through a solvent evaporation method. Additionally, to further enhance the preservation efficiency of edible films, various natural compounds, such as essential oils, bacteriocins, phenolic compounds, and other bioactive plant extracts are added to achieve greater antibacterial and antioxidant activity, enabling the films to meet specific protective requirements. Furthermore, the active compounds incorporated into the film are protected and released in a controlled manner to achieve increased shelf life (Yuan et al., 2022).

Polysaccharides, such as carboxymethyl cellulose (CMC) and various natural gums are widely recognized as promising materials for packaging film production due to their favorable thermal properties, biodegradability, abundance, and cost-effectiveness (Eshaghi et al., 2024). CMC, a water-soluble, colorless, tasteless, and non-toxic anionic derivative of cellulose, exhibits excellent film-forming capabilities. Films made from CMC are known for their relatively high mechanical strength and transparency, which make them particularly suitable for applications in the food industry (Keller, 2020). Tragacanth is a large polysaccharide molecule composed of acidic and anionic monosaccharides, along with some mineral salts, such as calcium, magnesium, and potassium, and a small amount of protein. This gum consists of a soluble part and a water-swelling insoluble part. The swellable insoluble part is often referred to as bassorin in most scientific references, while the soluble part is known as tragacanthin or tragacanthic acid. Numerous studies have been conducted to accurately identify the chemical structure and components of tragacanth. However, due to the complexity of its structure and the diversity and variation in its components across different species, the structure and composition of tragacanth gum vary depending on the plant species, time and place of growth, climatic changes, and collection methods. Tragacanth is resistant to acidic environments, and the viscosity and thickness of its solution change little within the pH range of 1 to 10 (Nazemi et al., 2023).

In recent years, algae have gained increasing attention as natural preservatives due to their rich nutritional profile, which includes polysaccharides, PUFAs, proteins, and antioxidants, such as carotenoids and phenolic compounds. Among these, *Dunaliella salina* stands out as one of the most halotolerant eukaryotic organisms, thriving in hypersaline environments, such as salt lakes, marshes, and brine pools near coastal areas. It is widely

recognized as the most important commercial source of natural β-carotene worldwide (Salimpour et al., 2019; Hejrani et al., 2017). *D. salina* produces both cis and trans isomers of β-carotene, with a notably higher antioxidant potential than synthetic β-carotene, which predominantly consists of trans isomers (Hyrslova et al., 2022; Bansal et al., 2009).

The common sea bass, belonging to the Serranidae family, is considered one of the most valuable and economically significant fishes in the Persian Gulf and is classified as a first-grade fish in the southern region of Iran (Millamena, 2002). Due to its desirable flesh taste, rapid growth, favorable feed conversion ratio, and high antioxidant properties, this fish has gained significant popularity as a marine aquaculture species worldwide (Hyrslova et al., 2022; Sathasivam et al., 2019). This study aimed to investigate the synergistic effects of a biocomposite film composed of CMC and tragacanth gum, enriched with alcoholic extract of *D. salina* algae, on the preservation and shelf-life extension of sea bass fillets. By evaluating physicochemical, microbiological, and sensory parameters during refrigerated storage, the research sought to develop an eco-friendly, antioxidant-rich active packaging solution that enhances product quality, reduces spoilage, and contributes to sustainable seafood preservation.

Materials and Methods

Preparation of tragacanth gum coating

Tragacanth gum was procured from the market. The gum was dissolved in mildly hot distilled water with continuous stirring. Glycerol (1%) was added as a plasticizer (Bhan et al., 2022).

Extraction of algae alcohol

To prepare the extracts, 8 g of *D. salina* powder from the National Algae Bank of Iran (INAC) was used in 200 mL flasks with pure ethanol for the 4% extract, and 4 g in 200 mL flasks for the 2% extract. The extracts were concentrated using a Heidolph WB rotary evaporator at 30 ˚C and stored at 4 ˚C in the dark.

Preparation of CMC solution

One gram of oral CMC from Sigma-Aldrich was dissolved in 100 mL of distilled water (1% w/v) under sterile conditions. Glycerol (0.4% w/v) was added as a softener, and the mixture was stirred for 10 minutes (Saeid Asr et al., 2021).

Preparation of edible film

The mixture of CMC, tragacanth gum, and *D. salina* algae extracts was stirred for 3 hours at room temperature using a magnetic stirrer for optimal dissolution. The mixture was sterilized and cast by pouring 100 mL into molds at 20 ˚C. Once dried, the films were removed and used to wrap fish fillets for evaluation (Rajaei & Shekarchizadeh, 2019). The experimental treatments used in this study are presented in Table 1.

Preparation of sea bass fillets

Fresh sea bass weighing between 700 and 800 g were purchased from the market and brought to the laboratory. The fish were eviscerated and filleted by hand to ensure uniformity. Skinless fillets were cut into squares measuring 7 cm by 7 cm with a thickness of 1 cm (Volpe et al., 2015). Finally, the fillet samples were packaged using smart biocomposite films and stored at 4 ˚C for 9 days.

Film tests

Antioxidant activity using DPPH (2,2-diphenyl-1-picrylhydrazyl)

Five mL of samples containing the CMC edible film and tragacanth gum without algae extract, and samples containing film and gum with 2% and 4% alcoholic algae extract (2×2 cm) were mixed with 2 mL of DPPH ethanol solution (0.2 mmol/L) and allowed to react in the dark for 30 min. The absorbance was recorded at 517 nm using a spectrophotometer. DPPH radical scavenging activity was calculated using Equation 1 (Cai et al., 2022):

1. $DPPH\ scavenging\ activity\ (\%)=(A1-A0)/(A1)\times 100$

Where, A1 is the absorbance value of the control (0.5 mL of distilled water mixed with 2 mL of DPPH ethanol solution) and A0 is the sample absorbance value (0.5 mL of each NVP solution mixed with 2 mL of DPPH ethanol solution).

Thickness

The thickness of the films was measured using a digital micrometer with an accuracy of 0.001 mm. Measurements were randomly taken and averaged at five points on each film. These values were then evaluated and analyzed in conjunction with the mechanical test results (Ojagh et al., 2010).

Humidity

Pieces of film measuring 3×3 mm were cut and weighed to determine their initial weight. The samples were then placed in an oven at 90 ˚C until a constant final dry weight was achieved. This final weight was considered the dry weight (Ojagh et al., 2010).

Turbidity

Film samples were cut into squares and placed inside a spectrophotometer. The absorption spectrum at 600 nm was recorded for each sample. Turbidity was calculated using the following Equation 2 (Peng & Li, 2014):

2. *Film turbidity=Absorbance at a wavelength of 600 nm/ Film thickness in mm*

Bass fillet test

pH

pH was determined using a CRISON pH meter (Barcelona, Spain) equipped with type 52-00 electrodes. A type 32-52 electrode was used to analyze penetration into fish fillets, with three repetitions performed (Volpe et al., 2015).

