



The influence of chitosan-carboxymethyl cellulose composite and bi-layer film and coatings on flavor quality and volatile profile of Asian sea bass during storage at refrigerator

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Abstract

This study investigated the effects of chitosan-carboxymethyl cellulose (CH-CMC) composite and bilayer coating and film on flavor quality of Asian sea bass (*Lates calcarifer*) fillets during storage at refrigerator for 16 days. In this experiment, different indexes including: sensory evaluation, trimethylamine nitrogen (TMA-N), total viable counts (TVC), free amino acids (FAAs), volatile organic compounds (VOCs), ATP related compounds, and K value were measured. Main reason for off-flavor of treated samples was mainly attributed to the significant inhibition of bacterial growth after death. The initial total viable count (TVC) (\log_{10} CFU/g) in the all samples of fillet was $1.21 \log_{10}$ CFU/g. TVC in sea bass fillet became lower than $7 \log_{10}$ CFU/g for BF (bi-layer film) samples throughout the entire storage. The results of FAAs showed that use CH-CMC reduced the accumulation of bitterness-taste. Treated samples decreased slowly with degradation of inosine- 5'-monophosphate (IMP) resulting higher umami intensity and overall acceptability. Synergistic effects of glutamic acid (Glu) and inosine- 5'- monophosphate (IMP) has inhibitory effects on bitter taste in samples. The VOCs including aldehydes, ketones, alcohols, and hydrocarbons were decreased by the CH-CMC coatings and film. Eight key flavor volatile organic compounds, including 3-methylbutanal, hexanal, E-2-hexanal, ethanol, 1-pentanol, 1-hexanol, 2,3 pentanedion, hydroxyl-2-butanone, were identified in all samples. Generally, these results suggested the advantageous potential for CH-CMC film in retarding formation of fishy odor and taste and improving flavor of Asian sea bass compared with coating during entire the storage time, because of good oxygen barriers properties of film and potential properties to crosslink with flavors in order to enhance functionalities of packaging material.

Keywords Asian sea bass · Biopolymers · Flavor quality · Volatile profile

Introduction

Asian sea bass (*Lates calcarifer*) is valuable cultured fish in the south of Iran because of its desirable taste, odor, and high nutritional value. Asian sea bass was cultured a broad variety of salinity (ranged from 0–56% salinity) [1]. High water value, bacterial activity, and *nutritional-rich* lead to a shorter shelf life of seafood [2]. Shelf life, defined as the time storage which a seafood product remain safe, is essential for assuring fish quality and protecting consumers from the effects

of degradation. Shelf life of seafood products is limited by changes in sensory characteristics, lipid oxidation, and microbiological assessment. The flavor (including taste and odor) of seafood was produced by chemical and bacterial interactions. None-volatile compounds (including nucleotides and free amino acids (FAAs)) and volatile organic compounds (VOCs) (secondary compounds generated from unsaturated fatty acids oxidation) cause to change of taste and odor in fish, respectively [3], which led to sensory rejection. A higher content of unsaturated fatty acids in seafood products cause to form more unsaturated volatile aldehydes, resulting in determining the specific aromas of flesh species. The decomposition of adenosine- 5'-triphosphate (ATP) with some enzymes is the most important factor in loss of freshness and quality of seafood and production of flavor nucleotides [4]. Free amino acids (FAAs) are precursors of volatile compounds for changing seafood odor. Also, FAAs can contribute to form important compounds

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to formation of taste in fish [5]. Few research have assessed in flavor compounds of fish during storage [6, 7]. Hence, research of amount in flavor compounds in sea bass fish during storage is useful in order to estimating of shelf life. Addition to, trimethylamine nitrogen (TMA-N) is derived from trimethylamine N-oxide (TMAO) by activity of foodborne bacteria and auto-enzymatic activity, which cause flavors loss in fish [8]. Generally, the FAAs and 5'-nucleotides were one of the main reasons providing umami-sweet taste of seafood.

The application of biopolymers coatings and films is a new method to protect its quality [9–12]. The application of suitable preservation methods can protect the quality of seafood products against lipid oxidation and bacteriological activity [13]. Various biopolymers have been used for extension of shelf life of fish quality by forming a thin layer of edible material [14] by two methods including edible coatings and edible films [15]. Among carbohydrate polymers, carboxymethyl cellulose and chitosan are popular candidates to be used as active packaging materials for improving some properties, especially antioxidant and antibacterial properties, compared with those of single component based coatings [16–18]. Carboxymethyl cellulose (CMC), as a natural carbohydrate polymer, is derived from cellulose [19]. Based on the results of different researchers, CMC has some problems such as non-antibacterial activity and poor moisture barrier properties [20, 21]. Therefore, improvement of these properties can perform through cross-linking with other polymer chains in the polysaccharide matrix to the coatings [18, 20]. Chitosan (CH), as a polycationic polysaccharide, is obtained from alkaline deacetylation of chitin which can be extracted from shells of crustaceans, cell walls of fungi, and other biological materials [22]. CH is an antioxidant and antibacterial hosting matrix against food born pathogen and free radicals for seafood coating purpose [23]. Some published studies showed that the functional properties of CMC improve through its combination with chitosan [16, 24]. The positive efficacy of chitosan coatings on improving sensory and maintain flavor quality were also observed in grass carp by Guan et al.[7]. Sekhon et al.[25] identified that plant extracts can retard of generation of off-flavor compound in hairtail fish balls. The influence of biopolymers blend on the reduction of flavor compounds in seafood have not been reported. Therefore, the aim of this study was the influence of edible CH and CMC coatings and film on the flavor composition and sensory analysis of Asian sea bass (*Lates calcarifer*).

Material and methods

Materials

Medium molecular weight chitosan ($\geq 92.0\%$ deacetylation) was purchased from the Tahrakaran Bahadorr Pars, Shiraz, Iran. Glycerol was obtained from the Duksan Pure Chemical

Co. (Sungkok-Dong, Korea). Carboxymethyl cellulose (medium viscosity) were purchased from the Sigma Company. All other chemicals were of analytical grade or of the highest grade available.

