



Effect of Carboxymethyl Cellulose Edible Coating Enriched with Summer Savory Extract on Quality Parameters of Spangled Emperor (*Lethrinus nebulosus*) Fillets During Refrigerated Storage

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Abstract

In this study, the effect of carboxymethyl cellulose (CMC) edible coating containing summer savory (*Satureja hortensis*) extract (SHE) on the quality changes of spangled emperor (*Lethrinus nebulosus*) fillets over a period of 9 days of refrigerated storage (4 ± 2 °C) was investigated. The compounds of *S. hortensis* extract were evaluated by GC/MS, and carvacrol (28.67%) was the main combination of the extract. According to the results of this study, microbial growth, lipid oxidation and protein degradation in the coated fillets were delayed compared to the control sample (uncoated). Also, texture hardness and sensory properties of the treated samples improved as compared to the control during refrigerated storage. Among treatments, CMC+ 1.5% SHE was the most effective treatment to maintain the quality of *L. nebulosus* fillets. Thus, carboxymethyl cellulose coating enriched with *S. hortensis* extract could be a promising method to maintain the quality of *L. nebulosus* fillets during refrigerated storage.

Keywords *Lethrinus nebulosus* · Edible coating · Carboxymethyl cellulose · *Satureja hortensis* · Quality parameters

Introduction

Spangled emperor (*Lethrinus nebulosus*) is one of the commercial species of fish in southern Iran, including Hormozgan province, considering the high rate of fishing of this fish among other Persian Gulf fishes and its high market penetration among the residents of the south of Iran; so, it is important to maintain the quality and identify the nutritional value and the principles of its processing. Fish flesh is highly susceptible to lipid oxidation and microbial spoilage due to its high levels of unsaturated fatty acids as well as the high levels of simple compounds that can be used for corrosive bacteria [51]. Refrigerated storage is a common method used in the centers of supply and sale or transfer of fish.

Storing fish in the refrigerator reduces the rate of enzymatic and microbial activities, but does not stop them completely, and unpleasant changes such as lipid oxidation and microbial spoilage occur slowly and cause the fish to spoil after a few days [44]. There are several methods to protect and enhance the shelf-life of foods, which among them films and coatings biodegradable are very promising methods [45]. Several studies have shown that edible coatings made from protein, polysaccharide and oil-containing materials help to increase shelf life and maintain the quality of fish [11, 27, 50, 55]. One of the most important cellulose-derived polysaccharides is carboxymethyl cellulose (CMC) that is used in the production of films and coatings. Some desirable properties of CMC are it being water soluble, odorless, tasteless, of high density, non-toxic, non-allergenic, flexible, with moderate strength, transparent, resistant to oil and fats and moderately resistant to moisture and oxygen transmission [31, 57]. The use of active packaging is a novel method for keeping food, especially fresh food, such as fresh meat; recent years, extensive researches has been done to generate and commercialize it [62]. Antioxidant and antibacterial compounds are used in both synthetic and natural forms such as organic acids, enzymes, bacteriosins and essential

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oils to eliminate or reduce food spoilage [5]. Natural products such as herbal extracts, as pure compounds, or standard extracts due to their chemical diversity provide a wide range for controlling microbial growth. Plant extracts also showed antifungal activity against a wide range of fungi, antioxidant and antimutagenic activities and inhibited food lipid oxidation [39]. Summer savory (*Satureja hortensis* L.) is a herbaceous plant of the family Lamiaceae, used as a spice and for flavoring and also used in traditional medicine for the treatment of infectious diseases. *S. hortensis* possesses a broad spectrum of biological activities, including antispasmodic, antidiarrheal, antinociceptive, anti-inflammatory, antioxidant and antimicrobial properties [1]. Therefore, the purpose of this study was to investigate the possibility of using active carboxymethyl cellulose coating in combination with summer savory extract in order to increase shelf life of refrigerated spangled emperor fillets through reducing oxidative and microbial activities and improvement of its qualitative-sensory characteristics.

Materials and Methods

Preparation of Extract and Coating solution

Summer savory (*Satureja hortensis*) plant was harvested from the Sar-e-Pul Zahab area in Kermanshah province (Kermanshah, Iran) in October 2015. The plant was washed with tap water and then was dried in an oven at 55 °C for 24 h. Then the leaves were separated from the stems and powdered by the mill. Extraction with some modifications was carried out according to the method described by Yuan et al. [61]. In order to remove chlorophyll, 200 g of powder was combined with chloroform (1:10, w/v) for 30 min. Then the mixture was blended with ethanol 80% (1:10, w/v) for 2 h at 40 °C using a magnetic stirrer. After filtering (Waterman No. 1) the extract, the solvent was evaporated under vacuum by a rotary evaporator. In order to completely evaporate the solvent, the concentrated extract was dried in an oven at 60 °C for 24 h. The sticky extract was stored in dark polyethylene cans in the refrigerator until use.

Carboxymethyl cellulose (CMC, Samchun, Korea) powder was blended with distilled water (1%, w/v) and heated at 85 °C by a magnetic stirrer to completely dissolve and obtain a clear solution. Then glycerol (50% v/w of CMC amount) as a plasticizer was added to the solution and stirred for 10 min. After the solution was cooled, summer savory extract at concentrations of 0.5, 1 and 1.5% (v/v) were added to carboxymethyl cellulose solution and stirred for 4 min [15].

GC–MS Analysis of Extract

The compositions of *S. hortensis* extract were analyzed using a GC–MS Agilent 7890-5975 system equipped with a HP-5 column (30 m × 0.25 mm × 0.25 μm). The column temperature was set at 40 °C for 1 min and then raised to 300 °C at rate of 5 °C/min and kept for 3 min. As the carrier gas, helium was used at a flow rate of 1 ml/min and split mode of 50:1. The ionization voltage and ionization source temperature were 70 eV and 280 °C, respectively. Identification of extract compounds was performed using retention times (R_t) index and comparison with indexes in reference books and articles and using information in the computer library.