Total basic volatile nitrogen (TVB-N)

TVB-N was determined according to the modified Kjeldahl micro distillation method described by Cobb et al. (Cobb et al., 1973). TVB-N values are expressed in milligrams of nitrogen per 100 g of sample, determined by micro-Kjeldahl, using the Equation 3 (Volpe et al., 2015; Cobb et al., 1973):

3. *(TVB-N) + {(V1-V20) N×100×14×50}/W×5*

Where, v1 is the volume (mL) of sulfuric acid (H_2SO_4) used for the sample, V2 is the volume (mL) of sulfuric acid used for the blank, N is the normality of sulfuric acid, and W is the weight of the sample (g)

Thiobarbituric acid (TBA)

TBA was measured by colorimetry. A 200 mg fish fillet sample was transferred to a 25 mL Erlenmeyer flask and then made up to volume with 1 butanol. Then, 5 mL of the above mixture was poured into dry capped tubes, and 5 mL of TBA reagent was added. The capped tubes were placed in a water bath at 95 ˚C for 2 hours and then cooled to room temperature. The absorbance value of AS at a wavelength of 530 nm was then read against distilled water as the control. The amount of TBA (mg of malondialdehyde (MDA)/ kg of fish meat) was obtained according to the Equation 4 (Mahasti Shotorbani et al., 2019):

4. TBA=AS-AB×50/200

Microbial test

Determination of the minimum inhibitory concentration (MIC)

The MIC of the smart films was determined using the agar disk diffusion method on Mueller-Hinton agar medium. Subsequently, 10 µL (equal 0.5 McFarland standard) of the *Escherichia coli* (ATCC 25922) and *Salmonella typhi* (ATCC 13311) microbial suspensions were inoculated onto the agar surface, and the bacteria were incubated at 37 ˚C for 24 hours.

Total microbial count (TVC)

In fish treatments, 10 g of Sea Bass fish fillet was removed under sterile conditions and mixed and homogenized with 90 mL of sterile physiological serum of 0.85% and then dilutions were prepared. One milliliter of the dilutions was cultured on plates containing PCA agar culture medium and kept in an incubator at 37 ˚C for 24–48 hours. For psychrophilic bacteria, it was kept in an incubator for 10 days at 7 ˚C. After the incubation period, the colonies were counted and, according to the dilution factor, their number was reported as log CFU/g (Mahasti Shotorbani et al., 2019; Hernández et al., 2009).

Sensory evaluation method

Sensory evaluation has been an important standard method for judging consumer acceptability of sea bass or sea bass fillets. This was assessed using the 5-point hedonic method described by Huang et al. (2021), with minor modifications. This method involved 30 evaluators, comprising 15 men and 15 women. The team members were asked to rate the fish fillet samples on appearance, overall acceptance, smell, and taste on a scale of 1 to 10, with a total possible score of 10 (Chu et al., 2023; Huang et al., 2021).

Statistical analysis

At least three repetitions of each experiment were performed, and all data were analyzed using SPSS software, version 26 (SPSS, IL, USA). The results were calculated using two-way analysis of variance (ANOVA), and comparisons between mean values were performed using Duncan's multiple range test. Differences at $P<0.05$ were considered significant (Li et al., 2022).

Results

Smart packaging

Antioxidant activity

Based on Figure 1, the antioxidant activity of the smart packaging films varied significantly across treatments. Films incorporating bioactive compounds—particularly those containing hydroalcoholic extracts of *D. salina* algae (T3 & T4)—exhibited the highest antioxidant capacity (P<0.05), indicating their potential to neutralize free radicals. In contrast, T2 or films lacking active ingredients showed significantly lower antioxidant activity (P<0.05).

Moisture

The results of moisture content analysis (Table 2) in the smart film samples indicated that the incorporation of the alcoholic extract of *D. salina* significantly reduced the moisture content of the films (P<0.05). Moreover, increasing the extract concentration from 2% to 4% led to a more pronounced decrease in moisture content (P<0.05).

Turbidity

The the incorporation of the alcoholic extract significantly increased film turbidity (Table 2). This increase was dose-dependent, with higher extract concentrations leading to greater turbidity (P<0.05).

Thickness

The incorporation of 2% *D. salina* alcoholic extract did not significantly affect the thickness (P>0.05). In contrast, the 4% concentration showed a notable increase in film thickness (P<0.05).

Bass fillet test

pH

Based on Figure 2, on day 0, all treatments exhibited relatively low pH values, indicating freshness and minimal spoilage. As storage progressed, the control group consistently recorded higher pH levels compared to the treated samples, particularly on days 6 and 9 (P<0.05). Treatments with active films and alcoholic extracts—especially those with 4% *D. salina* extract—effectively delayed pH elevation, maintaining significantly lower values throughout the storage period (P<0.05).

TVB-N

Based on the data presented in Figure 3, the TNB-N values of fish fillet samples showed a progressive increase over the 9-day storage period (P<0.05). On day 0, treatments containing the active film combined with 2% and 4% alcoholic extracts exhibited significantly lower TNB-N levels compared to the control group (P<0.05). By day 3, the treatment incorporating CMC film, tragacanth gum, and 4% alcoholic extract demonstrated the most effective inhibition of nitrogenous base formation (P<0.05). On day 6, the control group continued to show the highest index, while treatments with 2% and

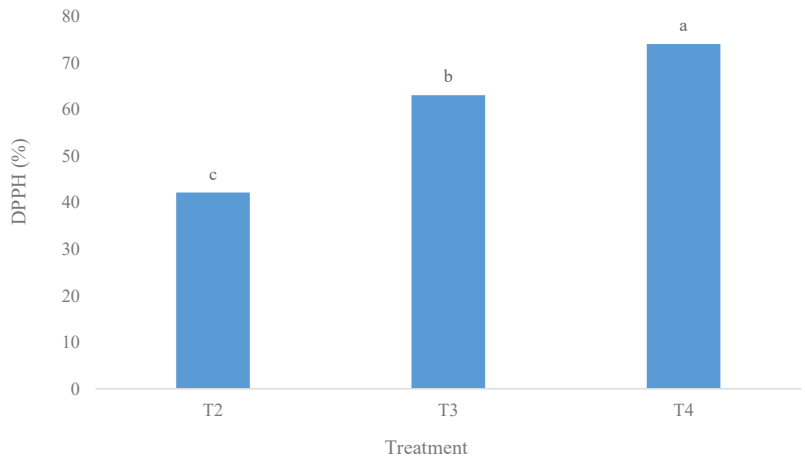

Figure 1. Antioxidant activity of smart packaging films

Note: Values are expressed as Mean±SD (n=3). Different lowercase letters indicate statistically significant differences (P<0.05).

Table 1. Research treatments

Treatment	Formulation
T1	Uncoated control
T2	Fish fillet coated with CMC film and tragacanth gum
T3	Fish fillet coated with CMC film, tragacanth gum, and 2% algae alcohol extract
T4	Fish fillet coated with CMC film, tragacanth gum, and 4% algae alcohol extract

4% algae extract maintained significantly lower TVB-N values (P<0.05). By day 9, the treatment containing the smart film and 4% alcoholic extract of *D. salina* algae exhibited the highest TNB-N level among the experimental groups, yet it remained significantly lower than the control (P<0.05).

TBA

T1 showed the highest TBA index, indicating the greatest lipid oxidation and resulting rancidity due to the lack of protective coatings. T2 was expected to have a lower TBA index compared to T1, suggesting some protection against oxidation provided by the edible film. T3 treatment was expected to demonstrate a further reduced TBA index, reflecting enhanced antioxidant protection from the 2% alcoholic extract of *D. salina* algae. T4 was anticipated to have the lowest TBA index, indicating the greatest reduction in lipid oxidation due to the higher concentration of algae extract providing increased antioxidant activity.