Preparation and treatment of fish samples

Asian sea bass (*Lates calcarifer*) (the average weight and size: 302 g; 45 cm) was freshly caught from Choebdeh farms in Abadan city, Khozestan province, Iran. The fish was immediately transported to the seafood processing laboratory and then stunned, gutted, peeled, washed with sterile water and filleted. Seven different treatments were used: 1) C: Control; 2) composite coating (CC) (mixture of chitosan (Medium molecular weight chitosan ($\geq 92.0\%$ deacetylation, Sigma Chemical Company)) and carboxymethyl cellulose (CMC) (Karen Pharmaceutical Company) solutions were prepared for immersing fillets for 30 s and then allowed to stand for a 2 min period followed by immersing again in the solution for 30 s); 3) bi-layer coating (BC) (chitosan solution were prepared for immersing fillets for 30 s and then allowed to stand for a 2 min period followed by immersing in the CMC solution for 30 s); 4) Bi-layer films (BF) (CMC solution (60 mL) were poured onto surface of rectangular plates; and after dried, chitosan solution (40 mL) were added on to surface of CMC film as second layer); 5) composite film (CF) (the solution of chitosan (40 mL) and CMC (60 mL) was blend and then poured on the surface of rectangular plates). For application of film for wrapping, the films were peeled off by hand from the plates after evaporation.

For formation of edible coating, the fillets were taken out from the solution and allowed to drain at 4 °C for 1 h. The samples packed in sterile polyethylene box and stored in a refrigerator for 16 days. Sensory evaluation, TMA-N content, TVC, FAAs, volatile organic compounds, ATP related compounds, and K value analysis were performed at 4-day intervals to assess the sea bass quality.

Sensory analysis

Changes in sensory attributes, including odor, color, and appearance, were measured by descriptive hedonic scale with 5 scores (5 = like extremely and 1 = dislike extremely). Sensory evaluation of samples were performed by a 12 panelists (7 male and 5 female, aged 30 and 33). Panelists had previously been trained according to ISO 8586 [26], experienced in fish freshness evaluation carried out the sensory analysis. Research activities supported by the Framework Programme should respect fundamental ethical principles, including those reflected in the Charter of Fundamental Rights of the European Union and take into account opinions of the European Group on Ethics in Science and New Technologies (EGE).

Determination of TMA-N and TVC

TMA-N muscle was measured according to the procedure proposed by AOAC [27]. For counting of bacteria, the pour plate method was used in a plate count agar (PCA) to count bacteria (total viable counts (TVC)) that were incubated at 37 °C for 24 h.

Determination of FAAs

Homogenization of 1 g of the specimen was performed in ten percent sulfosalicylic acid solution for 1 min. By centrifuging at 4 °C for 15 min at 13,000×g, filtering the supernatant was performed within filters (0.22- μ m) for measuring FAA utilizing an automatic amino acid analyzer.

Measurement of volatile compounds

To extract the fish volatile compounds, a solid-phase manual headspace microextraction tool was used within a fiber (50/30 μ m divinyl benzene-carboxy-polydimethylsiloxane), and a gas chromatography/mass spectrometer was used for analysis. Equilibration of the specimens was performed in headspace vials for 20 min at 60 °C, after exposure to Headspace-solid phase microextraction (HS-SPME) at the same temperatures. For 35 min, absorption of the volatile profile was considered in the headspace over to SPME fibers. In the splitless injector port, HS-SPME fibers were desorbed thermally for 5 min at the temperature of 250 °C. Using a Rxi-1MS capillary column, the compounds were separated in the extract. The carrier gas was helium (purity of more than 99.995%) at 1 mL/min flow rate. Initially, for 3 min the temperature of column was 35 °C, it was raised to 200 °C (the 10 °C/min rate), then to 260 °C (a 20 °C/min rate) after maintaining for 11 min until the program ends. The mass spectrometer's operation was in the electron effect mode with an electron energy of 70 eV and a source temperature of 230 °C. The spectral attainment was conducted in scanning state (m/z 30-500 amu scan range). A NIST search was performed to identify and quantify the volatile compounds utilizing the 2,4,6-trimethylpyridine internal standard.

ATP-related compounds analysis

The ATP-related compounds were extracted and determined based on the former technique with some modifications. By homogenizing the thawed specimens (2 g) 7.5 mL of pre-cooled 6% perchloric acid solution (v/v), then centrifuging was performed for 5 min (4 °C, 10,000 g). At the same conditions, extraction of the precipitate was reperformed. Collecting the supernatants, they were neutralized immediately by KOH solutions (1 and 10 mol L⁻¹) within the 6.5–6.8 ultimate pH ranges, after centrifuging for 5 min (4 °C,

3,000 g). The acquired supernatant was treated to 25 mL with cold distilled water, then to analyze the ATP-related compounds, filtering was performed through a 0.22- μ m filter membrane utilizing an HPLC armed with a Waters C18 column and a PDA detector. The isocratic elution process was employed with a mobile phase of 98% potassium phosphate buffer (0.05 M, pH 6.8) combined with 2% methanol. The detection wavelength and flow rates were respectively 254 nm and 1.0 mL min⁻¹. The ATP-based compounds were determined based on the standards peak area and retention time such as ADP, AMP, ATP, IMP, HxR, and Hx.

Statistical analysis

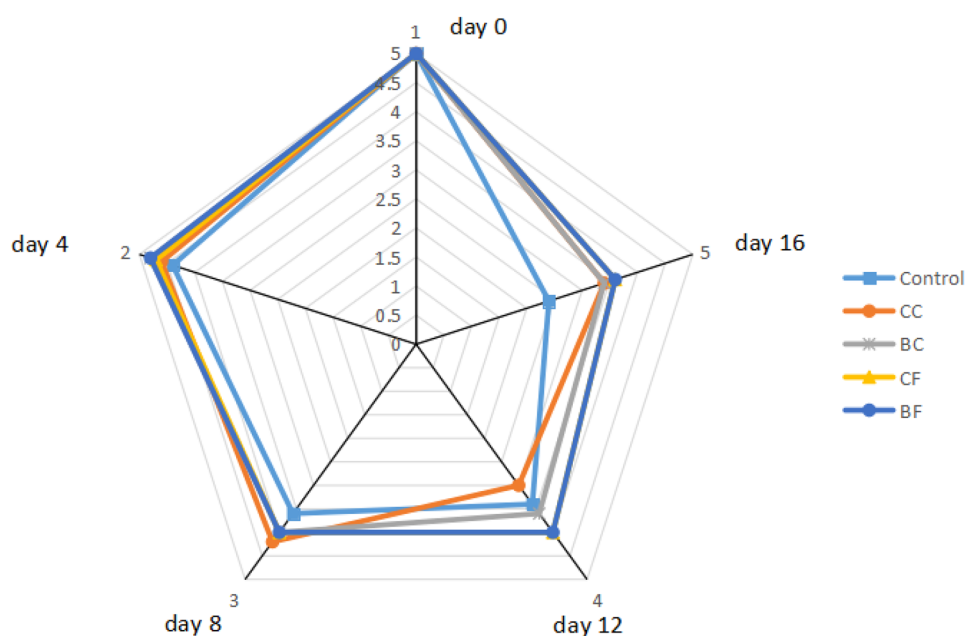
Statistical analysis was performed with SPSS software. The one-way analysis of variance (ANOVA) was used to compare the mean, and then Duncan's multiple range test was a statistical test for detecting significant differences.

