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ORIGINAL PAPER

Effect of Chitosan Coating Enriched with Ergothioneine on Quality Changes of Japanese Sea Bass (*Lateolabrax japonicas*)

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Abstract The effect of chitosan–ergothioneine coating (CHER) on the post-mortem quality and shelf life of Japanese sea bass (*Lateolabrax japonicas*) stored at 4±1 °C for 16 days was investigated. Four different treatments were used: control without coating, CH with chitosan coating, ER with 0.3 % ergothioneine immersion, and CHER with chitosan containing 0.3 % ergothioneine coating. Sea bass pH value, total volatile basic nitrogen, peroxide value, thiobarbituric acid-reactive substances, biogenic amines, color, texture profile, and microbiological and sensory qualities were measured. The results indicate that treatment with CHER coating inhibited increase of total volatile basic nitrogen, peroxide value, and TBA value; maintained tissue hardness; and reduced microorganism counts, such as *Pseudomonas*, compared with control treatment. The efficiency was better than that of ER or CH treatment. Furthermore, sea bass treated with chitosan–ergothioneine coating also exhibited a positive effect, causing low biogenic amine content, especially putrescine, cadaverine, and histamine. Sensory evaluation proved the efficacy of chitosan–ergothioneine coating by maintaining the overall quality of sea bass during the storage period. Our study suggests that chitosan–ergothioneine coating might be a promising candidate for maintaining sea bass quality and extending their shelf life.

Keywords Chitosan · Ergothioneine · *Lateolabrax japonicas* · Biogenic amine · Microbiological quality · Refrigerated storage

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Introduction

Japanese sea bass (*Lateolabrax japonicas*), as an euryhaline fish species, is widely reared in China, Japan, Korea ,and Taiwan and is one of the most important marine fish cultured in China, the yield of sea bass reached 122,964 tons, accounting for 12.8 % in the total marine cultured fish of China (Hu et al. 2013). Sea bass has white flesh, mild taste, and low fat content, which made them popular around the world. However, raw fish are usually more perishable than other fresh products, and sea bass only have a short shelf life of 8 days under refrigerated storage (Boyd et al. 1992). The spoilage of raw fish is caused by endogenous enzymes and microbial activities, resulting in protein degradation, lipid oxidation, or decomposition (Bohme et al. 2011).

In recent years, development of edible coatings has been based on the use of polysaccharides, protein, lipids, or their combination in various ways. Edible coatings could be applied as a barrier to reduce the transport of moisture and gas, creating a micromodified atmosphere around products (Vargas et al. 2008). Chitosan is the second most naturally abundant polysaccharide existing mainly in shells of crab and shrimp. It is a cationic amino-polysaccharide which shows good biocompatibility, biodegradability, antibacterial and antifungal activity, membrane-forming capacity, and nontoxic nature (Alishahi and Aider 2012). It has been used to maintain the quality of seafood such as Pacific white shrimp (Huang et al. 2012), Pacific oysters (Cao et al. 2009), salmon (Sathivel 2005), and Atlantic cod (Jeon et al. 2002).

Ergothioneine is a naturally rare amino acid and a native membrane-impermeable thiol compound. Application of ergothioneine as a common food additive is a challenge of scientists as well as an expectation of processers. It can be detected in specialty fungus (such as *Claviceps purpurea* and *Flammulina velutipes*), kidney, liver, black and red beans, and oat bran and can scavenge singlet oxygen, hydroxyl radical,

hypochlorous acid, and peroxyl radicals (Ey et al. 2007). In addition, Song et al. (2010) have reported that ergothioneine could protect against neuronal injury induced by cisplatin, to thereby reduce the side effects of antitumor agent. Previous studies have been primarily focus on application of ergothioneine for the melanosis prevention in shrimp (Encarnacion et al. 2012) and crab (Encarnacion et al. 2011), or as a color stabilizer in fish meat (Bao et al. 2010) and beef (Bao et al. 2008), but not used as natural preservatives in raw whole fish and also never combined with chitosan to preserve seafood. Thus, this study was conducted to evaluate the effect of chitosan and ergothioneine, applied individually and/or in combination, on the physiochemical quality, biogenic amino content, microbiological, and sensory attributes of sea bass during refrigerated storage.

Materials and Methods

Preparation of Sample and Treatment

Live Japanese sea bass used in this study were obtained from a local aquatic market in Jinzhou, China. The fish was 26–28 cm long, with a weight of 600–700 g. The fish were transferred to the Seafood Processing Laboratory of Bohai University within 0.5 h, killed by slurry ice, and then kept at 0 °C until use. Food-grade chitosan (deacetylated degree 95 %) was purchased from Zhejiang Fuli Biological Technology Co., Ltd. (Zhejiang, China). L-(+)-Ergothioneine of the genus *C. purpurea* was purchased from Sigma-Aldrich Co. (St. Louis, USA).