Preparation of Fish and Treatments

Fresh spangled emperor with an approximate weight of 400 ± 50 g was purchased from catchment of Arvand Free Zone in Khuzestan, Abadan (Abadan city, Khuzestan province, Iran). The fish were placed in icebox (1: 2 fish weight-to-ice ratio) and then were transferred to the fisheries laboratory of Khorramshahr Marine Science and Technology University within 1 h after purchasing. They were gutted and deheaded, and two fillets with a weight of approximately 200 g from each fish were prepared. The fillets were then divided into five groups: carboxymethyl cellulose (CMC) coating, CMC coating containing 0.5, 1 and 1.5% of *S. hortensis* extract (SHE): (CMC+ 0.5% SHE, CMC+ 1% SHE and CMC+ 1.5% SHE, respectively) and the control (uncoated). For each treatment, three replicates were considered. In order to prepare samples, the fillets were immersed in prepared solutions (4 °C) for 10 min and then were placed on sterile nets to drain for 10 min at 8–10 °C. Finally, samples were individually packed in sterile polyethylene pouches and were stored in a refrigerator (4 ± 2 °C) for 9 days. To determine the quality of fish fillets, the physicochemical analysis (pH, TVB-N, TBA, FFA, texture and color), microbial analysis (total viable count and total psychrophile count) and sensory evaluation were periodically performed for samples every three days.

Proximate Composition Analysis

The contents of moisture, crude protein, crude lipid and ash of spangled emperor were determined according to the standard method of AOAC [2].

Biochemical Analysis

The pH of the samples using the pH meter (Hanna instrument, Rumania) was determined based on the method of

Suvanich et al. [56]. Total volatile basic nitrogen (TVB-N, mg N/100 g sample) was measured according to the method described by Goulas and Kontominas [18]. Thiobarbituric acid (TBA, mg malondialdehyde [MDA]/kg sample) index was determined using the Siripatrawan and Noipha [53] method. The amount of free fatty acids (FFA, % oleic acid) was determined according to the method used by Flick et al. [12].

Microbial Analysis

For microbial analysis, 10 g of fish fillet sample was homogenized under sterile conditions with 90 ml phosphate buffer solution, and subsequent further serial decimal dilutions of this mixture (1 ml) were prepared using the same phosphate buffer solution (1:10). 1 ml of each dilution was added to the nutrient agar using the pour plate method. Total viable counts (TVC) were determined after incubating the plates at 32 °C for 48 h and counting the number of colony forming units. Psychrophilic bacteria count (PTC) was performed after 10 days of storage of the plates in the refrigerator at 7 °C. Microbial analysis was performed in three replications, and all counts were expressed as \log_{10} CFU/g [22, 49].

Color and Texture analysis

The surface color of the fillets of fish was evaluated by color meter (IMG-Pardazesh Cam-System XI, Dynamic Technology Tools Company, Iran) and using the CIE Lab system in the form of three indicators L^* , a^* and b^* and measuring 5 different points per sample. The L^* indicator determines lightness range from 0 (darkness) to 100 (lightness) and the a^* and b^* indices represent red/green (\pm) and yellow/blue (\pm) position, respectively [50].

To measure the texture parameters (texture profile analysis) of fish fillet, the samples were subjected to a pressure test using a texture analyzer (Model CT3, Brookfield, US) with probe profile of TA7 and load of 10 kg at room temperature. The force required to compress up to about 50% of the sample initial height was measured. The texture strength was calculated through the results of the amount of forces entered into the sample (g) and penetration depth (cm) [25]. Three texture parameters including hardness, adhesiveness and resilience were investigated.

Sensory Evaluation

The six-member trained panel evaluated the sensory attributes of fish samples in a completely randomized design. The sensory attributes of fillets based on a 5-point hedonic scale, in four sections texture (5: firm, 1: very soft), odor (5: extreme desirable, 1: extremely unacceptable/off-odors), color (5: natural color, 1: extreme discoloration) and general

acceptance (5: extremely acceptable, 1: extremely unacceptable), were evaluated. The critical score of the acceptance of fish samples was considered 4.0, and below it meant the rejection of the sensory attributes [40].

Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using the SPSS 16.0. Comparison of mean and significant difference at $p < 0.05$ level was performed using Duncan's multiple range test. The results were expressed as mean \pm standard deviation.

Results and Discussion

Analysis of the Chemical Composition of Extract

The compounds derived from the analysis of summer savory extract using GC/MS are shown in Table 1. Totally, 22 compounds were identified for summer savory extract and the most abundant component was carvacrol (28.67%). Pavela et al. [43] stated that carvacrol was the major component of ethanolic and hexanic extracts of *S. hortensis* (98% and 87.5%, respectively). In the study reported by Sajfirtova et al. [48], carvacrol was identified as the major combination of ethanolic (12.6%) and hexanic (20%) extracts of *S. hortensis*. Factors such as weather, geographical and seasonal conditions, plant maturity and extraction methods may affect the chemical composition and change it [32].

Effect of CMC Coating Enriched with Summer Savory Extract on Proximate Composition Changes

The main components of fish flesh are moisture, protein, lipid and ash. The proximate compositions of fish vary according to different factors such as nutrition, fish size, gender, age, season of catching and environment, and so, there are significant changes in the muscle composition of the fish [44]. The initial content of moisture, crude protein, crude lipid and ash of spangled emperor fillet was 79.50 ± 1.32 , 91.92 ± 1.63 , 1.26 ± 0.22 and 4.91 ± 0.38 , respectively, which is comparable to the results reported by El Shehawy et al. [10]. Table 2 shows the trend of variations of the approximate composition of spangled emperor fillets during refrigerated storage period.

The moisture content in all treatments decreased with increasing storage time, but this was not significant, and at the end of storage period, there were no significant differences ($p > 0.05$) between the treated and control samples in moisture content. Drip loss during storage may be one of the reasons for reducing moisture content. In addition, by