Results and discussion

Sensory evaluation

Figure 1 shows the results of total acceptance scores of control samples and treated samples during storage. Total acceptance of fish defined with color, odor, and overall appearance. Sensory scores ranged from 1 to 5. At the beginning of storage, sensory scores in all samples was 5, indicating high-quality. Overall quality of fish with score less than 3 defined with signs including putrid odor, no shiny color, and overall unacceptability, which led to unacceptable for human consumption. The time of sensory rejection of control sample was observed after 8 days of storage, and then this sample was totally unacceptable after 16 days with generation of ammonia and fishy odors. According to Olatunde et al. [28], sensory scores of Asian sea bass decreased during storage. Sensory scores of the treated samples were significantly lower than that of the control samples. The reason of sensory deterioration may be caused by increased TMA-N content, nucleotide metabolism, bacterial spoilage, and lipid oxidation, resulting to produce off-odor and off-taste compounds [28, 29]. Sensory attributes of treated samples with composite coating (CC) and bilayer coating (BC) with behave similarly during the storage period, without great changes. Composite film (CF) or bi-layer film (BF) fillets containing CH and CMC is more effective than CC and BC in controlling sensory scores throughout the storage time, suggesting that antioxidant activity, antibacterial activity, and barrier properties of CH-CMC film. It can be concluded, film is more effective than coating in controlling sensory attributes of fillets. Extended inhibition of the TVC and lipid

Fig. 1 Changes in sensory evaluation of Asian sea bass fillets during refrigerated storage. (Control=uncoated samples; CC=composite coating, BC=bilayer coating, CF=composite film; BF=bilayer film)



oxidation by wrapped samples might be prevented the off-odor development. Yu et al.[30] observed that raw grass carp treated with chitosan coatings had increasing acceptability than the control samples during storage at refrigerator. The results suggested that Asian sea bass fillets treated by CH-CMC could be successfully extended shelf life (moderately fresh) at the end of storage and to delay the loss of freshness.

Changes in TMA-N and TVC

Trimethylamine nitrogen (TMA-N) is one of the most important primary compounds to evaluate the off-odor and off-flavor in spoiled fish [30]. Changes in TMA-N value of Asian sea bass during storage for 16 days are shown in Fig. 2A. The initial TMA-N contents in all groups was 0.81 mg N/100 g of flesh, which indicated fish freshness. TMA-N content of Asian sea bass fillets was increased gradually during the entire storage time ($P < 0.05$) for both control and treated samples by endogenous enzymes activities and metabolic activity of spoilage microorganisms such as *Shewanella putrefaciens* and *Aeromonas* spp, which produced ammonia and primary, secondary, and tertiary amines [31, 32]. The final contents of TMA-N in control samples sharply increased to 7.21 mg N/100 g of flesh, indicating the rapid deterioration of sensory properties on untreated samples at the end of storage and causes consumer rejection. The TMA-N content of samples coated/or wrapped with the CH-CMC treated sample was consistent with decrease of fishy odor compounds because of antibacterial activity of them. This results was confirmed by Yu et al. [30]. The maximum acceptability level of TMA-N for sea bass are 5 mg N/100 g [33]. There was difference between composite and bilayer,

as well as between coating and film. The TMA-N value of Asian sea bass fillets treated with CH-CMC remained below 5 mg/100 g on the 16th day of storage.

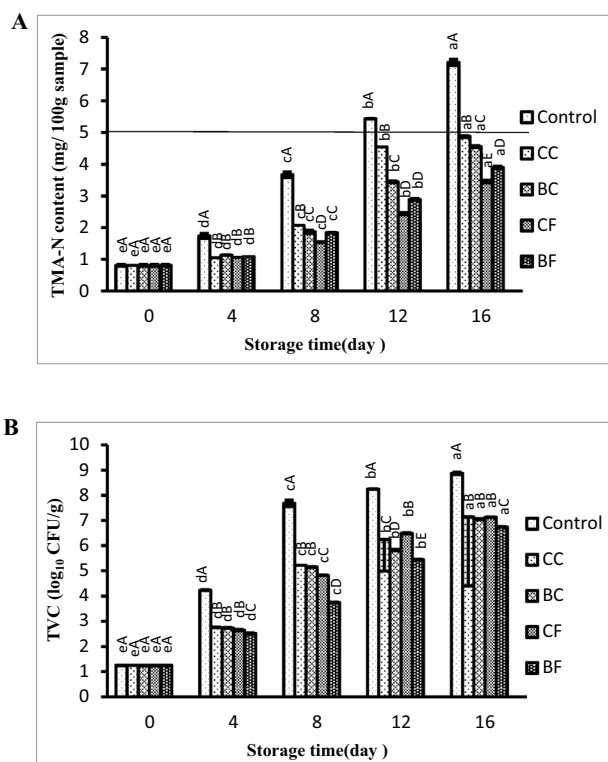


Fig. 2 Changes in TMA-V and TVC of Asian sea bass fillets during refrigerated storage. (Control=uncoated samples; CC=composite coating, BC=bilayer coating, CF=composite film; BF=bilayer film)

Table 1 Changes in FAAs content (mg/100 g) of control and coated Asian sea bass during storage at refrigerator. (Control = uncoated samples; CC = composite coating, BC = bilayer coating, CF = composite film; BF = bilayer film)