According to the Figure 4, among all treatments and days evaluated, the highest TBA index on day zero was observed in the treatment with CMC, tragacanth, and 4% alcoholic algae extract. The lowest and most favorable results were obtained from the treatment using the CMC film and tragacanth containing 4% alcoholic extract. On days 3, 6, and 9, the lowest results continued to be associated with the treatment using the CMC film and tragacanth with 4% alcoholic extract. Conversely, the highest index was consistently observed in the control treatment of the fish fillet without any film or extract.

Microbial test results

MIC

Based on Table 3, for *E. coli*, treatments T1 and T2 showed the highest MIC values (7.30 and 7.18 mg/mL, respectively), indicating lower inhibitory potency (P<0.05). In contrast, treatments T3 and T4 demonstrated significantly stronger antimicrobial activity, with MIC values of 5.29 and 4.47 mg/mL, respectively (P<0.05). A similar trend was observed for *Salmonella*, where T1 exhibited the highest MIC percentage (8.63%), followed by T2 (8.43%), T3 (8.13%), and T4 (7.44%). The progressive decrease in MIC values from T1 to T4 suggests that the formulation used in T4 was the most effective in inhibiting both pathogens (P<0.05).

TVC

By evaluating the results from the graph (Figure 5), we observed an expected increasing trend in microbial load over time. Among all the treatments, the sample containing the film and a 4% alcohol extract consistently showed the best results, maintaining the lowest microbial load. Specifically, it had 3.5 log CFU/g on day zero, an average of 4.9 log CFU/g on day 3, 7 log CFU/g on day 6, and an average of 6.8 log CFU/g after 9 days.

Table 2. Results of thickness, opacity, and moisture of smart packaging

Treatment	Humidity (%)	Turbidity (%)	Thickness (mm)
T2	33.642^{b}	2.402^{c}	0.632^{b}
T3	27.8^{c}	2.526^{b}	0.63^{b}
T4	25.303^{ed}	2.563^{a}	0.821^{a}

Note: Values are expressed as mean values (n=3). Different lowercase letters within each column indicate statistically significant differences (P<0.05).

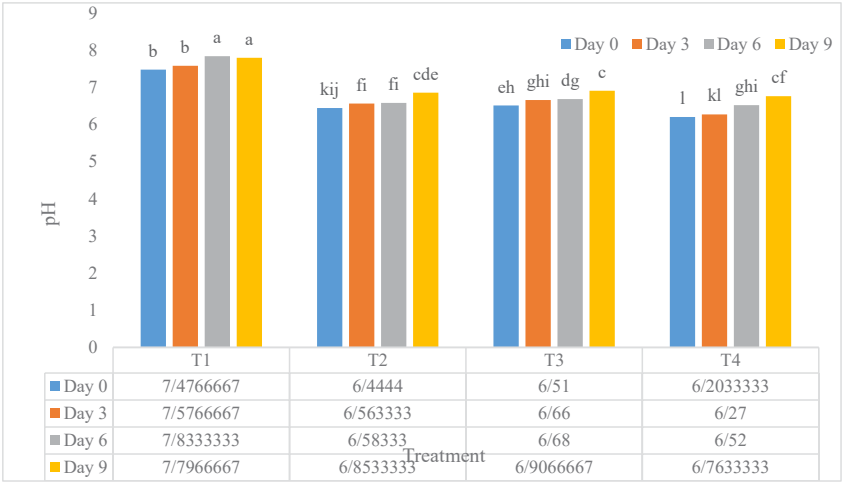

Figure 2. pH of fish fillets during 9-day storage

Note: Values are expressed as mean values (n=3). Different lowercase letters indicate statistically significant differences (P<0.05).

Psychrophilic bacteria

The changes in psychrophilic bacterial populations during the 9-day storage period are presented in Figure 6. The microbial load of psychrophilic bacteria increased over time, as anticipated. On day zero, the sample with CMC film and gum without extracts exhibited the highest microbial load, while the best result (an average of 3.1 log CFU/g) was observed in the treatment containing CMC film and gum with 2% algae alcohol extract. On day 3, this treatment again showed the best result, with an average of 4.1 log CFU/g. After 9 days of storage, treatments with 2% and 4% alcohol extracts of algae, combined with CMC film and gum, performed best, with average microbial loads of 5.7 and 5.8 log CFU/g, respectively.

Overall, these results suggest that the fillets were stored under good conditions, as indicated by the microbial loads, even when considering the control treatment. Most treatments, particularly those with alcoholic extracts of algae, demonstrated good antibacterial properties against psychrophilic microorganisms.

Sensory evaluation

The results of sensory evaluation, including color, appearance, taste, consistency, and smell scores, are summarized in Table 4. The CMC edible film and gum with 4% alcohol extract was consistently rated highest in terms of appearance on days 0, 3, and 9. The control treatment without film had the lowest appearance ratings on these days, while on day 6, the treatment with 2% alcohol extract and film had the lowest appearance results.

Regarding consistency, the control sample achieved the highest results on day 3. By day 6, the best consistency was observed in the treatment with film and 2% algae extract, and by day 9, the treatment with film containing 4% algae extract achieved the best consistency.

Table 3. The MIC results of smart films against selected pathogens

Treatment	*E. coli* (mg/mL)	*Salmonella* (%)
T1	7.30^{a}	8.63^{a}
T2	7.18^{a}	8.43^{b}
T3	5.29^{b}	8.13^{c}
T4	4.47^{c}	7.44^{d}

Note: Values are expressed as mean values (n=3). Different lowercase letters within each column indicate statistically significant differences (P<0.05).

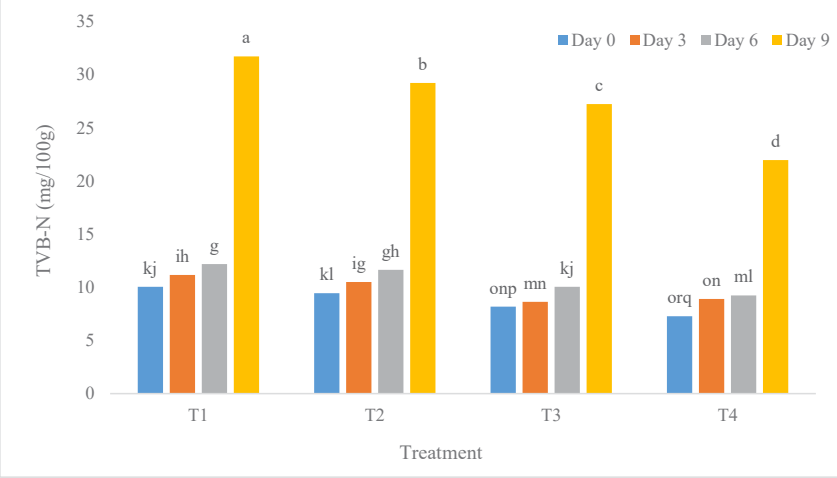

Figure 3. TNB-N values of fish fillets during 9-day storage

Note: Values are expressed as mean values (n=3). Different lowercase letters indicate statistically significant differences (P<0.05).