Chitosan solutions were obtained by dissolving the chitosan (2 %) and glycerol plasticizer (0.75 %) in acetic acid (1 %) for 1 h at room temperature with a magnetic stirrer to achieve complete dispersion. Fishes were divided into four groups of 15 each. Four different treatments were used: (1) control; (2) chitosan coating (CH); (3) ergothioneine immersion (ER) (0.3 %); and (4) chitosan-containing ergothioneine (0.3 %) coating (CHER). The fishes in the control treatment were immersed in distilled water for 10 min at 20 °C. Other fishes were dipped into the above coating solution for 10 min at 20 °C, respectively. The ratio of fish to immersing solution was maintained as closely as possible to one part by weight of fish to four of solution. After that, they were removed and permitted to drain for 0.5 h to form the stable coatings. The coating treatments were selected according to preliminary experiments in sea bass to assure adherence and steadiness of the coatings. Finally, fishes were packed in air-proof polypropylene pouches and stored at 4±1 °C for subsequent quality assessment. Each group repeated three times with three fishes at each sampling time. Biogenic amino content and microbiological and sensory attributes were performed at 4-day intervals to measure the quality of fish.

Chemical Analysis

pH Value

The values of pH were measured by mixing the fish samples (10 g) with 90 ml distilled water, and the mixture was stirred for 30 min. After being filtered, the pH values of the filtrate were measured using a digital pH meter (FE20, Mettler Toledo, Shanghai, China).

Total Volatile Basic Nitrogen (TVB-N)

The total volatile basic nitrogen values were determined with a Kjeltec 8400 (Foss, Sweden). The microdiffusion method was mensurated by distillation after adding MgO to the homogenized samples (Jeon et al. 2002). Total volatile basic nitrogen (TVB-N) values were expressed in milligrams nitrogen per 100 g samples.

Peroxide Value (PV)

The peroxide value was expressed as milliequivalents per kilogram fat, was determined by iodometric titration after an addition of acetic acid (A.O.C.S. 1994).

Thiobarbituric Acid-Reactive Substances (TBARS)

In this study, the thiobarbituric acid reactive substances of fish samples were evaluated by measuring the concentration of malonaldehyde (Botsoglou et al. 1994). Homogenized samples (200 mg) were absolutely dissolved in 25 ml of 1-butanol. Five milliliters of the solution were poured into a 20 ml stoppered test tube containing 5 ml of thiobarbituric reactive reagent. The mixture was incubated in the boiling water for 90 min followed by an ice bath for 10 min before measurement. The absorbance was read at 532 nm using a UV-Vis spectrophotometer.

Biogenic Amine Analysis

Biogenic amine analysis was done using the method of Hernandez-Jover et al. (1997). Fish muscle (5 g) was taken from the dorsal part of the fish fillet without skin and homogenized with 10 ml 0.6 M cold perchloric acid using a homogenizer (PRO200, Pro Scientific Inc., Oxford, USA) for 1 min, centrifuged at 10,000 rpm for 10 min at 4 °C, and filtered through Whatman no. 1 filter paper. The residue was extracted repeatedly, and the filtrates were combined and brought to 25 ml with 0.6 M perchloric acid. For analysis, 0.2 ml was taken to centrifuge tubes, and 40 μl of 2 M NaOH was added, followed by 60 μl saturated $NaHCO_3$, and mixed on a vortex mixer for 1 min. The 0.4 ml of 10 mg/mL dansyl chloride (DNS-Cl) solution prepared in acetone was added,

and the reaction solution was left at 40 °C for 30 min in darkness. The residual DNS-Cl was removed by adding 20 μl ammonia (25 %), and after 30 min, the mixture was adjusted to 1 ml with acetonitrile, centrifuged at 5,000 rpm for 5 min. The supernatant was filtered through 0.22 μm filters prior to HPLC analysis.

For the biogenic amine analyses, an Agilent reverse-phase column (C18, 5 μm, 4.6×250 mm) was used. Ammonium acetate and acetonitrile were used as mobile phases. The flow rate was 0.8 ml/min, and the temperature was 30 °C. The injection volume was 10 μl, and the detection was monitored at 254 nm.

Color Measurement

The surface color of sea bass fillets was measured with a WSC-S colorimeter (Shanghai Precision Instrument Co. Ltd., Shanghai, China). To analyze the L^* (black/white), a^* (red/green), and b^* (yellow/blue) values, three measurements were taken for each fillet. The color intensity is expressed by a chroma value (C^*ab), while hue (H^0ab) represents the purity of color, and these were respectively calculated according to the formula: $C^*ab=(a^{*2}+b^{*2})^{1/2}$ and $H^0ab=\arctan(a^*/b^*)$.

Texture Profile Analysis

The texture properties of fish samples were evaluated at room temperature using a TA-XT plus texture analyzer (Stable Micro Systems Ltd., Godalming, UK) equipped with a 5-mm diameter cylindrical probe (P/5). Texture profile analysis (TPA) was performed using the dorsal muscle above the lateral line of fish (1.5 cm×1.5 cm×1.0 cm) which were compressed twice to 75 % of the original height. The speed of probe was 2 mm s^{-1} during penetration. The parameters (hardness, cohesiveness, adhesiveness, springiness, chewiness, gumminess, resilience) were calculated based on definitions of Bourne (2002).