Storage time	groups	Lys	Ile	Glu	Asp	Ala	Tau	His	Gly	Arg	
Day 0		16.79±0.14	22.72±0.24	20.25±0.08	1.00±0.01	15.19±0.14	16.48±0.03	150.32±0.06	50.16±0.02	5.22±0.00	
Day 8	Control	40.88±0.57 ^a	42.10±0.42 ^a	24.82±0.29 ^a	5.56±0.18 ^a	19.83±0.17 ^a	42.17±0.02 ^a	184.96±0.36 ^a	70.30±0.06 ^b	15.23±0.01 ^a	
	CC	26.16±0.50 ^d	36.51±0.06 ^b	22.44±0.33 ^b	3.34±0.14 ^c	17.72±0.40 ^b	31.11±0.00 ^c	175.25±0.08 ^b	72.17±0.01 ^a	9.86±0.18 ^b	
	BC	25.43±1.10 ^d	35.43±0.10 ^{bc}	21.25±0.08 ^b	4.13±0.06 ^b	17.72±0.15 ^b	31.32±0.16 ^c	174.19±0.03 ^c	71.71±0.30 ^a	9.82±0.07 ^b	
	CF	36.29±0.66 ^b	35.35±0.38 ^{bc}	21.48±0.87 ^b	4.53±0.21 ^b	18.37±0.09 ^b	32.08±0.03 ^b	174.12±0.01 ^c	71.92±0.03 ^a	9.72±0.22 ^b	
Day 16	BF	34.07±0.03 ^c	34.32±1.24 ^c	21.86±0.31 ^b	4.53±0.01 ^b	18.08±0.17 ^b	32.04±0.02 ^b	175.36±0.06 ^b	72.08±0.03 ^a	10.02±0.00 ^b	
	Control	15.89±0.44 ^b	23.25±0.59 ^b	24.49±0.31 ^c	6.08±0.03 ^d	21.31±0.43 ^b	25.17±0.03 ^c	162.62±0.30 ^a	85.43±0.01 ^a	7.16±0.32 ^a	
	CC	21.24±0.66 ^a	29.29±0.29 ^a	25.95±0.38 ^{ab}	7.41±0.09 ^c	24.65±0.29 ^a	27.18±0.02 ^a	161.49±0.31 ^b	81.32±0.09 ^c	7.47±0.03 ^b	
	BC	20.78±0.66 ^a	29.02±0.55 ^a	27.02±0.27 ^a	8.29±0.10 ^b	24.68±0.09 ^a	27.12±0.01 ^a	160.46±0.32 ^c	81.92±0.85 ^c	7.02±0.02 ^b	
Taste attributes*	CF	20.05±0.29 ^a	29.76±0.18 ^a	25.23±0.57 ^{bc}	8.95±0.00 ^a	25.23±0.09 ^a	26.96±0.03 ^b	160.15±0.02 ^c	83.87±0.32 ^b	7.08±0.03 ^b	
	BF	20.25±0.08 ^a	29.68±1.14 ^a	27.11±0.06 ^a	8.30±0.07 ^b	25.50±0.49 ^a	26.95±0.09 ^b	159.89±0.33 ^c	82.14±0.01 ^c	6.59±0.08 ^b	
	Sweet/ Bitter (-)		Bitter (-)	Umami (+)	Umami (+)	Sweet (+)	Miscellaneous	Bitter (-)	Sweet (+)	Bitter/ Sweet (+)	
Storage time	groups	Ser	Thr	Phe	Val	Tyr	Asp	Pro	Leu	Met	Total
Day 0		3.26±0.03	3.10±0.01	0.34±0.00	4.21±0.00	2.12±0.00	1.05±0.00	3.02±0.00	3.21±0.00	4.01±0.00	322.51±0.17
Day 8	Control	8.30±0.05 ^a	10.00±0.00 ^a	2.02±0.00 ^c	7.23±0.00 ^a	8.05±0.01 ^a	3.08±0.03 ^d	6.03±0.00 ^c	4.08±0.02 ^d	6.02±0.00 ^e	500.70±1.78
	CC	5.17±0.03 ^b	6.57±0.04 ^b	3.33±0.16 ^{ab}	6.16±0.03 ^b	5.08±0.06 ^d	4.08±0.06 ^b	7.17±0.01 ^b	5.07±0.02 ^b	7.05±0.01 ^d	444.31±0.97
	BC	5.08±0.03 ^b	6.16±0.08 ^c	3.50±0.02 ^a	5.15±0.04 ^c	6.22±0.01 ^c	4.52±0.00 ^a	7.22±0.00 ^b	5.11±0.00 ^{ab}	7.12±0.01 ^c	441.12±1.28
	CF	5.06±0.01 ^b	6.06±0.02 ^c	3.20±0.04 ^b	5.47±0.00 ^d	6.43±0.10 ^b	3.87±0.05 ^c	7.61±0.03 ^a	5.16±0.00 ^a	7.23±0.01 ^b	453.99±0.66
Day 16	BF	5.07±0.00 ^b	6.18±0.03 ^c	3.37±0.06 ^{ab}	5.15±0.03 ^{ab}	6.54±0.01 ^b	4.14±0.00 ^b	7.13±0.19 ^b	4.95±0.03 ^c	7.42±0.00 ^a	452.35±1.49
	Control	12.18±0.04 ^a	8.84±0.16 ^a	2.93±0.28 ^b	8.12±0.01 ^a	13.65±0.18 ^a	4.08±0.03 ^d	7.16±0.03 ^d	4.46±0.07 ^d	8.05±0.00 ^e	430.17±1.08
	CC	7.23±0.00 ^b	8.13±0.04 ^b	4.55±0.00 ^a	7.85±0.16 ^b	12.13±0.03 ^b	4.53±0.01 ^c	8.16±0.01 ^b	6.22±0.00 ^b	8.50±0.02 ^b	453.17±1.34
	BC	7.07±0.02 ^b	7.75±0.36 ^b	4.16±0.04 ^a	7.13±0.03 ^c	9.39±0.07 ^c	6.03±0.00 ^a	8.30±0.05 ^b	6.41±0.00 ^a	8.49±0.02 ^b	451.11±0.16
Taste attributes*	CF	7.27±0.13 ^b	8.23±0.00 ^b	4.14±0.03 ^a	7.15±0.00 ^c	8.82±0.15 ^d	4.14±0.03 ^d	8.53±0.00 ^a	6.56±0.09 ^a	8.86±0.06 ^a	451.02±1.31
	BF	7.14±0.07 ^b	6.97±0.04 ^c	4.18±0.09 ^a	6.77±0.04 ^d	8.53±0.00 ^d	4.87±0.05 ^b	7.82±0.09 ^c	5.90±0.09 ^c	7.94±0.04 ^c	457.04±1.30
	Sweet (+)		Sweet (+)	Bitter (-)	Sweet/ Bitter (-)	Bitter (-)	Miscellaneous	Sweet/ Bitter (+)	Bitter (-)	Bitter/ Sweet/ sulfurous (-)	