In terms of taste, the sample containing the edible film and 4% alcoholic extract of algae had the best results on all test days. For smell, the sample containing 2% alcoholic extract scored the highest on day 0. On day 3, the film containing CMC plus gum achieved the best rating, while on days 6 and 9, the treatment with 4% alcoholic extract received the highest smell scores.

Discussion

The use of edible films and coatings has been extensively researched in recent years, particularly in the context of aquatic products. Studies have focused on coatings made from various chemicals, polysaccharides, cellulose, and plant-based compounds. The primary goal of this research was to extend the shelf life of aquatic or protein products while enhancing their antioxidant and antimicrobial properties. Another key objective was to improve organoleptic properties. This particular study aimed to enhance and prolong the shelf life of sea bass fish fillets using edible films. The films incorporate CMC and tragacanth gum, along with hydroalcoholic extracts of *D. salina* algae. Through these compounds, the research sought to boost the antioxidant and antimicrobial efficacy of the coatings, thereby offering a potential solution for preserving the quality and safety of fish fillets.

The antioxidant activity of the smart packaging films demonstrated a significant enhancement with the incor-

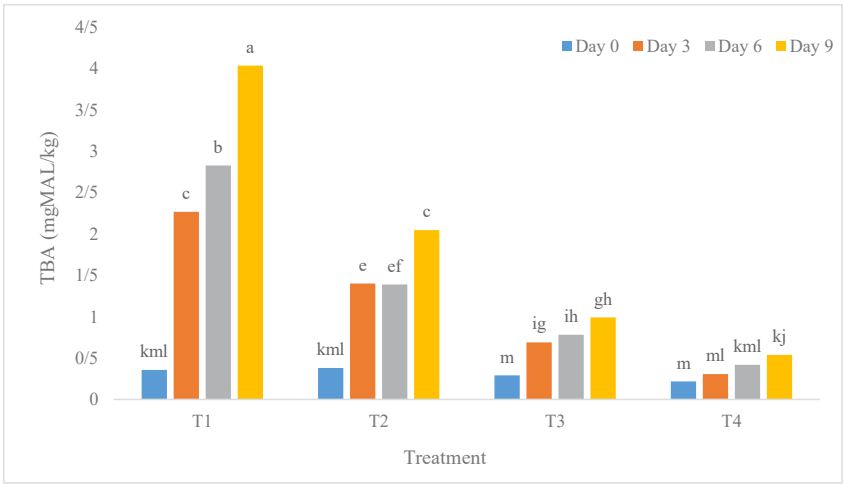

Figure 4. TBA values of fish fillets during 9-day storage

Note: Values are expressed as mean values (n=3). Different lowercase letters indicate statistically significant differences (P<0.05).

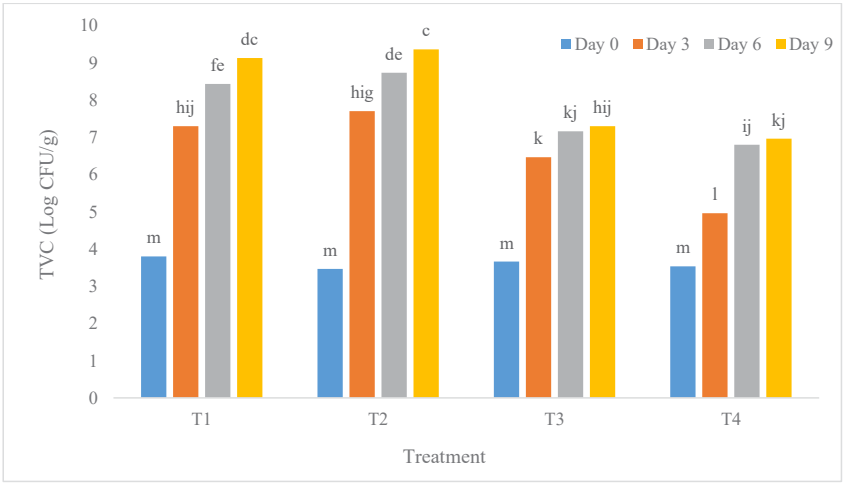

Figure 5. TVC of fish fillets during 9-day storage

Note: Values are expressed as mean (n=3). Different lowercase letters indicate statistically significant differences (P<0.05).

poration of hydroalcoholic extracts of *D. salina*. Among the tested formulations, the film containing 2.6 mL of extract exhibited the highest antioxidant capacity, with a statistically significant difference compared to other treatments.

The antioxidant activity of smart packaging films was significantly enhanced by the incorporation of hydroalcoholic extracts of *D. salina*. Among the tested treatments, the film containing 2.6 mL of extract showed the highest radical scavenging capacity, with a statistically significant difference compared to other formulations (P<0.05). This effect is attributed to the high content of carotenoids—particularly β-carotene—and phenolic compounds in *D. salina*, which are known to neutralize reactive oxygen species and delay lipid oxidation (singh et al., 2016). In study by Tan et al. (2024) the antioxidant properties of edible films made from CMC and starch were shown to increase from 84% to 91% using the DPPH method. Singh et al. (2016) demonstrated that carotene-enriched extracts of *D. salina* under stress conditions exhibited up to 57.5% free radical scavenging activity, confirming its potent antioxidant properties.

The moisture content of the smart packaging films decreased significantly with increasing concentrations of *D. salina* extract. Treatment T2 (without extract) exhibited the highest humidity level (33.642%), while T3 and T4—containing 2% and 4% extract, respectively—showed progressively lower values (27.8% and

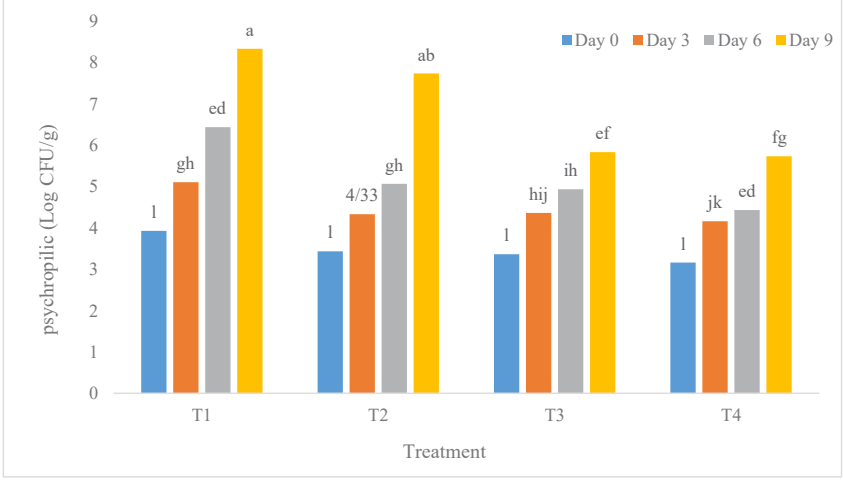

Figure 6. Psychrophilic bacteria of fish fillets during 9-day storage

Note: Values are expressed as mean values (n=3). Different lowercase letters indicate statistically significant differences (P<0.05).