Microbiological Analysis

All samples were analyzed for mesophilic bacteria, pseudomonad, enterobacteria, lactic acid bacteria, and yeasts counts. The samples were homogenized for 2 min with a BagMixer (Model 400, Interscience, France). Aerobic counts were determined on plate count agar (PCA, Aoboxing Bio-Tech, Beijing, China) by counting the number of colony-forming units after incubation at 35 °C for 48 h and expressed as $\log_{10}$CFU/g. Pseudomonads was counted on cephaloridin fucidin cetrimide agar (CFC, Aoboxing Bio-Tech, Beijing, China) and incubated at 30 °C for 48 h. Enterobacteria was enumerated on violet red bile glucose agar (VRBGA, Aoboxing Bio-Tech, Beijing, China) and incubated at 30 °C for 24 h. Lactic acid bacteria and yeasts were counted on de Man Rogosa Sharpe agar (MRS, Aoboxing Bio-Tech, Beijing, China) and Potato Dextrosa agar (PDA, Aoboxing Bio-Tech, Beijing, China), respectively. The incubation conditions were 37 °C for 3 days and 28 °C for 5 days, respectively.

Sensory Evaluation

On each day of sampling, Japanese sea bass with different treatments were decapitated, scaled, and eviscerated. The samples for sensory evaluation were prepared by steaming for 10–20 min at 98 °C. Salt (1.5 %) was added. After cooking, fish were cooled rapidly in ice water and drained on the stainless sieve for 5 min at 4 °C. The samples were placed on a stainless steel tray and covered with an aluminum foil before assessment. The sensory attributes based on odor and taste was evaluated by a sensory panel of five trained assessors, who were acquainted with fish consumption and had no allergies to fish meats. For scoring, nine-point hedonic scale was used to evaluate samples, where 9=like extremely to 1=dislike extremely, a sensory score of 4 was taken as the borderline of acceptability (Ojagh et al. 2010). Samples were presented in plates coded with random three-digit numbers.

Statistical Analysis

The experiment followed a completely randomized design ($n=3$). Dates were subjected to one-way analysis of variance. Mean separations were performed by Duncan's multiple range test (SAS version 8.1). Differences at $p<0.05$ were considered significant.

Results and Discussion

Effect of Chitosan Coating Enriched with Ergothioneine on Chemical Properties

Changes in the pH value of Japanese sea bass over storage time are shown in Fig. 1a. The pH value of all treatments gradually decreased in the first 8 days. No significant differences were observed between control and ER samples, but lower pH value was found in CH and CHER samples. The initial reduction in pH value may be the joint results of pH value of immersion solutions and the formation of lactic acid from glycogen in fish meat (Li et al. 2012). The pH value of control sea bass increased after 8 days of storage while CH and CHER sea bass experienced a slight increase during the same period. The lowest levels of pH value were recorded in CH and CHER samples at the end of storage. The rising pH value later during storage was due to accumulation of alkaline compounds, such as ammonia and trimethylamine mainly derived from microbial action during fish muscle spoilage

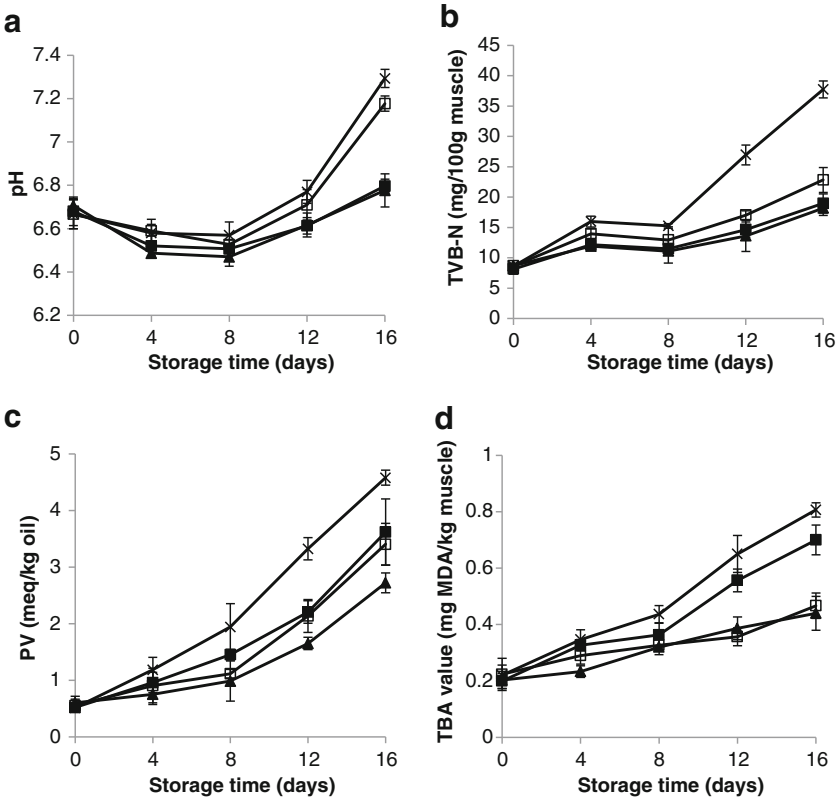

Fig. 1 Changes in pH value (**a**), TVB-N (**b**), peroxide value (**c**), and TBARS (**d**) of Japanese sea bass treated with control (*multiplication sign*), CH (*black square*), ER (*white square*), and CHER (*black triangle*) stored at 4 °C for 16 days. Each data point is the mean of three replicate samples. *Vertical bars* represent standard deviation of means