Table 1 Chemical composition of *Satureja hortensis* extract using GC–MS method

No.	Compound	Area (%)
1	Carvacrol	28.67
2	Cyclotrisiloxane, hexamethyl-	17.26
3	Octasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl-	16.08
4	Benzo[h]quinoline, 2,4-dimethyl-	6.27
5	Camphene	3.72
6	^a Ethanol, 1-(2-butoxyethoxy)	3.60
7	2-(Acetoxymethyl)-3-(methoxycarbonyl)biphenylene	3.39
8	Benzonitrile, m-phenethyl-	3.04
9	Tetrasiloxane, decamethyl-	2.98
10	Silicic acid, diethyl bis(trimethylsilyl) ester	2.24
11	4-Methyl-2-trimethylsilyloxy-acetophenone	2.05
12	Benzene, 2-[(tert-butyl dimethylsilyloxy)-1-isopropyl-4-methyl-	1.74
13	Benzo[thiophene-3-carboxylic acid, 4,5,6,7-tetrahydro-2-amino-6-ethyl-, ethyl ester	1.70
14	2-Methyl-7-phenylindole	1.07
15	1H-Indole-2-carboxylic acid, 6-(4-ethoxyphenyl)-3-methyl-4-oxo-4,5,6,7-tetrahydro-, isopropyl ester	0.83
16	1,1,1,3,5,5,5-Heptamethyltrisiloxane	0.80
17	1-Benzazirene-1-carboxylic acid; 2, 2,5a-trimethyl-1a-[3-oxo-1-butenyl] perhydro-; methyl ester	0.77
18	5-Methyl-2-phenylindolizine	0.74
19	Silane, 1,4-phenylenebis(trimethyl-	0.71
20	N-methyl-1-adamantaneacetamide	0.68
21	Methyltris(trimethylsilyloxy)silane	0.68
22	Heptasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11,13,13-tetradecamethyl-	0.66

^aMay be the solvent in the GC column

increasing the storage time of the fillets in the refrigerator, proteolytic enzymes affect proteins and convert them to amino acids and cause the protein to lose its water-binding capacity [9]. The measurement results of crude protein in all samples showed a decreasing trend during storage. At the end of storage period, there were no significant differences ($p > 0.05$) between the different samples in the crude protein content. Reducing the protein content during storage can be attributed to the denaturation of proteins and the production of simple polypeptide chains and the conversion of nitrogen to volatile compounds. All of these processes are caused due to inappropriate conditions of storage temperature and the concentration of enzymes [9]. The lipid and ash contents of all sample groups increased with increasing storage time. At the end of storage period, there were no significant differences ($p > 0.05$) between the different samples in the lipid and ash values. The inverse relationship between the amount of moisture and lipid has already been proven [9]. The results of this study were in agreement with the study of Ehsany et al. [9] who reported that the moisture and protein contents of silver carp decreased during icing and freezing (–24) storage, while the content of lipid and ash increased with time.

Effect of CMC Coating Enriched with Summer Savory Extract on pH Changes

Generally, the natural pH of live fish is just above 7.0, typically about 7.3, which after the death of the fish and it entering to the rigor mortis stage and conversion of glycogen to lactic acid falls markedly [28]. The process of pH changes is shown in Fig. 1a. The initial pH values of samples of control, CMC, CMC+ 0.5% SHE, CMC+ 1% SHE and CMC+ 1.5% SHE were 6.89, 6.85, 6.74, 6.78 and 6.74, respectively. pH value was significantly increased over time due to production of alkaline compounds such as ammonia, trimethylamine and volatile basic compounds produced by either endogenous or microbial enzymes [35]. Ozyurt et al. [42] stated that pH values higher than 7.10 represent decomposition in fish. The control and CMC groups exceeded the acceptable limit of pH value at third day of storage, while the pH value of samples treated with summer savory extract exceeded such limit at sixth day of storage. At the end of storage, pH values were 7.56, 7.26, 7.40, 7.30 and 7.30 for control, CMC, CMC+ 0.5% SHE, CMC+ 1% SHE and CMC+ 1.5% SHE, respectively. At the end of storage, pH values of CMC, CMC+ 1% SHE, CMC+ 1.5% SHE were significantly ($p < 0.05$) less than the control sample. This

Table 2 Changes in proximate composition of *Lethrinus nebulosus* fillets treated with carboxymethyl cellulose (CMC) coating enriched with *satureja hortensis* extract (SHE) during refrigerated storage

Proximate composition parameter (%)	Storage time (days)			
	0	3	6	9
Moisture				
Control	79.50 ± 1.32 ^{aA}	79.16 ± 1.60 ^{aA}	78.66 ± 1.89 ^{aA}	78.25 ± 0.35 ^{aA}
CMC	78.66 ± 1.25 ^{aA}	77.83 ± 1.04 ^{aA}	78.33 ± 1.15 ^{aA}	77.25 ± 1.25 ^{aA}
CMC+ 0.5% SHE	79.83 ± 1.44 ^{aA}	79.25 ± 0.35 ^{aA}	79.00 ± 1.73 ^{aA}	79.33 ± 0.76 ^{aA}
CMC+ 1% SHE	79.50 ± 0.50 ^{aA}	78.66 ± 1.04 ^{aA}	78.66 ± 0.28 ^{aA}	78.33 ± 2.30 ^{aA}
CMC+ 1.5% SHE	78.25 ± 0.75 ^{aA}	79.25 ± 0.35 ^{aA}	78.33 ± 1.04 ^{aA}	77.50 ± 0.86 ^{aA}
Protein^a				
Control	91.92 ± 1.63 ^{aA}	90.33 ± 1.93 ^{aAB}	88.72 ± 1.49 ^{aAB}	87.52 ± 1.73 ^{aB}
CMC	88.46 ± 0.53 ^{aA}	84.63 ± 0.25 ^{bB}	88.36 ± 0.40 ^{aA}	88.22 ± 0.50 ^{aA}
CMC+ 0.5% SHE	89.87 ± 2.48 ^{aA}	90.20 ± 0.41 ^{aA}	89.78 ± 3.06 ^{aA}	88.07 ± 2.68 ^{aA}
CMC+ 1% SHE	91.39 ± 2.63 ^{aA}	90.41 ± 2.13 ^{aA}	78.84 ± 2.35 ^{aAB}	86.10 ± 0.11 ^{aB}
CMC+ 1.5% SHE	88.41 ± 0.48 ^{aAB}	91.59 ± 1.27 ^{aA}	87.80 ± 3.21 ^{aB}	87.07 ± 0.76 ^{aB}
Lipid^a				
Control	1.26 ± 0.22 ^{bA}	1.92 ± 0.79 ^{abA}	1.80 ± 0.33 ^{bA}	2.23 ± 0.73 ^{aA}
CMC	1.88 ± 0.89 ^{abA}	1.72 ± 0.73 ^{bA}	2.10 ± 0.24 ^{abA}	2.43 ± 0.70 ^{aA}
CMC+ 0.5% SHE	1.23 ± 0.34 ^{bC}	3.12 ± 0.44 ^{aA}	2.33 ± 0.12 ^{abB}	2.23 ± 0.20 ^{aB}
CMC+ 1% SHE	1.41 ± 0.14 ^{bB}	1.41 ± 0.40 ^{bB}	2.53 ± 0.37 ^{aA}	2.41 ± 0.84 ^{aA}
CMC+ 1.5% SHE	2.36 ± 0.10 ^{aA}	2.38 ± 0.80 ^{abA}	2.33 ± 0.39 ^{abA}	2.75 ± 0.41 ^{aA}
Ash^a				
Control	4.91 ± 0.38 ^{aA}	5.25 ± 1.25 ^{aA}	6.00 ± 1.00 ^{aA}	6.33 ± 0.57 ^{aA}
CMC	5.00 ± 0.00 ^{aA}	6.00 ± 1.00 ^{aA}	5.50 ± 0.50 ^{abA}	5.33 ± 1.52 ^{aA}
CMC+ 0.5% SHE	4.50 ± 0.50 ^{aC}	5.00 ± 1.00 ^{abC}	6.00 ± 0.00 ^{aAB}	6.75 ± 0.75 ^{aA}
CMC+ 1% SHE	4.62 ± 0.62 ^{aA}	4.50 ± 0.50 ^{aA}	5.00 ± 1.00 ^{abA}	5.00 ± 0.00 ^{aA}
CMC+ 1.5% SHE	4.75 ± 0.75 ^{aA}	5.00 ± 1.00 ^{aA}	4.50 ± 0.50 ^{bA}	6.00 ± 1.00 ^{aA}