All data were expressed as mean ± standard deviation. Mean values within the same day with different superscripts are significantly different (P < 0.05)

Abbreviations: Lys: Lysine; Ile: Isoleucine; Glu: Glutamic acid; Asp: Aspartic acid; Ala: Alanine; His: Histidine; Tau: Taurine; Gly: Glycine; Arg: Arginine; Ser: Serine; Thr: Threonine; Phe: Phenylalanine; Val: Valine; Tyr: Tyrosine; Asp: Asparagine; Pro: Proline; Leu: Leucine; Met: Methionine

*Taste attributes (+: pleasant, -: unpleasant) (Liu and Qin, 2016)

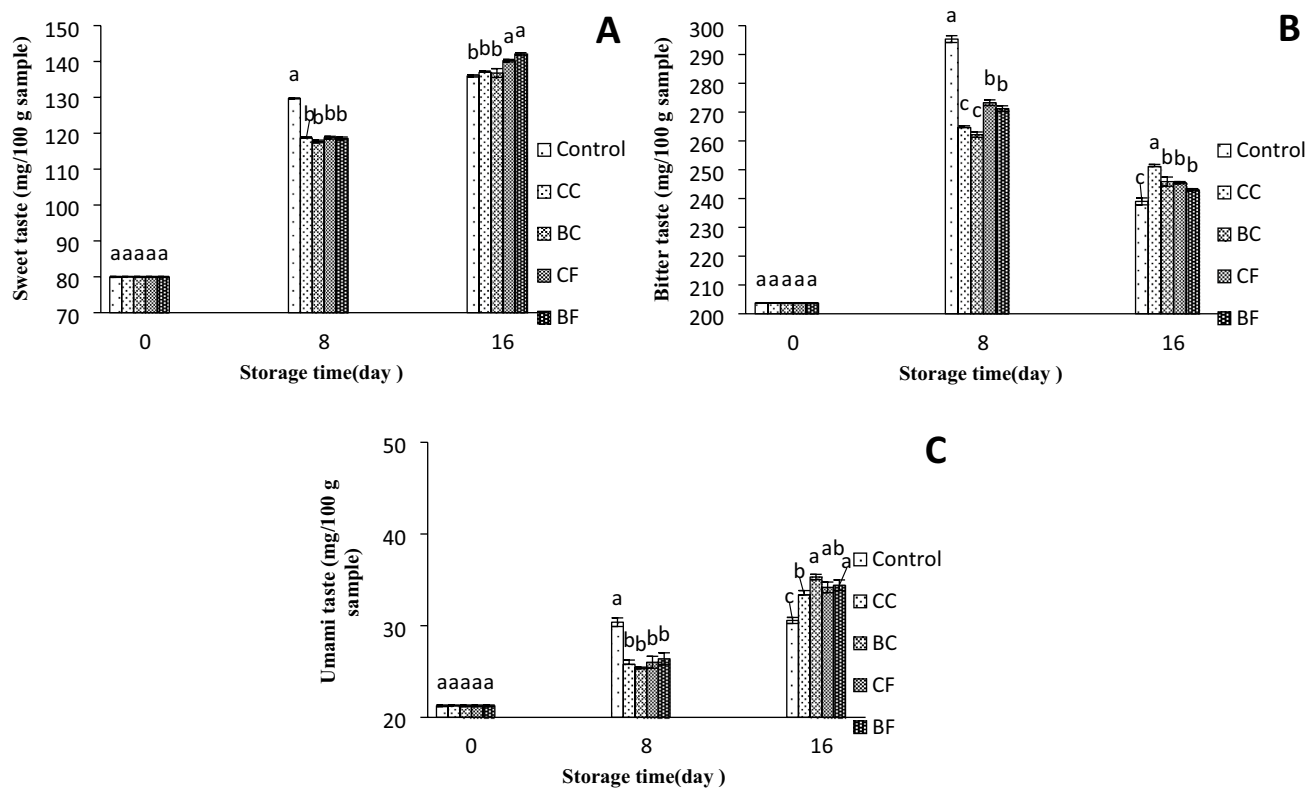


Fig. 3 Content of sweet amino acids (a), bitter amino acids (b), and umami amino acids (c) in control and treated samples. (Control = uncoated samples; CC = composite coating, BC = bilayer coating, CF = composite film; BF = bilayer film)

The coating and wrapped process efficiency of Asian sea bass fillet samples on the total viable counts (TVC) during storage at the refrigerator are shown in Fig. 2B. The initial TVC value in the Asian sea bass fillet were $1.21 \log_{10}$ CFU/g, indicates that the Asian sea bass was of higher quality. The TVC increased exponentially over storage time ($P < 0.05$). By the day 16 of storage, the TVC count in CC, BC, and CF became more than $7 \log_{10}$ CFU/g, which is higher than the maximally recommended limit in raw fish. BF did not achieve this count to the end of 16 days storage time. From the bacteriological point of view, control samples were acceptable up to 8-day storage. Treated samples (CC, BC, CF, and BF) led to a slower reduction in TVC in Asian sea bass compared to the control samples. There was no significant ($P < 0.05$) difference between the CC and BC, but there was significant different between the CF and BF. See Fig. 2.