(Jeon et al. 2002). Our results are similar with Lu et al. (2012), who showed the same change trend in pH value of Japanese sea bass across the storage. However, other studies have reported the continuously rising pH value in sardine under the icing with rosemary extract treatment during chilled storage (Ozyurt et al. 2012).

The TVB-N value throughout the storage time in the control and coated sea bass is shown in Fig. 1b. The highest TVB-N value was observed in the control samples; it reached 37.7 mg N/100 g flesh at the end of storage, followed by 26.9 mg N/100 g flesh at the 12th day of storage. From the viewpoint of Gimenez et al. (2002), a level of more than 25 mg N/100 g flesh was considered as an unacceptable value in fish and fishery products. For all of the coated sea bass, the TVB-N was less than 25 mg N/100 g flesh, which indicates that the coated fish maintained freshness during storage. Both control and coated fish showed an increasing trend in TVB-N during the initial 4 days, then slightly decreased in the following 4 days, and again increased towards the end of storage. For up to 16 days of storage, the TVB-N in the CHER sample was slightly lower than that in the CH and ER fishes. Nevertheless, there was no significant difference between the CHER and CH samples ($p > 0.05$). In this study, the CHER coating significantly reduced the TVB-N of sea bass as compared with control sample and retard fish spoilage and quality deterioration. Similar superior effects of the edible coating treatments in TVB-N have been observed in other fishes (rainbow trout, sardine, large yellow croaker) (Ojagh et al. 2010; Ozyurt et al. 2012; Li et al. 2012). The relatively low TVB-N in the CHER sea bass could be due to the synergistic effect of chitosan coating combined with ergothioneine treatment.

Lipid deterioration is often one of the main causes of a short shelf life of fish and fish products. Lipid oxidation in fish depends on many factors such as the species, storage temperature, lipid content, etc. The lipid content of Japanese sea bass was about 1.42±0.13 g/100 g muscle, which was higher than that (0.45–0.65 g/100 g muscle) of haddock and cod. Aubourg and Medina (1999) reported that different lipid damage was detected between the fish species, showing higher lipid oxidation (PV and TBA value) and hydrolysis in haddock than in cod. Lipid oxidation primary products, as hydroperoxides, can be evaluated by peroxide value (PV). As shown in Fig. 1c, the PV value increased progressively throughout the storage reaching 3.62, 3.40, 2.72, and 4.58 meq kg^{-1} of lipids for CH, ER, CHER, and control samples, respectively. It is obvious that the PV trend did not reflect the decomposition of peroxides to secondary products that characterize the later phase of lipid oxidation. Bao et al. (2008) reported that ergothioneine in mushroom extract could effectively suppress lipid oxidation of big-eye tuna meat. Moreover, chitosan coating is useful in retarding the production of peroxides in rainbow trout fillets (Ojagh et al. 2010) and herring fillets (Jeon et al. 2002) stored in refrigeration conditions. In the present study, CHER treatment led to a significantly lower peroxide value than that in other

samples, due to a synergistic effect of CH and ER. However, there were no significant variations between CH and ER samples.

TBA value increased in all samples during the 16-day storage period (Fig. 1d). Although the rate of increase was initially generally constant in all the treatments, values increased at an accelerated rate between 8 and 16 days storage period in control and CH samples. A smaller acceleration was observed between 8 and 16 days storage period in ER and CHER samples. Total increase in TBA value in CH samples was 250 %. In contrast, relatively small increases in the TBA value of samples treated with ER were recorded. Values remained relatively steady throughout the storage and were 109 % only higher than initial values when the experiment was terminated. The results indicated that the chitosan was less effective in retarding the formation of malonaldehyde, compared with ergothioneine. Ergothioneine showed the radical scavenging activity via hydrogen donating and reducing power, thereby terminating the propagation (Ey et al. 2007). In the present study, also the TBA value was significantly lower ($p<0.05$) in samples coated with CHER than in samples coated with CH samples, but no significant difference was observed in CHER and ER samples. This can be attributed to either a more rapidly reduced free radical or decreased lipids oxidation (Encarnacion et al. 2012), which was due to the effect of ergothioneine on fish fillets.

Effect of Chitosan Coating Enriched with Ergothioneine on Biogenic Amine Contents

Biogenic amine content of fish can be used to evaluate the freshness, and extent of spoilage since biogenic amines are witnessed at low levels in fresh fish, and their presence is related to microbiological spoilage (Ozyurt et al. 2012). The type and amount of biogenic amine formed during storage depends on many factors, such as fish species, microbial flora, temperature, packaging, and use of antimicrobial agents. Table 1 shows the formation of biogenic amines in Japanese sea bass stored in different treatment conditions.