Table 4. Results of sensory evaluation of fish fillets during 9-day storage

Parameters	Treatment	Day 0	Day 3	Day 6	Day 9
Color	T1	8.66^{a}	5.831^{ef}	3.401^{ih}	2.703^{ij}
	T2	5.261^{gf}	7.107^{cd}	4.162^{gh}	2.162^{j}
	T3	8.304^{ab}	5.503^{f}	3.663^{ih}	5.861^{ef}
	T4	6.132^{edf}	6.135^{edf}	6.901^{ced}	7.164^{cbd}
Appearance	T1	3.801^{hgi}	6.01^{de}	4.105^{hg}	1.732^{j}
	T2	6.764^{dbc}	5.502^{fe}	4.132^{hg}	2.864^{i}
	T3	5.965^{de}	4.404^{fg}	3.701^{hgi}	3.502^{hgi}
	T4	7.36^{abc}	7.361^{abc}	8.1^{a}	7.701^{ab}
Taste	T1	5.532^{fg}	4.2^{ki}	2.762^{klj}	1^{m}
	T2	6.733^{cde}	4.901^{hg}	4.301^{hi}	2.062^{mc}
	T3	5.831^{fge}	3.862^{hig}	2.935^{klj}	3.403^{kij}
	T4	7.13^{bcd}	7.131^{bcd}	8.001^{ab}	7.73^{abc}
Consistency	T1	7.9^{a}	7.431^{ab}	3.561^{ij}	1.301^{k}
	T2	6.301^{def}	6.232^{ef}	4.030^{ih}	2.703^{j}
	T3	6.632^{bf}	4.111^{ih}	6.531^{ca}	3.766^{ij}
	T4	5.001^{gh}	5.007^{gh}	6.9^{ff}	7.365^{ae}
Smell	T1	2.6^{k}	5.5^{hg}	2.836^{jk}	1.162^{l}
	T2	6.4^{dg}	6.231^{dg}	4.564^{hi}	1.93^{lk}
	T3	8.333^{ab}	6.131^{feg}	3.662^{ji}	4.301^{i}
	T4	5.831^{fg}	5.831^{fg}	8.5^{ab}	8.032^{be}

Note: Values are expressed as mean values (n=3). Different lowercase letters indicate statistically significant differences (P<0.05).

25.303%). This reduction in moisture can be attributed to the hydrophobic nature of bioactive compounds, such as carotenoids and lipids present in *D. salina*, which interfere with water retention in the polymer matrix. According to Singh et al. (2016), *D. salina* contains significant amounts of β-carotene and lipophilic antioxidants that can reduce the water-binding capacity of biopolymer films. Additionally, the incorporation of microalgal extracts may lead to increased cross-linking or matrix densification, further limiting moisture absorption (Kevin et al., 2023).

The turbidity of the smart packaging films increased significantly with higher concentrations of *D. salina* extract, rising from 2.402% in the T2 film to 2.563% in T4. This increase is attributed to the presence of suspended bioactive compounds, such as carotenoids, proteins, and polysaccharides, which scatter light and reduce film transparency. Soiklom et al. (2025) demonstrated that adding *Ascophyllum nodosum* extract to alginate-based films led to a 13% decrease in transparency compared to control samples. This reduction was attributed to the presence of phenolic compounds, pigments, and suspended solids in the algal extract, which scattered incident light and disrupted the uniformity of the polymer matrix.

In terms of thickness, the film containing 4% extract (T4) showed a marked increase to 0.821 mm compared to 0.630 mm in the T3 (P<0.05). This change is likely

due to the structural contribution of algal biomass and its interaction with the polymer matrix, which enhances film density and swelling. Soiklom et al., (2025) showed that the incorporation of *Spirogyra* sp. extract into chitosan-based films significantly increased film thickness, which they attributed to the accumulation of algal solids and matrix swelling. Do Nascimento et al. (2021) demonstrated that the addition of *Brassica oleracea capitata* extract had no significant effect on the thickness of indicator films composed of green banana starch, gelatin, alginate, and the extract. This finding contrasts with the results of our study.

Changes in pH are one of the key quality indicators of fish spoilage. Generally, the pH of live fish muscle ranges between 6.6 and 7.0, but after death, depending on factors, such as season, species, and other variables, it fluctuates between 6.0 and 7.0 (Stamatis & Arkoudelos, 2007).

As shown in Figure 2, the pH of fish fillets increased progressively during the 9-day storage period, rising from an initial value of 6.32±0.04 on day 0 to 7.18±0.06 by day 9. This upward trend was statistically significant. This increase may be attributed to the production of compounds, such as trimethylamine and dimethylamino ammonia by spoilage bacteria, as well as protein degradation and the release of volatile nitrogenous bases (Goulas & Kontominas, 2005). The most pronounced pH rise was recorded in the control group (T1). These results clearly demonstrate the effect of the hydroalcoholic algal extract in slowing down the pH increase in white bass fillets. Studies have shown that the use of edible coatings containing plant extracts can inhibit pH elevation in fish and beef during storage by suppressing enzymatic activity, microbial growth, and protein degradation, which otherwise lead to the accumulation of alkaline compounds. Moreover, the presence of plant extracts in edible films and coatings can alter their permeability to carbon dioxide gas (Majid et al., 2010). The inhibitory effect of algal extract on pH elevation in fish fillets may be attributed to its bioactive compounds, particularly polyphenols and antioxidant pigments, which suppress microbial growth and enzymatic activity responsible for protein degradation and the release of alkaline nitrogenous compounds (Liu et al., 2025). Afrin et al. (2023) demonstrated that the application of seaweed extracts significantly slowed the pH increase in tilapia fillets during refrigerated storage.

TVB-N is widely used as an indicator of seafood quality, as it is directly associated with microbial growth and the formation of key metabolic compounds, such as ammonia, trimethylamine, diethylamine, and methylamine (Maghami et al., 2019). The TVB-N values of fish fillets increased progressively over the 9-day storage period, indicating ongoing microbial and enzymatic spoilage. The increase in TVB-N levels in the samples may be attributed to endogenous enzymatic activity and microbial metabolism, leading to the production of ammonia and biogenic amines, such as trimethylamine and methylamine (Kakaei & Shahbazi, 2016). The control sample (T1) exhibited the highest TVB-N level on day 9, reaching 28.42±0.73 mg N/100 g, which significantly exceeded the acceptable freshness threshold. In contrast, the sample treated with 4% algal extract (T4) showed the lowest TVB-N, with a final value of 17.86±0.58 mg N/100 g. Since TVB-N is primarily generated through bacterial degradation of fish muscle, higher total viable counts in the control sample indicate a greater degree of spoilage. The lower TVB-N levels observed in samples packaged with hydroalcoholic algal extract may be attributed to a faster reduction in bacterial populations, a diminished capacity of bacteria to perform oxidative deamination of non-protein nitrogenous compounds, or a combination of both mechanisms (Fan et al., 2008). Afrin et al. (2023) reported that fillets treated with a 2% alcoholic extract of *Padina tetrastromatica* exhibited a final TVB-N value of 1.63 mg N/100 g after 4 weeks of refrigerated storage. This low level indicates effective inhibition of spoilage-related nitrogenous compound formation, highlighting the preservative potential of the algal extract compared to other treatments. Ahmadi and Shurmasti (2020) demonstrated that fish fillets treated with a CMC-based multiplot film containing mint extract exhibited significantly lower TVB-N accumulation during 9 days of refrigerated storage. The TVB-N value in treated samples increased moderately from 12.55 to 17.94 mg N/100 g, whereas the control group reached 51.11 mg N/100 g, indicating pronounced spoilage. These results confirm the preservative effect of the mint-enriched film in retarding microbial degradation and nitrogenous compound formation. Cai et al. (2022) evaluated the effect of CMC edible film containing Nostoc extract and sodium on salmon fillets. They analyzed antioxidant properties using the DPPH method at concentrations of 100 and 200 mg/mL. The results indicated high inhibition of free radicals, with 70% inhibition at 200 mg/mL and 62% at 100 mg/mL. The CMC film with *Nostoc* was effective in reducing pH and the TVB-N index.