All values were mean ± standard deviation from three replicates

Different small letters in the same column indicate significant differences between means ($p < 0.05$)

Different capital letters in the same row indicate significant differences between means ($p < 0.05$)

^aProtein, lipid and ash contents were measured based on dry weight

phenomenon could be related to the antimicrobial properties of CMC coating and summer savory extract in reducing the microbial growth (Fig. 2).

Effect of CMC Coating Enriched with Summer Savory Extract on TVB-N Changes

TVB-N is one of the most important indicators of sea food quality [26]. Figure 1b shows variations in the TVB-N value of spangled emperor fillets during refrigerated storage. The initial value of TVB-N in control, CMC and CMC samples containing 0.5, 1 and 1.5% of summer savory extract was 13.30, 14.00, 17.73, 16.80 and 17.50 mg N/100 g, respectively, and at the end of storage period, reached to 55.53, 53.90, 55.82, 46.20 and 45.73 mg N/100 g, respectively. The amount of TVB-N in all sample groups significantly increased during storage. The increase in TVB-N value is due to the activity of bacteria and endogenous enzymes of fish flesh [54]. The acceptable level of TVB-N has been reported for human consumption to be 25–35 mg N/100 g

sample, but this varies among different species [21]. In the present study, the TVB-N value of the control and CMC+ 0.5% SHE samples (37.80 and 38.50 mg/100 g, respectively) was more than acceptable limit range of 25–35 mg/100 g on day 6, while the amount of TVB-N in the samples treated with CMC, CMC+ 1% SHE and CMC+ 1.5% SHE exceeded the acceptable limit at day 9 of storage. At the end of storage, CMC+ 1.5% SHE group had the lowest of TVB-N value, although there were no significant differences ($p > 0.05$) compared to other treated and control samples. Less values of TVB-N in samples treated with summer savory extract can be due to the reduction of spoilage bacterial population, which can be attributed to the antimicrobial properties of the extract.

Effect of CMC Coating Enriched with Summer Savory Extract on TBA Changes

Lipid oxidation refers to the oxidation of unsaturated fatty acids in the muscle of the fish, resulting in the unpleasant

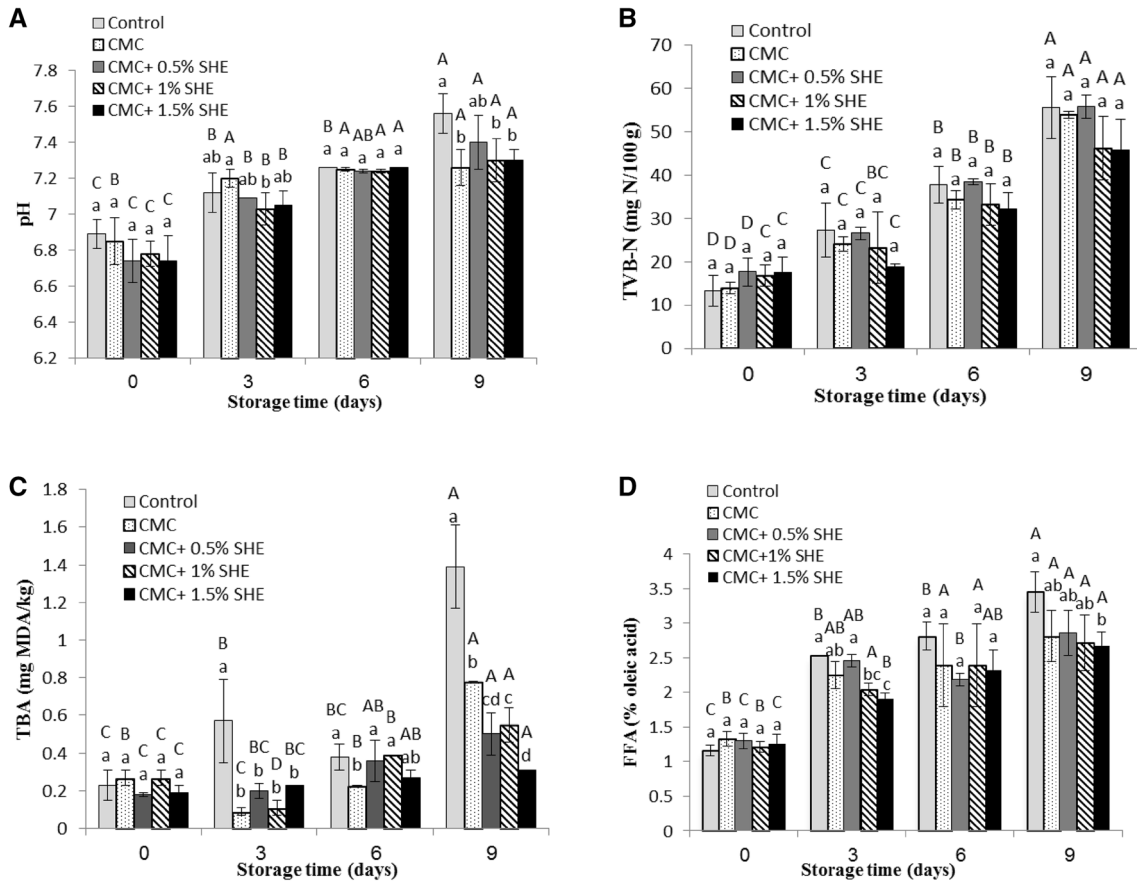


Fig. 1 Changes in chemical parameters pH (a); TVB-N (b); TBA (c); and FFA (d) of *Lethrinus nebulosus* fillets stored under different conditions during refrigerated storage. (CMC: carboxymethyl cellulose,