Spermine and spermidine were the two higher concentrations found in sea bass under different treatments during the initial storage, which is due to the presence of two amines as natural constituents of living cells. These two amines fluctuated throughout the whole storage period. Similar trend in both spermine and spermidine levels in sea bass is reported for some sea fish species including sardine (Ozyurt et al. 2012) and Mediterranean hake (Baixas-Nogueras et al. 2001). Histamine is the causative agent for fish poisoning, and putrescine and cadaverine potentiate the toxicity of histamine. The highest histamine concentration was observed in the control group (28.41±1.53 mg/kg), and a lower level of histamine in chitosan group (4.21±1.10 mg/kg) suggests that chitosan inhibits the growth of bacteria with histidine decarboxylase activity. Alak et al. (2011) investigated the effect of chitosan on biogenic amine formation in Atlantic bonito fillets stored at 4 °C. They found that chitosan have potentially inhibition effects on histamine, putrescine, and cadaverine accumulation in bonito muscle. Similarly, chitosan coating enriched with ergothioneine did significantly inhibit the formation of histamine in sea bass during storage period in the present study. These could be attributed that both chitosan and ergothioneine have antimicrobial and antioxidant properties. Similar to spermine and spermidine, tyramine changed unsteadily throughout the whole storage. Tryptamine was not detected in the initial 8 days, and no significant difference was observed in tryptamine between the treatments stored at 4 °C from day 12 to day 16 ($p>0.05$), which can be attributed to the fact that a low storage temperature can inhibit the formation of tryptamine. Phenylethylamine was not found during the storage of sea bass at refrigerated temperature (4 °C).

Effect of Chitosan Coating Enriched with Ergothioneine on Color

The appearance of food products is of major importance to consumers, both from the point of view of acceptability and preference. Therefore, color plays a decisive role when evaluating the quality of the product at the point of sale. The color is influenced by both muscle structure characteristics and pigment concentrations (Gines et al. 2004). Different values obtained after application of CHER, compared with the control treatment, are shown in Table 2. From this table, no significant difference in L^* value was observed in CHER samples compared with CH or ER samples from day 0 to day 12. On day 16, there was no significant difference in L^* value between ER and CHER samples, but CHER sample was higher than CH sample ($p<0.05$), it is mainly attributed to the inhibition of ergothioneine on postharvest melanosis formation in fish products and also the synergistic effect of CH combined with ER treatment (Encarnacion et al. 2011). The value of b^* showed a significant correlation with lipid oxidation development, reflecting an evolution toward grey-blue tones as the muscle aged. Similar result was obtained in the previous research, in which the increased b^* value was observed stored at 0 °C for 16 days (Li et al. 2011). C^*ab significantly decreased with days of storage, indicating a reduction in color intensity. Regarding coordinates a^* and H^0ab, no significant correlations were observed over the period of storage in the present study.

Effect of Chitosan Coating Enriched with Ergothioneine on Texture

TPA was carried out to determine the effect of chitosan combined with ergothioneine treatment and storage for 16 days on the texture of fish muscle. The results of texture

Table 1 Changes in biogenic amines concentration (milligrams per kilogramg muscle) of Japanese sea bass coated with chitosan+ergothioneine stored at 4 °C for 16 days