The TBA index is widely used to assess the degree of lipid oxidation in fish, based on the quantification of MDA content as a primary oxidation product. The TBA values of fish fillets increased significantly over

the 9-day storage period. The increasing trend of this index during storage may be attributed to the rise in free iron and other pro-oxidants within the fish muscle. The primary products of lipid oxidation are hydroperoxides, which are unstable compounds and do not directly contribute to off-flavors in fish. However, during the secondary stage of autoxidation, hydroperoxides are further oxidized into aldehydes and ketones—among them MDA—which are responsible for undesirable taste and odor in the final product (Fan et al., 2008). The control sample (T1) exhibited the highest TBA level on day 9, reaching 2.47±0.09 mg MDA/kg, which reflects advanced oxidative spoilage. In contrast, the fillets treated with 4% algal extract (T4) showed the lowest TBA value of 1.03±0.05 mg MDA/kg. This can be attributed to the high antioxidant activity of bioactive compounds present in the algae, such as polyphenols and carotenoids. These compounds act as free radical scavengers, inhibiting lipid peroxidation. Additionally, the incorporation of algal extracts into edible films may improve oxygen barrier properties, further reducing oxidative stress on the fish muscle during storage (Soiklom et al., 2025). Similar results were observed by Afrin et al. (2023) and Ahmadi et al. (2020), who reported that the incorporation of plant and seaweed extracts into edible films significantly reduced lipid oxidation in fish fillets during storage.

The MIC values of smart films against *E. coli* and *Salmonella* varied significantly among treatments, indicating differences in antimicrobial efficacy. Treatment T4, containing the highest concentration of algal extract, exhibited the strongest inhibitory effect, with the lowest MIC values of 4.47 mg/mL for *E. coli* and 7.44% for *Salmonella*.

Both TVC and psychrophilic bacteria increased significantly over the 9-day storage period. The increased growth of mesophilic and psychrophilic bacteria in controls indicates the presence of sufficient oxygen to support the growth of these microorganisms. In contrast, the reduced bacterial growth rate observed in smart packaging films is attributed to their oxygen barrier properties (Indumathi et al., 2019), as well as the antimicrobial activity of algal extract compounds incorporated into the film matrix.

Marine algae are rich in bioactive constituents, such as phlorotannins, PUFAs, terpenoids, and sulfated polysaccharides, which exhibit broad-spectrum antimicrobial effects by disrupting bacterial membranes, inhibiting enzymatic systems, and interfering with nutrient uptake (Reinhardt, 2024). A study was conducted by Nowruzi et al. (2023) that investigated the effect of phycoerythrin as an antimicrobial and antioxidant compound that increased the shelf life of Nile tilapia (*Oreochromis niloticus*). The results of this study showed the effect of phycoerythrin on *Salmonella* bacteria during cold storage. The results of this study were consistent with the antibacterial test analysis of the extract in our research.

Overall, our study's findings were consistent with those of other researchers, and they aligned closely with international standards for the tested parameters. Through comprehensive sensory, chemical, biochemical, physical, and microbiological analyses, it was determined that the CMC and gum tragacanth film, along with 2% and 4% hydroalcoholic extracts of *D. salina*, significantly enhanced the shelf life of sea bass fillets. Sea bass fish is highly perishable. Thus, it is essential to develop edible films that inhibit microbial growth at cold temperatures and delay spoilage. Among the treatments, the best results were observed with a film containing 4% alcoholic extract. Although the use of edible films and coatings with various plant extracts is expanding as biodegradable active packaging, further research is needed for the production and commercialization of this modern method.

Ethical Considerations

Compliance with ethical guidelines

This study was approved by the research ethics committee of North Tehran Branch, Islamic Azad University, Tehran, Iran (Code: IR.IAU.TNB.REC.1404.428).

Funding

This research did not receive any grant from funding agencies in the public, commercial, or non-profit sectors.

Authors' contributions

Conceptualization, Supervision, Project Administration, and Resources: Zhaleh Khoshkhoo; Methodology: Amirhossien Ebrahimi, Afshin Akhondzadeh Basti and Marjaneh Sedaghati; Investigation, formal analysis, data curation, visualization, and writing the original draft: Amirhossien Ebrahimi; Review and editing: Zhaleh Khoshkhoo, Afshin Akhondzadeh Basti, and Marjaneh Sedaghati.

Conflict of interest

The authors declared no conflict of interest.

Acknowledgments

The authors would like to thank the Laboratory of North Tehran Branch, Islamic Azad University, Tehran, Iran, for providing the laboratory facilities used in this study.

References

Shahrier, J., Rasul, G., Afrin, F., Islam, R., & Shah, A. K. M. A. (2023). Extension of shelf life of Nile tilapia (Oreochromis niloticus) fillets using seaweed extracts during refrigerated storage. *Food Science & Nutrition, 11*(11), 7430–7440. [DOI:10.1002/fsn3.3673] [PMID]

Ameur, A., Bensid, A., Ozogul, F., Ucar, Y., Durmus, M., & Kulawik, P., et al. (2022). Application of oil-in-water nanoemulsions based on grape and cinnamon essential oils for shelf-life extension of chilled flathead mullet fillets. *Journal of The Science of Food and Agriculture, 102*(1), 105–112. [DOI:10.1002/jsfa.11336] [PMID]

Ahmadi, Z., & Khademi Shurmasti, D. (2020). [Effects of Mentha spicata L. extract in carboxymethyl cellulose-oleic acid composite coating on the shelf life of fish fillets during cold storage (Persian)]. *Iranian Journal of Medicinal and Aromatic Plants Research, 36*(5), 724-733. [DOI:10.22092/ijmapr.2020.343042.2797]

Bansal, M. P., & Jaswal, S. (2009). Hypercholesterolemia induced oxidative stress is reduced in rats with diets enriched with supplement from Dunaliella salina algae. *American Journal of Biomedical Science and Research, 1*(3), 196-204. [Link]

Bhan, C., Asrey, R., Meena, N. K., Rudra, S. G., Chawla, G., & Kumar, R., et al. (2022). Guar gum and chitosan-based composite edible coating extends the shelf life and preserves the bioactive compounds in stored Kinnow fruits. *International Journal of Biological Macromolecules, 222*(Pt B), 2922–2935. [DOI:10.1016/j.ijbiomac.2022.10.068] [PMID]

Cai, M., Zhong, H., Li, C., Aliakbarlu, J., Zhang, H., & Cui, H., et al. (2022). Application of composite coating of Nostoc commune Vauch polysaccharides and sodium carboxymethyl cellulose for preservation of salmon fillets. *International Journal of Biological Macromolecules, 210,* 394–402. [DOI:10.1016/j.ijbiomac.2022.05.051] [PMID]