SHE Satureja hortensis extract). Different small letters indicate significant differences ($p < 0.05$) between treatments. Different capital letters indicate significant differences as storage time ($p < 0.05$)

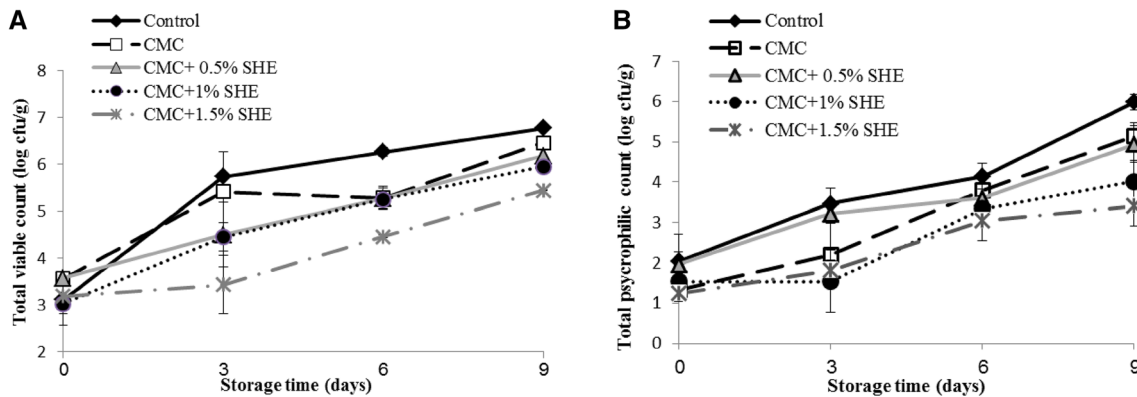


Fig. 2 Changes in a total viable count (TVC) and b psychrotrophic bacteria counts (PTC) of *Lethrinus nebulosus* fillets stored under different conditions during refrigerated storage. (CMC carboxymethyl cellulose, *SHE Satureja hortensis* extract)

odor and taste in the fish and shortening of its shelf life. TBA is an index of lipid oxidation based on the malonaldehyde (MDA) content, which is the secondary product of the oxidation of unsaturated fatty acids [4]. Results of changes in TBA values of spangled emperor fillets during

refrigerated storage are shown in Fig. 1c. The initial TBA content varied between 0.18 and 0.27 mg MDA/kg. With increasing storage time, TBA content increased in all sample groups. The TBA value in the treated samples, especially CMC+ 1.5% SHE, was significantly ($p < 0.05$)

lower compared to the control sample at the end of storage period. A similar study was reported by Aksu and Ozer [1], who stated that the highest concentration (500 ppm) of summer savory extract compared to other concentrations had the greatest effect on reducing the TBA amount of beef at 4 °C. Less TBA value in the samples treated with extract can be attributed to compounds such as thymol and carvacrol, phenolic compounds with hydroxyl groups (OH) that have the ability to remove free radicals and cause lower levels of malondialdehyde in the samples treated with extract [36]. Reducing the amount of thiobarbituric acid in some storage days may be due to reduced hydroperoxide values and the reaction between malondialdehyde and proteins, amino acids and glycogen, which reduces malondialdehyde value and subsequently reduces the TBA value [17, 46]. At the end of storage period, the TBA content of control, CMC, CMC+ 0.5% SHE, CMC+ 1% SHE and CMC+ 1.5% SHE was 1.39, 0.78, 0.50, 0.55 and 0.31 mg MDA/kg, respectively. The acceptable limit of TBA value in fish meat is considered 1–2 mg MDA/kg [19]; the TBA content in the control group was beyond acceptable level at day 9 of storage. Meanwhile, the TBA content of treated sample groups did not exceed 1–2 mg MDA/kg until the end of storage period.

Effect of CMC Coating Enriched with Summer Savory Extract on FFA Changes

Glycerides, glycolipids and phospholipids are hydrolyzed by lipase enzymes and converted to free fatty acids, which later are transformed into aldehydes and ketones. Hydrolysis of lipid alone does not reduce the quality, taste and odor of the product, but FFA have a direct negative effect on the taste of fish flesh and exacerbates lipid oxidation [3, 20]. Changes in the FFA values of the spangled emperor fillets during refrigerated storage (4 ± 2 °C) are shown in Fig. 1d. The initial FFA value of the various groups ranged between 1.16 and 1.33% of oleic acid. With the increase in the storage time, the FFA value was increased in all sample groups, and at the end of storage period, the FFA content in the groups of control, CMC, CMC+ 0.5% SHE, CMC+ 1% SHE and CMC+ 1.5% SHE reached to 3.45, 2.81, 2.86, 2.72 and 2.67%, of oleic acid, respectively. At the end of storage period, CMC+ 1.5% SHE group significantly ($p < 0.05$) had the least FFA value compared to the control. Probably the antioxidant properties of the ethanolic extract of summer savory are due to its phenolic compounds. Based on the GC/MS analysis of summer savory extract in our study, carvacrol was the main compound (28.67%) of the extract, which is a very active natural antioxidant [38].