BAs	Treatment	Days of storage				
		0	4	8	12	16
SPM	Control	21.40±0.37 ab	27.32±0.83 c	25.46±1.22 b	16.74±1.32 b	11.45±0.86 c
	CH	20.53±1.16 b	29.10±1.45 c	28.32±1.43 a	22.30±0.30 a	16.23±1.36 a
	ER	20.82±0.87 b	31.53±1.37 b	24.67±0.58 b	18.09±0.39 b	11.34±0.43 c
	CHER	22.56±0.42 a	34.63±0.73 a	23.06±1.84 b	14.23±0.74 c	13.47±0.37 b
SPD	Control	11.18±0.43 a	13.75±0.31 b	17.32±0.73 b	17.04±0.15 a	10.15±0.12 a
	CH	11.51±0.75 a	15.23±0.83 a	16.34±0.14 b	12.73±0.47 b	9.04±0.17 b
	ER	11.64±0.38 a	15.28±0.45 a	19.10±1.17 a	11.14±0.38 c	6.97±0.46 c
	CHER	11.47±0.42 a	14.28±0.57 ab	19.45±0.40 a	12.51±0.26 b	8.41±0.55 b
PUT	Control	0.40±0.07 a	4.76±0.46 a	15.39±0.73 a	23.80±1.14 a	37.23±2.14 a
	CH	0.48±0.12 a	2.14±0.13 c	5.36±0.07 c	12.67±0.78 b	17.42±0.74 bc
	ER	0.43±0.04 a	2.73±0.11 b	6.38±0.24 b	11.63±0.32 b	18.53±1.29 b
	CHER	0.51±0.04 a	2.43±0.08 bc	5.94±0.33 bc	11.45±0.20 b	15.13±0.54 c
CAD	Control	ND	1.48±0.16 a	4.60±0.74 a	9.15±2.15 a	14.70±1.79 a
	CH	ND	0.34±0.05 b	3.63±0.80 a	7.35±1.47 a	11.14±1.32 b
	ER	ND	0.37±0.08 b	4.34±1.13 a	7.41±0.89 a	12.45±1.64 ab
	CHER	ND	0.41±0.04 b	3.48±0.41 a	7.28±1.36 a	10.30±1.37 b
HIM	Control	ND	1.25±0.14	9.74±0.75 a	15.46±2.31 a	28.41±1.53 a
	CH	ND	ND	1.37±0.15 b	2.79±0.90 b	4.21±1.10 b
	ER	ND	ND	1.64±0.08 b	2.84±0.63 b	5.40±0.89 b
	CHER	ND	ND	1.13±0.04 b	2.17±0.74 b	3.52±0.71 b
TRM	Control	ND	ND	0.22±0.03 c	0.64±0.02 a	1.38±0.13 a
	CH	ND	ND	0.28±0.01 a	0.63±0.07 a	1.32±0.04 a
	ER	ND	ND	0.27±0.02 ab	0.58±0.12 a	1.34±0.07 a
	CHER	ND	ND	0.24±0.02 bc	0.56±0.04 a	1.30±0.12 a
2-PHE	Control	ND	ND	ND	ND	ND
	CH	ND	ND	ND	ND	ND
	ER	ND	ND	ND	ND	ND
	CHER	ND	ND	ND	ND	ND
TYM	Control	1.34±0.13 a	0.84±0.11 a	0.57±0.03 c	0.72±0.11 ab	1.12±0.21 ab
	CH	1.20±0.08 a	0.63±0.04 b	0.89±0.04 b	0.63±0.07 b	1.24±0.15 a
	ER	0.74±0.04 b	0.87±0.09 a	1.33±0.14 a	0.81±0.09 a	0.86±0.19 bc
	CHER	1.28±0.10 a	0.54±0.03 b	0.47±0.02 c	0.75±0.07 ab	0.79±0.08 c

Values are the mean of three replications±standard deviation. Means in same column with different letters are significantly different ($p<0.05$)

SPM spermine, *SPD* spermidine, *PUT* putrescine, *CAD* cadaverine, *HIM* histamine, *TRM* tryptamine, *2-PHE* 2-phenylethylamine, *TYM* tyramine, *ND* not detected

measurements (hardness, gumminess, chewiness) are summarized in Table 3. The control sea bass had the fastest softening rate, losing about 59.1 % of their hardness in about 16 days. The hardness of sea bass treated with CH, ER, or CHER also decreased but to a lesser extent. CHER, CH, or ER treatment exhibits significantly higher hardness than that in the control sample at the end of storage, but there were no significant differences among CH, ER, and CHER treatments. The average values for gumminess and chewiness displayed significant day-to-day changes with the storage time. The death of fish brings about autolysis, which resulted in muscle soften and less elastic, and the process subsequently accelerated by microbial activity. In the present study, CH, ER, or CHER treatment has the ability to slow down the loss of hardness, gumminess, and chewiness by inhibiting microbial activity. Feng et al. (2012) found that texture

Table 2 Changes in color of Japanese sea bass coated with chitosan+ergothioneine stored at 4 °C for 16 days

Treatments	*L**	*a**	*b**	*C*ab*	H^0ab
0 days					
Control	57.23±1.32 a	−2.42±0.38 a	−3.56±0.47 a	4.30±0.15 a	0.60±0.05 a
CH	56.71±1.63 a	−1.72±0.24 bc	−3.79±0.15 a	4.16±0.07 a	0.43±0.14 bc
ER	57.21±0.87 a	−1.30±0.29 c	−3.20±0.68 a	3.45±0.43 b	0.39±0.03 c
CHER	57.14±1.20 a	−2.18±0.45 ab	−3.40±0.40 a	4.04±0.30 a	0.57±0.06 ab
4 days					
Control	54.48±1.79 a	−1.85±0.21 a	−2.78±0.05 a	3.34±0.24 b	0.59±0.07 a
CH	54.69±1.21 a	−1.16±0.43 b	−2.54±0.73 a	2.79±0.11 c	0.43±0.04 a
ER	55.16±2.04 a	−1.79±0.15 ab	−3.07±0.21 a	3.55±0.15 ab	0.53±0.14 a
CHER	55.75±1.37 a	−2.15±0.46 a	−3.13±0.38 a	3.80±0.05 a	0.60±0.11 a
8 days					
Control	52.13±1.55 a	−1.47±0.19 b	−1.96±0.52 a	2.45±0.25 b	0.64±0.14 b
CH	53.43±0.39 a	−0.75±0.14 c	−2.15±0.19 a	2.28±0.42 b	0.34±0.06 c
ER	54.15±1.75 a	−2.64±0.51 a	−1.68±0.23 a	3.13±0.10 a	1.00±0.12 a
CHER	54.04±2.19 a	−1.69±0.24 b	−2.17±0.76 a	2.75±0.21 ab	0.66±0.02 b
12 days					
Control	50.63±2.31 a	−2.16±0.21 a	−1.54±0.42 ab	2.65±0.46 a	0.95±0.08 a
CH	51.37±1.54 a	−1.38±0.11 b	−1.73±0.12 ab	2.21±0.31 a	0.67±0.10 ab
ER	53.35±1.20 a	−1.73±0.32 ab	−1.30±0.30 b	2.16±0.15 a	0.93±0.21 a
CHER	53.46±1.17 a	−1.27±0.25 b	−1.89±0.17 a	2.28±0.12 a	0.59±0.17 b
16 days					
Control	48.27±1.05 c	−1.14±0.16 a	−0.76±0.24 a	1.42±0.14 a	1.01±0.25 a
CH	49.57±0.72 bc	−1.58±0.31 a	−0.91±0.17 a	1.82±0.04 a	1.05±0.19 a
ER	51.08±0.54ab	−1.30±0.21 a	−0.82±0.19 a	1.54±0.36 a	1.01±0.11 a
CHER	52.30±1.78 a	−1.26±0.22 a	−1.10±0.21 a	1.67±0.27 a	0.85±0.06 a