Changes in free amino acids (FAAs)

Table 1 shows FAAs values of control and treated samples during storage at refrigerator. Eighteen FAAs were identified in Asian sea bass fillet. Histamin is a major FAAs in control and treated Asian sea bass, followed by Glycine, Isoleucine, Glutamic acid, and Taurine. In confirmation

of this result, Shiau et al. [34] reported that white-fleshed fishes have higher histidine value comparison with others. Although, Konosu et al. [35]; and Calanche et al. [36] observed that predominant FAA of white muscle fish was Taurine. The Histamin content of all groups ranged from 36.94% to 46.80% of total FAAs contents. Similar results were found in previous studies in sea bass [37]. However, Yu et al. [30] reported that histidine was high at raw grass carp. FAAs effect on taste preferences (such as umami, bitter, and sweet) to rejection or acceptance of seafood products [5]. In this study, some FAAs such as Gly, Ala, Ser, Pro, Arg, and Thr were contributor to sweet taste of foods. The presence of Asp and Glu were the most abundant free amino acids in seafood led to umami taste. Also, Val, Met, Ile, Leu, His, Phe, Lus, and Tyr imparted bitter taste. In addition, FAAs can cause non-enzymic *Maillard browning reaction in seafood led to change of color flesh, which produced some volatile flavor compounds* [38].

Histidine, lysine, arginine, alanine, taurine, and isoleucine of all samples increased up to day 8 ($P < 0.05$) and the abrupt reduction was obtained at day 16 (Table 1). Similar results in raw grass carp were reported by Yu et al. [30]. Rigor-resolution of fish fillets can decrease the shelf life of fish due to activity of proteolytic enzymes, which effect on degradation of proteins. The enzymatic degradation of

proteins was caused an increase of FAAs content of fillets during storage [39]. On the other hand, the reduction of total FAAs values of samples after 16 days storage at refrigerator could be due to a higher bacterial growth. Bacterial growth depend on water content of muscle and a source of energy such as glycogen, peptides, and free amino acids [36]. FAAs are precursors of biogenic amines due to microbial deamination and decarboxylation of amino acids [40] such as histamine produced from histidine by bacterial metabolism. This experiment displayed, among all of the samples, treated samples had higher total FAAs content due to the antibacterial activities of CH for Asian sea bass fillet, as it was seen for water loss and bacterial counts. A lower bacterial count in coated sea bass fillet was observed compared to control samples (Fig. 2B). Aspartic acid, alanine, glutamic acid, and glycine have positive impact on taste characteristics of seafood and provide umami-sweetness taste [41]. There was no significant different in glycine content of control and coated samples. Glutamic acid and aspartic acid values of treated samples was higher than control samples at the end of storage. All samples significantly increased the alanine content of fillets, but the lowest alanine content was belonging to control samples. The obtained contents of sweet and umami amino acids of all samples significantly increased at the end of storage while value of bitter amino acids of samples increased within first 8 days and then decreased significantly (Fig. 3). The bitter and sweet amino acids were most abundant in all samples. Highest contents of bitter and sweet amino acids were observed in CC and BF, respectively. Overall, this study showed that FAAs content of sea bass fillet changed in the sensory attributes, such as taste, odor, color. The bitter amino acids content of Asian sea bass were high, but total FAAs and some FAAs with sweet taste was decreased bitter tastes by edible chitosan-CMC-based coating and film.

Changes of volatile organic compounds in Asian sea bass

As shown in Table 2, there was 29 volatile organic compounds (VOCs) including aldehyde, ketones, and alcohols, with small amounts of hydrocarbons in both control and treated samples. Polyunsaturated acids (PUFAs) of fish contribute to generation of VOCs by the activity of lipoxygenase, also may be produced by undergo auto-oxidation [42]. However, lipoxygenase oxidation seems to be the most important cause of the formation of VOCs at fresh fish. Distinction between enzymatic oxidation and auto-oxidative is not clear, because both methods can cause to produce of these compounds [43]. Aldehydes, as secondary oxidation products, have low odor and flavor thresholds than ketones and alcohols [44]. According to Frankel [45], some aldehydes such as hexanal, (E)-2-hexenal, and 2,4-heptadienals,

are markers for lipid oxidation. It was found that the hexanal (that come from oxidation of n-6 fatty acids (linoleic acid)) was the most predominant compound compared with other compounds in aldehydes, for production of a greater intensity of fishy odor [46]. Furthermore, other compounds of aldehydes such as Heptanal, octanal, and (E)-2-decenal can also effect to generation of fishy odor [47]. Alcohols levels were generally higher in the samples, followed by aldehydes and ketones. Combination of volatile aldehydes with alcohol compounds cause to decrease smell/odor of freshwater fish [48]. In this study, higher abundances of alcohol belonged to 1-hexanol. Ketones and hydrocarbons have insignificant effect on the production of fishy odor due to their own high threshold. Similar results were reported by Zhou et al. [47]. The coated/wrapped samples markedly decreased the contents of ketones and hydrocarbons because of lower metabolic substances of *Pseudomonas* [49]. These results recommended that alcohols largely contributed to the odor and flavor of Asian sea bass. These results indicated that treated samples was effective to prevent the production of VOCs in Asian sea bass, probably due to biopolymers as antioxidant agents, which further delayed oxidative processes and retarded spoilage. The production of fishy odor related to the identified volatile compounds (aldehydes and alcohols), resulting lipid oxidation [46]. CMC can significantly decrease of the oxidation of lipid of fillets due to oxygen barrier properties [20]. However, CMC as biopolymers needs to improve of antioxidant properties [50]. CMC coatings have the potential to combine with other biopolymers. A low abundance of off-odor compounds in treated samples were probably attributed to the antibacterial effects of the active packaging. According to Parlpani et al. [49], a low spoilage bacteria count of treated samples was also caused to generation little VOCs, because of partial metabolism of these bacteria including *Pseudomonas* spp, *Shewanella* spp, and *Enterobacteriaceae*.

Changes in ATP related compounds and K value

Figure 4a, b, c, d, e, and f shows changes in concentration of ATP related compounds, including adenosine-5'-triphosphate (ATP), adenosine-5'-diphosphate (ADP), adenosine-5'-monophosphate (AMP), inosine-5'-monophosphate (IMP), inosine (HxR), and hypoxanthine (Hx) in Asian sea-bass fillets stored at refrigerator, respectively. The amount of adenine nucleotides and its breakdown products in the muscle of fish are reliable index of seafood freshness. At the beginning of the storage, ATP, ADP, and AMP contents in the all samples was 1.5, 0.74, and 1.61 $\mu\text{mol/g}$, respectively. The ATP, ADP, and AMP values of all samples decreased throughout storage, which is higher than control ($P < 0.05$). After death of fish, ATP, ADP, and AMP contents rapidly decreased due to activity of endogenous autolytic enzymes.