Effect of CMC Coating Enriched with Summer Savory Extract on Microbial Changes

Most fresh and several lightly preserved seafoods are destroyed due to microbial activity. [28]. An assessment of the shelf life and post-processing contamination of fishery products using the total viable count (TVC) of bacteria is a useful method [29]. As shown in Fig. 2a, initial TVC of the spangled emperor fillets ranged from 3.03 to 3.58 log CFU/g. Over time, the TVC of all samples significantly increased and this trend was more intensive in the control sample. From day 6 of storage, TVC of the control sample was significantly ($p < 0.05$) higher than that the treated samples. Among the treatments, CMC+ 1.5% SHE had the greatest ($p < 0.05$) effect on reducing TVC compared to control. Antimicrobial properties of summer savory extract are due to polyphenolic compounds including phenolic acids and flavonoids [30]. In this study, based on data from the analysis of summer savory extract by GC/MS, carvacrol was the major combination of the extract, which as the inhibitor prevents the growth of different pathogens. Carvacrol appears to interact with membrane, dissolves into the phospholipid bilayer and gets aligned between the fatty acid chains, separating the fatty acid chains of the phospholipids, and creates channels that result in allowing ions to leave the cytoplasm [58, 59]. Gram-negative psychrophilic bacteria (PTC) are the main group of microorganisms responsible for the corruption of fresh fish during refrigerated storage [49]. At the beginning of the storage period, PTC of the samples of control, CMC, CMC+ 0.5% SHE, CMC+ 1% SHE and CMC+ 1.5% SHE was 2.04, 1.33, 1.96, 1.54 and 1.24 log CFU/g, respectively. With increasing storage time, PTC of different samples significantly ($p < 0.05$) increased (Fig. 2b). At the end of the storage period, the TVC and PTC of different sample groups did not exceed 7 log CFU/g, the recommended acceptable limit for the fish and fish products [24]. The samples of CMC+ 1.5% SHE and control had the lowest and highest PTC of 5.99 and 3.41 log CFU/g, respectively. Carvacrol as a main compound of *S. hortensis* extract in our study is capable of destroying the outer membrane of gram-negative bacteria, resulting in the release of polysaccharides and increased permeability of the cytoplasmic membrane relative to ATP [37]. A similar study reported by Choulitoudi et al. [7] stated that CMC coating enriched with extract and essential oil of *Satureja thymbra* (L.) reduced the bacterial load of gilthead seabream (*Sparus aurata*) fillets stored at 0 °C.

Effect of CMC Coating Enriched with Summer Savory Extract on Color Changes

The appearance of seafood in terms of product acceptance and selection is a very important parameter for the

consumer. Thus, color plays an important role in evaluating product quality from the point of view of sales. The color of fish muscle is affected by two factors: the structural characteristics of the muscle and the concentration of pigments [14, 16]. Changes in the color parameters of different samples of spangled emperor fillets during refrigerated storage period are shown in Table 3. Color parameters L^* , a^* and b^* in all of the groups showed a decreasing trend with increasing storage time. Color changes in fish muscle during storage are attributed to the oxidation of proteins with hemo groups such as hemoglobin and myoglobin [6]. Richards et al. [47] also expressed that probably hemoglobin is to be a catalyst for lipid oxidation due to its auto-oxidant ability. The highest L^* value was found in the control group, meanwhile CMC+ 1.5% SHE-treated group showed the significantly ($p < 0.05$) lowest L^* value compared with the control group. Siripatrawan and Harte [52] stated that increasing the concentration of green tea extract to chitosan film results in increased opacity and reduced film lightness. Many plant extracts often have the dark color that may affect the color of the final product, which depends on the color of the extract and its concentration [13].

Generally, the a^* value (red/green) of treated and control samples decreased with increasing storage time. At the end of storage period, there were no significant differences ($p > 0.05$) between treatment groups and the control in the a^* value. The behavior of red-green coordinate (a^*) in foods

depends on a variety of aspects, such as the technology used and the composition. Factors such as the structural integrity of the food, the content of pigment and its disposition (water or lipid-soluble) and the availability of surface water affect this coordinate [60]. Several researchers have investigated the effect of different antioxidants on meat and meat products and found that meat oxidation reduced the a^* value [23, 34]. Regarding coordinate b^* , there were significant differences ($p < 0.05$) between the treatment groups and control at the end of storage period. The difference in the b^* coordinate may be due to pH, oxidation, water activity, etc., in the food matrix, which has the most impact on this coordinate in many foods [8]. Similar study has been reported by Özalp Özen et al. [41].

Effect of CMC Coating Enriched with Summer Savory Extract on Texture Profile Analysis

Changes in tissue properties of the spangled emperor fillets during refrigerated storage period (4 ± 2 °C) are shown in Table 4. As the storage time increased, values of hardness, adhesiveness and resilience significantly ($p < 0.05$) changed for all samples. Hardness was decreased during storage ($p < 0.05$). In the control sample, hardness decreased sharply after day 3 of storage, and at the end of storage period, the lowest ($p < 0.05$) hardness value was found in the control group. Reduction in fillet hardness is probably

Table 3 Changes in the color parameters of *Letrinus nebulosus* fillets treated with carboxymethyl cellulose (CMC) coating enriched with *satureja hortensis* extract (SHE) during refrigerated storage