Values are the mean of three replications±standard deviation. Means in same column with different letters are significantly different ($p<0.05$)

CH chitosan, *ER* ergothioneine, *CHER* chitosan+ergothioneine, *C*ab* chroma value, H^0ab hue value

properties (especially hardness, gumminess, and chewiness) of black sea bream was closely related with microbial activity under refrigeration condition. In our study, texture changes had close correlation with microbial activity, biogenic amines, peroxide value, and TBARS along the storage time.

Effect of Chitosan Coating Enriched with Ergothioneine on Microbiological Characteristics

The effects of different treatments compared with control on the counts of mesophilic bacteria, psychrophilic bacteria, *Pseudomonas*, and lactic acid bacteria are shown in Table 4. With all treatments, the CFU for all of the studied microbial groups were increased as a function of time. The low levels of all the microorganisms at the beginning of storage indicate the raw material is excellent quality. In this work, 7 log cfu g^{-1} of mesophilic bacteria were used as a limit to evaluate microbial spoilage, which is based on other studies (Jeon et al. 2002; Ojagh et al. 2010; Li et al. 2012), the control sample exceeded the acceptability limits from the 12th day of the study, but treated samples were all below 7 log cfu g^{-1} over the period of storage. The most important organisms usually responsible for spoilage of fish are gram-negative, psychrophilic bacteria, particularly belonging to the Pseudomonaceae family, which are to some extent resistant to low temperatures due to a special cell membrane structure and the presence of cold resistant compounds (Bahmani et al. 2011). The lactic acid bacteria (LAB) constitutes substantial part of the natural microflora of fish, and in this study, LAB counts increased throughout the storage period, but was low since LAB grow slowly at refrigeration temperatures.

It is evident from this study that treatment with CH and CHER was more effective in reducing microbial counts than ER and control. The antibacterial effect found with coating solutions containing ergothioneine was to be expected. In our study, ergothioneine possess significant antibacterial effects, slightly lower than chitosan. We also found chitosan–ergothioneine exhibited superior antimicrobial activity compared with either chitosan or ergothioneine in coating. The synergistic effect may be due to the chitosan coating, which isolates the products from environments, reducing loss of ergothioneine, rendering it more effective in inhibiting microbial growth, and maintaining the keeping quality of sea bass.

Table 3 Changes in texture profile analysis of Japanese sea bass coated with chitosan+ergothioneine stored at 4 °C for 16 days

TPA	Treatment	Days of storage				
		0	4	8	12	16
Hardness	Control	274.14±18.21 a	227.13±17.35 a	187.05±9.63 a	143.23±8.28 b	112.25±7.18 b
(N)	CH	278.51±13.47 a	234.42±8.42 a	165.32±5.28 bc	158.24±11.50 ab	132.43±5.63 a
	ER	273.28±14.83 a	217.59±10.18 a	159.10±6.65 c	153.37±8.19 ab	130.54±4.80 a
	CHER	281.90±9.52 a	231.72±11.05 a	173.64±4.73 b	163.18±5.04 a	141.73±9.24 a
Gumminess	Control	163.73±12.73 a	84.82±7.36 b	71.42±6.39 b	54.31±5.39 b	38.29±4.73 b
(N)	CH	171.24±8.20 a	98.10±11.29 ab	82.40±9.21 ab	75.28±9.28 a	58.35±2.81 a
	ER	165.01±14.73 a	113.29±8.46 a	80.93±4.28 ab	72.39±7.51 a	55.41±6.30 a
	CHER	169.41±10.34 a	108.57±10.10 a	87.21±7.42 a	74.05±2.28 a	63.04±1.27 a
Chewiness	Control	131.53±8.38 a	80.53±2.78 b	67.29±5.45 a	47.41±5.32 c	32.83±1.37 b
(N mm)	CH	142.73±11.34 a	94.18±6.46 a	74.75±3.31 a	67.05±2.91 ab	48.03±4.87 a
	ER	136.20±10.74 a	92.37±7.57 a	71.23±6.17 a	72.83±2.54 a	51.37±3.25 a
	CHER	138.56±5.48 a	98.21±3.27 a	78.54±9.36 a	64.39±1.74 b	52.19±3.41 a