Cobb III, B. F., Alaniz, I., & Thompson JR, C. A. (1973). Biochemical and microbial studies on shrimp: Volatile nitrogen and amino nitrogen analysis. *Journal of Food Science, 38*(3), 431-436. [DOI:10.1111/j.1365-2621.1973.tb01447.x]

Chu, Y., Ding, Z., & Xie, J. (2024). The application of ice glazing containing D-sodium erythorbate combined with vacuum packaging to maintain the physicochemical quality and sweet/umami non-volatile flavor compounds of frozen stored large yellow croaker (Pseudosciaena crocea). *Food Research International (Ottawa, Ont.), 175,* 113657. [DOI:10.1016/j.foodres.2023.113657] [PMID]

do Nascimento Alves, R., Santos Lima, T. L., da Silva Chaves, K., & de Albuquerque Meireles, B. R. L. (2021). Biodegradable films with Brassica oleracea capitata extract as a quality indicator in sheep meat. *Journal of Food Processing and Preservation, 45*(1). [DOI:10.1111/jfpp.14997]

dos Santos, E. A., Ribeiro, A. E. C., Barcelos, T. T., da Rocha Neves, G. A., Monteiro, M. L. G., & Marsico, E. T., et al. (2022). Shelf life of sodium-reduced ready-to-eat fish product made with by-products from fish and fruit processing subjected to high-intensity ultrasound. *InnovativeFoodScience&EmergingTechnologies, 78*, 103021. [Link]

Eshaghi, R., Mohsenzadeh, M., & Ayala-Zavala, J. F. (2024). Bio-nanocomposite active packaging films based on carboxymethyl cellulose, myrrh gum, TiO2 nanoparticles and dill essential oil for preserving fresh-fish (Cyprinus carpio) meat quality. *International Journal of Biological Macromolecules, 263*(Pt 2), 129991. [DOI:10.1016/j.ijbiomac.2024.129991] [PMID]

Fan, X. J., Liu, S. Z., Li, H. H., He, J., Feng, J. T., & Zhang, X., et al. (2019). Effects of Portulaca oleracea L. extract on lipid oxidation and color of pork meat during refrigerated storage. *Meat Science, 147,* 82–90. [DOI:10.1016/j.meatsci.2018.08.022] [PMID]

Genç, İ. Y., Esteves, E., Anibal, J., & Diler, A. (2013). Effects of chilled storage on quality of vacuum packed meagre fillets. *Journal of Food Engineering, 115*(4), 486-494. [DOI:10.1016/j.jfoodeng.2012.09.007]

Goulas, A. E., & Kontominas, M. G. (2005). Effect of salting and smoking-method on the keeping quality of chub mackerel (Scomber japonicus): Biochemical and sensory attributes. *Food Chemistry, 93*(3), 511-520. [DOI:10.1016/j.foodchem.2004.09.040]

Hejrani, T., Sheikholeslami, Z., Mortazavi, A., & Davoodi, M. G. (2017). The properties of part baked frozen bread with guar and xanthan gums. *Food Hydrocolloids, 71,* 252-257. [DOI:10.1016/j.foodhyd.2016.04.012]

Hernández, M. D., López, M. B., Álvarez, A., Ferrandini, E., García, B. G., & Garrido, M. D. (2009). Sensory, physical, chemical and microbiological changes in aquacultured meagre (Argyrosomus regius) fillets during ice storage. *Food Chemistry, 114*(1), 237-245. [DOI:10.1016/j.foodchem.2008.09.045]

Hosseini, S. F., & Gómez-Guillén, M. C. (2018). A state-of-the-art review on the elaboration of fish gelatin as bioactive packaging: Special emphasis on nanotechnology-based approaches. *Trends in Food Science & Technology, 79,* 125-135. [DOI:10.1016/j.tifs.2018.07.022]

Huang, J., Zhou, Y., Chen, M., Huang, J., Li, Y., & Hu, Y. (2021). Evaluation of negative behaviors for single specific spoilage microorganism on little yellow croaker under modified atmosphere packaging: Biochemical properties characterization and spoilage-related volatiles identification. *Lwt, 140,* 110741. [DOI:10.1016/j.lwt.2020.110741]

Hyrslova, I., Krausova, G., Mrvikova, I., Stankova, B., Branyik, T., & Malinska, H., et al. (2022). Functional properties of Dunaliella salina and its positive effect on probiotics. *Marine Drugs, 20*(12), 781. [DOI:10.3390/md20120781] [PMID]

Indumathi, M. P., Saral Sarojini, K., & Rajarajeswari, G. R. (2019). Antimicrobial and biodegradable chitosan/cellulose acetate phthalate/ZnO nano composite films with optimal oxygen permeability and hydrophobicity for extending the shelf life of black grape fruits. *International Journal of Biological Macromolecules, 132,* 1112–1120. [DOI:10.1016/j.ijbiomac.2019.03.171] [PMID]

Kakaei, S., & Shahbazi, Y. (2016). Effect of chitosan-gelatin film incorporated with ethanolic red grape seed extract and Ziziphora clinopodioides essential oil on survival of Listeria monocytogenes and chemical, microbial and sensory properties of minced trout fillet. *LWT-Food Science and Technology, 72,* 432-438. [DOI:10.1016/j.lwt.2016.05.021]

Keller, J. (2020). Sodium carboxymethylcellulose (CMC). In *Food hydrocolloids* (pp. 43-109). CRC Press. [Link]

Kevin,A., Raya, I., Natsir, H., Anshar,A. M., Musa, B., & Mayasari, E. (2023). Effects of Microencapsulated Dunaliella Salina Algae on Sensory Evaluations, Omega-3 Fatty Acid and Nutritional Compositions Value of Sago Bagea Cookies. *Iranian Journal of Chemistry and Chemical Engineering, 42*(10), 3409-3421. [DOI:10.30492/ijcce.2023.562402.5601]

Lahreche, T., Durmuş, M., Kosker, A. R., Uçar, Y., Boga, E. K., & Hamdi, T. M., et al. (2022). Effects of different plant (marjoram and olive leaf) extracts on quality characteristics of red and ordinary muscles of vacuum–packaged tuna–like fillets. *Applied Food Research, 2*(1), 100034. [DOI:10.1016/j.afres.2021.100034]

Lanzarin, M., Ritter, D. O., Novaes, S. F., Monteiro, M. L. G., Almeida Filho, E. S., & Mársico, E. T., et al. (2016). Quality index method (QIM) for ice stored gutted Amazonian Pintado (Pseudoplatystoma fasciatum× Leiarius marmoratus) and estimation of shelf life. *LWT, 65,* 363-370. [DOI:10.1016/j.lwt.2015.08.019]

Li, Y., Tang, X., & Zhu, L. (2022). Bilayer pH-sensitive colorimetric indicator films based on zein/gellan gum containing black rice (Oryza sativa L.) extracts for monitoring of largemouth bass (Micropterus salmoides) fillets freshness. *International Journal of Biological Macromolecules, 223,* 1268-1277. [DOI:10.1016/j.ijbiomac.2022.10.273] [PMID]