Color parameter	Storage time (days)			
	0	3	6	9
L^*				
Control	59.14 ± 0.92 ^{aA}	57.15 ± 1.85 ^{aAB}	57.94 ± 0.03 ^{aAB}	55.99 ± 0.56 ^{aB}
CMC	59.08 ± 0.10 ^{aA}	56.45 ± 1.46 ^{aB}	56.60 ± 0.52 ^{bB}	55.34 ± 0.80 ^{abB}
CMC+ 0.5% SHE	59.81 ± 0.33 ^{aA}	56.98 ± 0.51 ^{aB}	56.30 ± 1.13 ^{bcBC}	54.80 ± 1.14 ^{abC}
CMC+ 1% SHE	59.66 ± 0.27 ^{aA}	56.23 ± 2.15 ^{aB}	55.75 ± 0.66 ^{bcB}	54.60 ± 0.17 ^{abB}
CMC+ 1.5% SHE	59.95 ± 0.22 ^{aA}	55.70 ± 1.29 ^{aB}	55.23 ± 0.40 ^{cB}	54.10 ± 0.96 ^{bB}
a^*				
Control	4.60 ± 0.65 ^{aA}	4.42 ± 0.72 ^{cdA}	3.80 ± 0.44 ^{cA}	3.82 ± 0.93 ^{aA}
CMC	5.56 ± 1.23 ^{aA}	4.20 ± 0.39 ^{dA}	3.86 ± 0.44 ^{cA}	4.49 ± 1.29 ^{aA}
CMC+ 0.5% SHE	5.27 ± 0.45 ^{aA}	5.26 ± 0.64 ^{bcA}	5.07 ± 0.49 ^{bA}	4.75 ± 0.28 ^{aA}
CMC+ 1% SHE	5.70 ± 0.72 ^{aAB}	6.40 ± 0.28 ^{aA}	6.07 ± 0.53 ^{aAB}	5.08 ± 0.47 ^{aB}
CMC+ 1.5% SHE	5.94 ± 0.36 ^{aA}	5.51 ± 0.50 ^{abA}	5.11 ± 0.40 ^{bA}	4.83 ± 0.92 ^{aA}
b^*				
Control	22.71 ± 1.35 ^{aA}	22.11 ± 0.58 ^{abAB}	22.25 ± 0.27 ^{aAB}	21.03 ± 0.46 ^{cB}
CMC	23.12 ± 0.49 ^{aA}	22.57 ± 0.36 ^{abAB}	22.62 ± 0.01 ^{aAB}	22.04 ± 0.20 ^{bB}
CMC+ 0.5% SHE	23.89 ± 0.11 ^{aA}	22.06 ± 1.20 ^{abB}	22.35 ± 0.21 ^{aB}	22.21 ± 0.19 ^{bB}
CMC+ 1% SHE	22.80 ± 0.02 ^{aA}	21.85 ± 0.78 ^{bA}	22.64 ± 1.10 ^{aA}	22.27 ± 0.16 ^{bA}
CMC+ 1.5% SHE	23.95 ± 0.12 ^{aA}	23.31 ± 0.03 ^{aB}	22.97 ± 0.26 ^{aC}	23.19 ± 0.08 ^{aBC}

All values were mean ± standard deviation from three replicates

Different small letters in the same column indicate significant differences between means ($p < 0.05$)

Different capital letters in the same row indicate significant differences between means ($p < 0.05$)

Table 4 Changes in the texture profile of *Lethrinus nebulosus* fillets treated with carboxymethyl cellulose (CMC) coating enriched with *satureja hortensis* extract (SHE) during refrigerated storage

Texture parameter	Storage time (days)			
	0	3	6	9
Hardness (g)				
Control	2414.50 ± 386.50 ^{aA}	2192.70 ± 230.36 ^{aA}	606.75 ± 98.25 ^{bB}	538.00 ± 67.00 ^{bB}
CMC	2534.50 ± 256.50 ^{aA}	2053.00 ± 251.00 ^{aAB}	1823.00 ± 343.00 ^{aB}	1476.00 ± 391.55 ^{aB}
CMC+ 0.5% SHE	2853.50 ± 80.50 ^{aA}	2363.50 ± 423.50 ^{aAB}	1845.20 ± 506.76 ^{aB}	1662.00 ± 300.23 ^{aB}
CMC+ 1% SHE	2868.00 ± 22.00 ^{aA}	1948.50 ± 125.50 ^{aB}	1803.50 ± 334.50 ^{aB}	1596.30 ± 203.67 ^{aB}
CMC+ 1.5% SHE	2423.00 ± 216.50 ^{aA}	1929.20 ± 351.25 ^{aAB}	1827.00 ± 344.00 ^{aB}	1243.50 ± 93.32 ^{aC}
Adhesiveness (mJ)				
Control	0.17 ± 0.07 ^{aB}	0.30 ± 0.10 ^{aAB}	0.16 ± 0.05 ^{aB}	0.40 ± 0.10 ^{aA}
CMC	0.15 ± 0.05 ^{aB}	0.27 ± 0.07 ^{abAB}	0.17 ± 0.07 ^{aB}	0.40 ± 0.10 ^{aA}
CMC+ 0.5% SHE	0.10 ± 0.00 ^{aC}	0.28 ± 0.06 ^{abA}	0.20 ± 0.00 ^{aB}	0.31 ± 0.02 ^{aA}
CMC+ 1% SHE	0.10 ± 0.00 ^{aB}	0.16 ± 0.05 ^{abB}	0.18 ± 0.03 ^{aB}	0.42 ± 0.07 ^{aA}
CMC+ 1.5% SHE	0.13 ± 0.05 ^{aB}	0.15 ± 0.05 ^{bB}	0.15 ± 0.05 ^{aB}	0.45 ± 0.05 ^{aA}
Resilience				
Control	0.12 ± 0.05 ^{aAB}	0.12 ± 0.01 ^{abAB}	0.18 ± 0.04 ^{aA}	0.10 ± 0.00 ^{abB}
CMC	0.16 ± 0.05 ^{aA}	0.10 ± 0.04 ^{abAB}	0.15 ± 0.02 ^{aA}	0.06 ± 0.02 ^{bB}
CMC+ 0.5% SHE	0.15 ± 0.02 ^{aA}	0.07 ± 0.01 ^{bB}	0.08 ± 0.02 ^{bB}	0.08 ± 0.01 ^{bB}
CMC+ 1% SHE	0.15 ± 0.02 ^{aA}	0.16 ± 0.01 ^{aA}	0.05 ± 0.00 ^{bB}	0.13 ± 0.00 ^{aA}
CMC+ 1.5% SHE	0.15 ± 0.04 ^{aA}	0.16 ± 0.06 ^{aA}	0.06 ± 0.02 ^{bB}	0.09 ± 0.02 ^{abAB}

All values were mean ± standard deviation from three replicates

Different small letters in the same column indicate significant differences between means ($p < 0.05$)

Different capital letters in the same row indicate significant differences between means ($p < 0.05$)

due to increase in microbial load, which makes the muscle softer and less elastic (Fig. 2). The muscle texture of the fish depends on intrinsic biological factors such as muscle fiber density, including fat and collagen level, as well as microbial and autolytic processes caused by the death of the fish, which induce degradation of myofibrillar protein integration and ultimately soften muscle [13]. Our results showed that, at the end of storage period, the hardness of the treated samples was significantly ($p < 0.05$) higher than the control sample. This suggests that CMC coating and summer savory extract have been able to reduce the activities of endogenous enzymes and microbial of spangled emperor fillets. Other properties of fibrous proteins and their function, such as water holding capacity, gelation, cohesion/adhesion and elasticity, are also effective on the muscle texture of fish [33]. For resilience and adhesiveness parameters, there were no significant differences ($p > 0.05$) between treated samples and the control at the end of storage period. However, there were significant differences ($p < 0.05$) between CMC+ 1% SHE group and those treated with CMC and CMC+ 0.5% SHE in resilience value.