Table 2 Changes of volatile organic compounds in control and coated Asian sea bass during storage at refrigerator

Compounds	Day 0	Day 8					Day 16				
		Control	CC	BC	CF	BF	Control	CC	BC	CF	BF
<i>Aldehydes</i>											
3-methylbutanal	1.20	3.22	3.13	3.42	3.12	3.41	4.21	3.52	3.75	3.42	3.52
2-methylbutanal	–	–	–	–	–	–	0.11	0.16	–	–	–
Hexanal	5.22	8.23	7.23	6.23	6.12	6.13	21.23	17.23	17.10	17.03	16.01
(<i>E</i>)-2-hexanal	0.98	1.03	1.10	1.11	1.06	1.08	5.23	2.02	–	–	–
(<i>E,E</i>)-2,4-heptadienal	0.09	0.12	0.01	0.03	0.02	–	0.24	0.16	0.18	–	–
Heptanal	0.08	0.17	0.10	0.08	0.06	0.09	0.21	–	–	–	–
Octanal	0.01	0.20	0.15	0.16	–	–	0.42	–	–	–	–
Butanal	–	–	–	–	–	–	0.15	–	0.25	0.26	–
Nonanal	0.75	0.95	0.55	0.45	0.51	0.53	1.02	0.98	0.85	0.84	0.72
<i>Alcohols</i>											
Ethanol	0.81	3.04	1.06	1.52	1.85	1.74	4.52	2.26	2.74	2.41	2.32
1-pentanol	1.06	5.02	2.03	2.06	2.45	2.82	6.52	3.21	3.45	4.02	5.02
1-hexanol	8.12	18.23	12.23	11.02	12.06	12.41	13.23	12.02	11.02	11.23	11.52
2-butoxyethanol	0.37	1.12	–	–	–	–	3.21	0.89	0.75	0.65	0.64
1-heptanol	0.01	0.11	0.10	0.09	0.12	0.10	1.52	0.19	0.20	0.12	0.26
1-decanol	0.08	1.62	0.70	0.52	0.54	0.64	3.23	1.65	1.42	1.06	1.72
1-dodecanol	0.04	0.51	0.42	0.25	0.64	0.12	0.89	0.54	0.65	0.42	0.43
1-penten-3-ol	0.65	1.06	0.99	0.98	0.85	0.97	5.23	1.06	1.52	1.42	4.75
1-octen-3-ol	0.01	0.12	–	–	–	–	–	–	–	–	0.21
(<i>Z</i>)-2-pentenol	0.05	0.74	–	–	–	–	–	–	–	–	–
<i>Ketons</i>											
2,3-pentanedione	1.33	2.52	1.98	1.87	1.87	1.46	4.23	3.26	3.41	3.19	3.11
3-pentanone	0.07	0.32	–	–	–	–	0.52	–	–	–	–
Hydroxyl-2-butanone	1.11	2.32	1.52	1.85	1.98	1.78	5.23	3.23	3.12	3.75	3.96
2,3-octanedione	0.05	0.84	–	–	–	–	1.12	–	–	0.48	–
3-pentanone	0.01	0.16	0.18	0.21	0.24	0.20	3.52	2.06	2.08	1.98	1.75
6-methyl-5-heptan-2-one	0.08	0.28	0.15	0.19	0.18	0.16	0.84	0.21	0.23	0.28	0.34
<i>Hydrocarbons</i>											
Methylbenzene (toluene)	0.37	0.98	0.57	0.54	0.46	0.42	2.25	2.45	1.65	1.71	1.65
Ethylbenzene	0.07	2.42	2.25	2.42	2.12	2.03	5.12	3.29	3.03	3.41	3.15
Dimethylbenzene	0.16	0.98	0.52	0.42	0.62	0.98	2.55	1.98	1.66	1.52	1.21
Ethylbenzene	0.10	0.62	–	–	–	–	1.52	–	–	–	–

(Control: uncoated samples; CC: composite coating, BC: ilayer coating, CF: composite film; BF: bilayer film)

Treated groups indicated a significantly ($P < 0.05$) higher ATP, ADP, and AMP values than the control samples, which might be attributed to the reduction of activities of endogenous autolytic enzymes or bacterial activity. IMP is produced through decomposition of AMP by AMP-deaminase [51]. Among 5'-nucleotide, IMP is responsible for umami taste [6]. IMP was gradually decreased which caused to change to flavor of seafood and a loss freshness [52]. IMP with Glutamic acid was caused meaty-flavor. The initial IMP level of all samples was $9.24 \mu\text{mol/g}$. The amount of IMP was significantly ($P < 0.05$) decreased in all samples. IMP content of treated samples slowly decreased compared with control

samples because of slowly degraded to Inosine (HxR) and Hypoxanthin (Hx), indicating that treated samples decreased related enzymes activity. According to Itoh & Kimura [53], reduction of IMP can led to progressive loss of the desirable fresh seafood flavor. IMP content of treated samples dramatically *increases* and reached the maximal content at 16th day because of rapid metabolic rate and rapid consumption of ATP. Similar results were resulted by Shi et al. [39]. Therefore, this results indicated that biopolymers may result in variations in the accumulation of IMP in Asian sea bass fillets. The Hx can used to detection of freshness index in seafood when the population of spoilage bacteria should be