Liu, X., Cheng, D., Zhu, F., Tang, H., Zhang, L., Liu, Y., & Yang, N. (2025). Incorporation of algae extract in bilayer coating and its preservation effect on cold storage golden pompano. *LWT,* 118140. [DOI:10.1016/j.lwt.2025.118140]

Maciel, V. B. V., Contini, L. R. F., Yoshida, C. M. P., & Venturini, A. C. (2020). Application of edible biopolymer coatings on meats, poultry, and seafood. In *Biopolymer Membranes and Films* (pp. 515-533). Elsevier. [DOI:10.1016/B978-0-12-818134-8.00021-3]

Hazavehei ha,Y., Mahasti, S. P., & Khoshkhoo, Z. (2019). [Effect of edible gelatin coating based on Dunaliella salina alge essential oil on physicochemical and microbial characteristics of rainbow trout fish burger during refrigerated storage (Persian)]. *Journal of Food Science and Technology(Iran), 16*(89), 125-137. [Link]

Majid, A., Mohaddesse, M., Mahdi, D., Mahdi, S., Sanaz, S., & Kassaiyan, N. (2010). Overview on Echinophora platyloba, a synergistic anti-fungal agent candidate. *Journal of Yeast and Fungal Research, 1*(5), 88-94. [Link]

Millamena, O. M. (2002). Replacement of fish meal by animal by-product meals in a practical diet for grow-out culture of grouper Epinephelus coioides. *Aquaculture, 204*(1-2), 75-84. [DOI:10.1016/S0044-8486(01)00629-9]

Maghami, M., Motalebi, A. A., & Anvar, S. A. A. (2019). Influence of chitosan nanoparticles and fennel essential oils (Foeniculum vulgare) on the shelf life of Huso huso fish fillets during the storage. *Food Science & Nutrition, 7*(9), 3030-3041. [DOI:10.1002/fsn3.1161]

Nazemi, Z., Sahraro, M., Janmohammadi, M., Nourbakhsh, M. S., & Savoji, H. (2023). A review on tragacanth gum: A promising natural polysaccharide in drug delivery and cell therapy. *International Journal of Biological Macromolecules, 241,* 124343. [DOI:10.1016/j.ijbiomac.2023.124343]

Nowruzi, B., & SA, A. A. (2023). Effect of phycoerythrin on antimicrobial activity and shelf-life extension of the Nile Tilapia (Oreochromis niloticus) at refrigerator temperature. *Archives of Razi Institute, 78*(6), 1811. [PMID]

Ojagh, S. M., Adeli, A., Abdollahi, M., Kazemi, M., & Habibi, M. (2017). Effect of ZnO nanoparticles on the physico-mechanical properties of agar/kappa carrageenan bilayer film. *Innovative Food Technologies, 5*(1), 13-23. [DOI:10.22104/jift.2017.500]

Peng, Y., & Li, Y. (2014). Combined effects of two kinds of essential oils on physical, mechanical and structural properties of chitosan films. *Food Hydrocolloids, 36,* 287-293. [DOI:10.1016/j.foodhyd.2013.10.013]

Rajaei, E., & Shekarchizadeh, H. (2019). [Investigation of physical and mechanical properties of edible film prepared from opopanax gum (Commiphora guidottii) (Persian)]. *Journal of Food Science and Technology(Iran), 16*(91), 323-335. [Link]

Reinhardt, I. (2024). Novel bioactive compounds from marine algae: Promising candidates for natural product-based drug development. *Journal of Pharmacognosy & Natural Products, 10,* 325. [Link]

Saeid Asr, E., Naghibi, S., Mojaddar Langroodi, A., Moghaddas Kia, E., Meshkini, S., & Ehsani, A. (2021). Impact of carboxymethyl cellulose coating incorporated with rosemary essential oil and sodium acetate on the quality and shelf life of rainbow trout fillet. *Journal of Aquatic Food Product Technology, 30*(1), 16-30. [DOI:10.1080/10498850.2020.1850586]

Salimpour, M., Khoshkhoo, Z., & Emtiazjoo, M. (2019). The investigation of production of ice cream containing Donalila salina alga powder. *Journal of Food Science and Technology (Iran), 16*(90), 271-282. [Link]

Sathasivam, R., Radhakrishnan, R., Hashem, A., & Abd_Allah, E. F. (2019). Microalgae metabolites: A rich source for food and medicine. *Saudi Journal of Biological Sciences, 26*(4), 709-722. [DOI:10.1016/j.sjbs.2017.11.003] [PMID]

Singh, P., Baranwal, M., & Reddy, S. M. (2016). Antioxidant and cytotoxic activity of carotenes produced by Dunaliella salina under stress. *Pharmaceutical Biology, 54*(10), 2269–2275. [DOI:10.3109/13880209.2016.1153660]

Sobhan, A., Muthukumarappan, K., & Wei, L. (2022). A biopolymer-based pH indicator film for visually monitoring beef and fish spoilage. *Food Bioscience, 46,* 101523htt [DOI:10.1016/j.fbio.2021.101523]

Soiklom, S., Siri-Anusornsak, W., Petchpoung, K., Soiklom, S., & Maneeboon, T. (2025). Development of bioactive edible film and coating obtained from Spirogyra sp. extract applied for enhancing shelf life of fresh products. *Foods, 14*(5), 804. [DOI:10.3390/foods14050804]

Stamatis, N., & Arkoudelos, J. (2007). Quality assessment of Scomber colias japonicus under modified atmosphere and vacuum packaging. *Food Control, 18*(4), 292–300. [DOI:10.1016/j.foodcont.2005.10.009]

Tan, X., Sun, A., Cui, F., Li, Q., Wang, D., Li, X., & Li, J. (2024). The physicochemical properties of Cassava Starch/Carboxymethyl cellulose sodium edible film incorporated of Bacillus and its application in salmon fillet packaging. *Food Chemistry: X, 23,* 101537. [DOI:10.1016/j.fochx.2024.101537] [PMID]

Tocher, D. R., Betancor, M. B., Sprague, M., Olsen, R. E., & Napier, J. A. (2019). Omega-3 long-chain polyunsaturated fatty acids, EPA and DHA: Bridging the gap between supply and demand. *Nutrients, 11*(1), 89. [DOI:10.3390/nu11010089] [PMID]

Volpe, M. G., Siano, F., Paolucci, M., Sacco, A., Sorrentino, A., & Malinconico, M., et al. (2015). Active edible coating effectiveness in shelf-life enhancement of trout (Oncorhynchus-mykiss) fillets. *LWT-Food Science and Technology, 60*(1), 615-622. [DOI:10.1016/j.lwt.2014.08.048]

Yuan, D., Hao, X., Liu, G., Yue, Y., & Duan, J. (2022). A novel composite edible film fabricated by incorporating W/O/W emulsion into a chitosan film to improve the protection of fresh fish meat. *Food Chemistry, 385,* 132647. [DOI:10.1016/j.foodchem.2022.132647] [PMID]

Zhao, R., Guan, W., Zhou, X., Lao, M., & Cai, L. (2022). The physiochemical and preservation properties of anthocyanidin/chitosan nanocomposite-based edible films containing cinnamon-perillaessential oil pickering nanoemulsions. *Lwt, 153,* 112506. [DOI:10.1016/j.lwt.2021.112506]

This Page Intentionally Left Blank