Effect of CMC Coating Enriched with Summer Savory Extract on Sensory Evaluation

The sensory evaluation results of the spangled emperor fillets during refrigerated storage period are shown in Table 5. The

sensory scores of all samples were decreased with the passage of time. A score of 4.0 is considered as the borderline limit of acceptability of fish samples for human consumption [40]. The control and treated samples received unacceptable scores for texture, color, odor and general acceptance on day 6 and 9 of storage, respectively. Changes in sensory properties are attributable to the results of microbial and chemical analysis. Clearly, the antioxidant and antimicrobial properties of summer savory extract could improve the sensory quality of spangled emperor fillets during storage. At the end of storage period, treated samples had higher sensory scores compared to the control sample and there were significant differences ($p < 0.05$) between the control and those treated with CMC+ 1.5% SHE and CMC+ 1% SHE groups.

Conclusion

The results of this study showed that coated treatments with and without of summer savory extract reduced microbial growth and oxidative activities of fatty acids, retarded protein denaturation and improved the texture hardness and sensory properties of *L. nebulosus* fillets during refrigerated storage. The natural extract due to its natural pigments, caused some color differences between coated and non-coated samples. Among treatments, CMC coating containing 1.5% followed by 1% of *S. horstensis* extract had

Table 5 Sensory evaluation of *Lethrinus nebulosus* fillets treated with carboxymethyl cellulose (CMC) coating enriched with *Satureja hortensis* extract (SHE) during refrigerated storage

Sensory parameter	Storage time (days)			
	0	3	6	9
Texture				
Control	4.94 ± 0.09 ^{aA}	4.60 ± 0.09 ^{bB}	3.10 ± 0.25 ^{bC}	1.94 ± 0.19 ^{cD}
CMC	4.88 ± 0.09 ^{aA}	4.55 ± 0.09 ^{bB}	4.11 ± 0.19 ^{aC}	2.33 ± 0.17 ^{bD}
CMC+ 0.5% SHE	4.94 ± 0.09 ^{aA}	4.66 ± 0.00 ^{abB}	4.05 ± 0.09 ^{aC}	3.05 ± 0.09 ^{aD}
CMC+ 1% SHE	4.88 ± 0.09 ^{aA}	4.66 ± 0.00 ^{abA}	4.13 ± 0.23 ^{abB}	3.13 ± 0.23 ^{aC}
CMC+ 1.5% SHE	5.00 ± 0.00 ^{aA}	4.77 ± 0.09 ^{aB}	4.13 ± 0.11 ^{aC}	3.06 ± 0.11 ^{aD}
Odor				
Control	4.77 ± 0.19 ^{aA}	4.60 ± 0.09 ^{aA}	3.27 ± 0.38 ^{bB}	1.88 ± 0.19 ^{bC}
CMC	4.66 ± 0.00 ^{aA}	4.66 ± 0.00 ^{aA}	4.05 ± 0.09 ^{abB}	1.99 ± 0.33 ^{bC}
CMC+ 0.5% SHE	4.77 ± 0.09 ^{aA}	4.66 ± 0.16 ^{aA}	4.11 ± 0.19 ^{abB}	1.99 ± 0.33 ^{bC}
CMC+ 1% SHE	4.77 ± 0.19 ^{aA}	4.61 ± 0.19 ^{aA}	4.33 ± 0.00 ^{abB}	2.71 ± 0.09 ^{aC}
CMC+ 1.5% SHE	4.77 ± 0.09 ^{aA}	4.60 ± 0.09 ^{aA}	4.22 ± 0.25 ^{abB}	2.49 ± 0.16 ^{aC}
Color				
Control	4.88 ± 0.09 ^{aA}	4.55 ± 0.19 ^{aA}	3.66 ± 0.33 ^{bB}	2.55 ± 0.19 ^{cC}
CMC	4.94 ± 0.09 ^{aA}	4.66 ± 0.16 ^{aA}	4.05 ± 0.25 ^{abB}	2.82 ± 0.28 ^{bcC}
CMC+ 0.5% SHE	4.88 ± 0.19 ^{aA}	4.60 ± 0.09 ^{aA}	4.05 ± 0.25 ^{abB}	2.94 ± 0.25 ^{abcC}
CMC+ 1% SHE	4.77 ± 0.19 ^{aA}	4.71 ± 0.09 ^{aA}	4.16 ± 0.28 ^{abB}	3.16 ± 0.16 ^{abC}
CMC+ 1.5% SHE	4.88 ± 0.19 ^{aA}	4.77 ± 0.09 ^{aA}	4.22 ± 0.19 ^{abB}	3.27 ± 0.09 ^{aC}
Total acceptance				
Control	4.94 ± 0.09 ^{aA}	4.66 ± 0.16 ^{aB}	3.55 ± 0.09 ^{bC}	1.99 ± 0.16 ^{cD}
CMC	4.94 ± 0.09 ^{aA}	4.66 ± 0.00 ^{aB}	4.00 ± 0.00 ^{aC}	2.41 ± 0.08 ^{bD}
CMC+ 0.5% SHE	4.86 ± 0.24 ^{aA}	4.77 ± 0.09 ^{aA}	4.16 ± 0.16 ^{abB}	2.33 ± 0.33 ^{bcC}
CMC+ 1% SHE	4.94 ± 0.09 ^{aA}	4.83 ± 0.17 ^{aA}	4.12 ± 0.10 ^{abB}	2.94 ± 0.25 ^{aC}
CMC+ 1.5% SHE	4.94 ± 0.09 ^{aA}	4.83 ± 0.00 ^{aA}	4.22 ± 0.19 ^{abB}	3.16 ± 0.16 ^{aC}

All values were mean ± standard deviation from three replicates

Different small letters in the same column indicate significant differences between means ($p < 0.05$)

Different capital letters in the same row indicate significant differences between means ($p < 0.05$)

the greatest effect on maintaining the quality of spangled emperor fillets. Efficacy of the coating incorporated with summer savory extract is due to compounds of the extract especially carvacrol as the major compound (28.67%), which gives the antimicrobial and antioxidant properties to the extract. Thus, carboxymethyl cellulose coating enriched with summer savory extract could be a promising method to maintain the quality of spangled emperor fillets during refrigerated storage.

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