Values are the mean of three replications±standard deviation. Means in same row with different letters are significantly different ($p<0.05$)

Table 4 Changes in microbial counts ($\log_{10}$ colony-forming units per gram) of Japanese sea bass coated with chitosan+ergothioneine stored at 4 °C for 16 days

Days at 4 °C	Control	CH	ER	CHER
Mesophilic bacteria				
0	2.17±0.13 a	2.22±0.05 a	2.15±0.12 a	2.21±0.08 a
4	3.82±0.07 a	3.37±0.09 b	3.46±0.07 b	3.27±0.23 b
8	5.64±0.21 a	4.36±0.12 bc	4.63±0.20 b	4.11±0.18 c
12	7.19±0.18 a	4.83±0.11 bc	5.14±0.25 b	4.52±0.09 c
16	7.75±0.09 a	5.79±0.16 c	6.24±0.13 b	5.23±0.11 d
Psychrophilic bacteria				
0	1.81±0.06 a	1.82±0.04 a	1.76±0.15 a	1.85±0.11 a
4	2.90±0.27 a	2.63±0.06 ab	2.71±0.07 ab	2.52±0.22 b
8	3.74±0.15 a	3.34±0.07 bc	3.50±0.28 ab	3.11±0.12 c
12	4.78±0.09 a	3.90±0.16 bc	4.12±0.22 b	3.78±0.04 c
16	5.81±0.22 a	4.57±0.19 c	4.89±0.13 b	4.35±0.10 c
Pseudomonas				
0	1.47±0.13 a	1.42±0.12 a	1.48±0.05 a	1.50±0.17 a
4	2.46±0.08 a	2.04±0.11 b	2.32±0.09 a	1.82±0.05 c
8	3.16±0.04 a	2.37±0.07 c	2.76±0.14 b	2.47±0.21 c
12	3.90±0.23 a	2.93±0.18 b	3.13±0.20 b	2.82±0.15 b
16	4.83±0.11 a	3.41±0.15 bc	3.56±0.24 b	3.24±0.11 c
Lactic acid bacteria				
0	1.82±0.15 a	1.87±0.03 a	1.81±0.11 a	1.86±0.07 a
4	2.31±0.04 a	2.25±0.07 ab	2.24±0.04 ab	2.14±0.12 b
8	2.85±0.22 a	2.47±0.22 b	2.53±0.06 ab	2.53±0.13 ab
12	3.63±0.25 a	2.87±0.11 b	2.94±0.09 b	2.83±0.20 b
16	4.13±0.07 a	3.22±0.14 b	3.37±0.13 b	3.15±0.18 b

Values are the mean of three replications±standard deviation. Means in same row with different letters are significantly different ($p<0.05$)

Effect of Chitosan Coating Enriched with Ergothioneine on Sensory Attributes

The general acceptability based on odor and taste of sea bass fillets decreased as the storage period in all the treatments (Fig. 2). On the basis of judgments made by sensory panel members, the control samples were unacceptable after 8 days of storage. However, sea bass in CH, ER, and CHER did not exhibit these characteristics even on day 16; higher general sensory scores were observed in ER samples compared with CH samples during storage; the CHER samples were acceptable and in marketable condition and recorded a sensory scores of 7.4 after 12 days of storage. The sensory evaluation results were correlated with chemical analyses and microbial changes, suggesting that CHER was effective in retarding sea bass sensory deterioration.

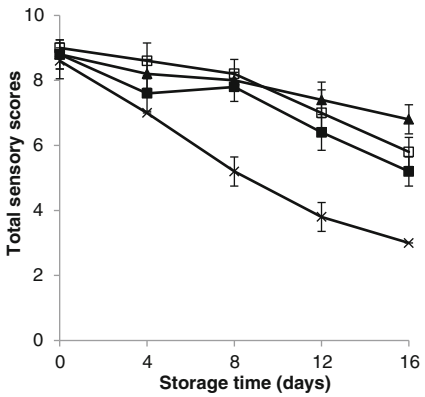

Fig. 2 Changes in sensory attributes of Japanese sea bass treated with control (*multiplication sign*), CH (*black square*), ER (*white square*), and CHER (*black triangle*) stored at 4 °C for 16 days. Each data point is the mean of three replicate samples. *Vertical bars* represent standard deviation of means

Conclusions

Improvement of sea bass storage quality by the chitosan–ergothioneine coating treatment involved the maintenance of tissue hardness and sensory quality, reduction of microbial counts, inhibition of peroxide value, total volatile basic nitrogen, and TBA value compared with control. Chitosan–ergothioneine-coated samples also exhibited lower levels of biogenic amine content during the storage period. The antioxidant, antimicrobial, and gas barrier effects of chitosan–ergothioneine coating have a broad potential application in the storage of fish and fish products.

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