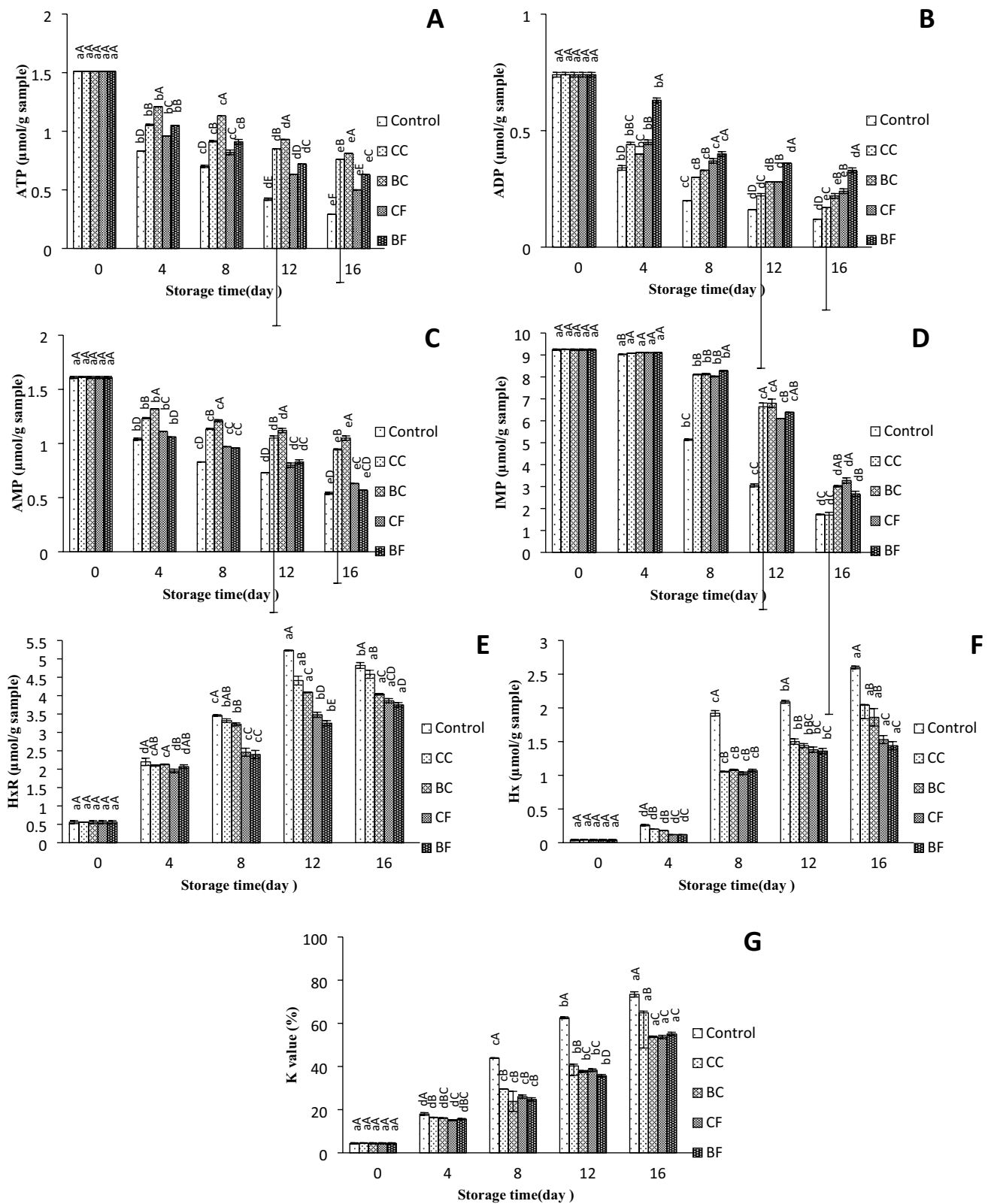


Fig. 4 Changes in ATP related compounds and K value of Asian sea bass fillets during refrigerated storage. (Control=uncoated samples; CC=composite coating, BC=bilayer coating, CF=composite film; BF=bilayer film)

less than 10^6 cfu/g [54]. Production of HxR and Hx cause to off-flavor in fish muscle led to decrease the freshness of fillets. Accumulation of Hx and HxR in fish fillets cause to produce a bitter taste [54]. Initial HxR and Hx levels samples was 0.56 and 0.04 μ mol/g. Hx levels in the treated samples decreased significantly, which was probably correlated to the reduction of autolytic enzyme activities (5-nucleotidase) and bacteria enzymes (inosine nucleotidase) produced by including *Pseudomonas* spp., *S. putrefaciens*, and *P. phosphoreum* [55]. Slowly increasing of Hx level of treated samples was consistent with antibacterial activity of chitosan, resulting to inhibit effectively the increase of bacterial count. This result showed that decomposition of IMP and production of Hx in fish muscle can lead to progressive loss of a desirable flavor and exhibit a bitter taste.

K value, as a freshness index of fish, was originally determined due to sum of concentration of ATP and its breakdown products.⁵⁴ K values of fresh, moderately, and spoilage fish are below 20%, between 20–60% and higher than 60%, respectively [56]. The initial K value of all samples was 4.36%, indicating Asian sea bass was fresh (Fig. 4g). The K value of treated samples was higher compared to control samples. This could be explained by the antibacterial properties of CH to minimize 5-nucleotidase activity, resulting inhibited breakdown of IMP. Similar study was observed by Li et al. [57], who indicated effect of chitosan coatings containing natural preservatives on the reduction of adenine nucleotides and their related compounds. Thus, this study indicated that application of polysaccharide or natural additives with inhibiting the growth of bacteria could provide to improve the shelf life of fish fillets. K value of control samples exceeded 60% at 12 days of storage period, which rejected acceptability of Asian sea bass fillets. By day 16 of storage, K value in Asian sea bass fillet became more than 60% for CC samples. K value of BC, CF, and BF samples did not reach the maximum acceptable level at 16 days of storage period. Therefore, it can be concluded that K value cannot be a suitable indicator to determine changes in nucleotide degradation products and shelf life of Asian sea bass fillets. Also, the results of study indicated that samples treated with BC, CF, and BF were effective in inhibiting the decomposition of ATP and preserving better seafood quality.

Conclusion

This study showed that, with using chitosan- carboxymethyl cellulose composite and bi-layer coating and film, flavor intensity decreased, to the point of undesirability for many consumers. Moreover, the umami, bitter, and sweet tastes of fillet depend on the free amino acids composition. In raw Asian sea bass, the predominant compounds in formation

off-flavor was bitter amino acids, alcohols, and Hx. The chitosan- carboxymethyl cellulose composite and bi-layer coating and film of Asian sea bass significantly decreased the amount of volatile profile and prevented fishy odor generation during entire storage time. Overall, the film was better than ones coating in reducing slowly FAAs, VOCs, and nucleotide metabolism of fillets at the end of storage.

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Declarations

Conflict of interest We wish to confirm that there are no known conflicts of interest associated with this publication. The authors declared that they have no conflicts of interest to this work.

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