



Effect of sodium alginate-based edible coating containing different anti-oxidants on quality and shelf life of refrigerated bream (*Megalobrama amblycephala*)

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ABSTRACT

The effect of alginate-based edible coating containing Vitamin C (Vc) and tea polyphenols (TP) for shelf-life extension of bream (*Megalobrama amblycephala*) was evaluated over a 21-day storage at refrigerated temperature (4 ± 1 °C). Bream were left untreated (CK), or were treated with alginate–calcium coating (T1), alginate–calcium coating incorporating 5% Vc (T2), or alginate–calcium coating incorporating 0.3% TP (T3). The fish samples were analyzed periodically for water loss, microbiological (total viable count), chemical (pH, total volatile basic nitrogen (TVB-N), thiobarbituric acid (TBA), *K*-value) and sensory characteristics. The results indicated that coating treatments retarded the decay of fish compared to uncoated bream. T2 more efficiently inhibited the growth of total viable counts than did T1 or T3 ($p < 0.05$). Coating treatments predominantly reduced chemical spoilage, reflected in TVB-N, pH, and TBA, retarded water loss ($p < 0.05$) and increased the overall sensory quality of fish compared to uncoated bream.

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1. Introduction

Bream (*Megalobrama amblycephala*) is native to China, distributed originally to affiliated lakes of middle and lower reaches of the Yangtze River in China. In 1964 it was domesticated successfully, and gradually extended due to aquaculture. Now it has become one of the main farmed freshwater species in China. Endowed with excellent biological characteristics for rearing (fast growth rate, easy cultivation and high feed efficiency ratio), its farming in China is currently increasing. Statistical data show that more than 1,000,000 tons of bream were caught in China in 2009. Bream has high nutritional values, so it has been shown to be a convenient specie for commercial production, aiding in the diversification of freshwater aquaculture. It also has become a popular edible fish in China now. However, bream is usually more perishable than most other foodstuffs. Each year about 50,000 tons of bream are wasted. After it is harvested, its storage period is limited. Though low temperature can delay the rate of fish deterioration and also extend the shelf life of fish to some extent, the quality of fish muscle will still deteriorate during cold storage. Microbial activity and enzymes contained in fish tissues also degrade the muscle protein resulting in the quality loss of bream.

Deterioration of fish muscle mostly occurs in the fat-containing portions. The proportion of unsaturated fatty acids in bream fat is approximately 70%. These fatty acids are affected by the environmental oxygen that oxidizes and spoils the fish meat (Kilinceker, Dogan, & Kucukoner, 2009). So taking some measures to delay the decline of fish quality and extend the preservation life of fish through inhibiting, or retarding the growth of microorganisms and reducing the rate of lipid oxidation is necessary. Coating the foods with edible materials has been researched as an effective method to improve the food quality (Matuska, Lenart, & Lazarides, 2006).

Hydrophilic edible films are good barrier for oxygen and carbon dioxide and possess suitable mechanical properties at low relative humidity. Many studies have shown that edible coatings made of protein, polysaccharide, and oil-containing materials help to prolong the shelf life and preserve the quality of fish (Artharn, Prodpran, & Benjakul, 2009; Fan, Sun & Chen, 2009; Jeon, Kamil, & Shahidi, 2002; Sathivel, 2005; Stuchell & Krochta, 1995). But there is little literature about bream preservation so far.

Alginate is a salt of alginic acid, a polymer of D-mannuronic acid and L-guluronic acid, and is isolated from brown algae (Lu, Liu, & Ye, 2009). Alginate has unique colloidal properties and can form strong gels or insoluble polymers through cross-linking with Ca^{2+} by post-treatment of CaCl_2 solution. Such biopolymer-based films can keep good quality and prolong shelf life of foods by increasing water barrier, preventing microbe contamination, maintaining the flavor,

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reducing the degree of shrinkage distortion and retarding fat oxidation. Alginate is a GRAS substance (FDA). Coating fish, shrimp, scallop and pork with sodium alginate showed that it can prolong their shelf life, reducing thawing loss, cooking loss, weight loss and maintaining the functional properties during frozen storage (Wanstedt, Seideman, & Donnelly, 1981; Wang, Liu, & Teng, 1994; Yu, Li, & Xu, 2008; Zeng & Xu, 1997). Moreover, the coatings may serve as carriers for antimicrobial compounds and antioxidant in order to maintain high concentrations of preservatives on the surface of foods. A few antimicrobial agents and antioxidant have been incorporated into edible coatings to suppress quality changes during storage (Chidanandaiah, Keshri & Sanyal, 2009; Fan, Chi & Zhang, 2008; Haque, Shon, & Williams, 2009; Kang, Jo, & Kwon, 2007). Furthermore, to meet consumers demands for safe foods, numerous studies are currently focused on using natural ingredients instead of synthetic preservatives (Gennadios, Milford, & Hanna, 1997). Vitamin C (Vc) and Tea polyphenols (TP) are both well-known natural anti-oxidants. They play an important role in fat oxidation and enzyme inhibition and demonstrate potential for their use as the preservatives and the anti-oxidants in food industry especially in the field of the preservation of manufactured meat. Vc can scavenge singlet oxygen and reduce oxygen- and carbon-centered radicals, resulting in dehydro-ascorbic acid formation (Gregory, 1996). Vc can also chelated metal ions. TP is a kind of Polyhydroxy organic matter extracted from tea leaves; it is a good hydrogen donor, can remove the original free radicals, resulting in the formation of relative stable free radical intermediate substances.

This research was to identify whether the addition of anti-oxidants into alginate–calcium coating solutions can keep good quality and extend the shelf life of bream through suitable coating formulations.

2. Materials and methods

2.1. Materials

Breams, (*Megalobrama ambycephala*; weight and length: 381.34 ± 35.30 g and 29.19 ± 1.78 cm, respectively) were purchased from a local market (Huilongguan market) in Beijing, China and transported to the laboratory alive, then immediately stunned, scaled, gutted and washed in water.

Food-grade sodium alginate (Ri-xing Seaweed Industrial Co., Ltd., Qingdao City, China) was used for the coating formulations. Calcium chloride (Yuanli Chemical Co., Ltd., Tianjin City, China) was used to induce the cross-linking reaction. Vc (Biodee Biotechnology co., Ltd., Beijing, China) and TP (Keyi Chemical Co., Ltd., Zhengzhou City, China) were added as anti-oxidants.

2.2. Preparation of the coating-forming solutions and treatments

Bream were divided into four coating formulations to which the following treatments were randomly assigned.

CK: control, untreated.

T1: 1.5% sodium alginate/10% glycerin

T2: 1.5% sodium alginate/10% glycerin/5% Vc

T3: 1.5% sodium alginate/10% glycerin/0.3% TP

Sodium alginate solution was prepared by mixing 30 g of alginate with 1000 ml of distilled water and stirred at a controlled temperature of 80 °C until the mixture became clear. 500 ml solution containing nothing or 100 g Vc or 6 g tea polyphenols and 200 ml glycerin were mixed with the prepared sodium alginate solution and stirred thoroughly. Then the well-mixed solution was made up to 2000 ml with distilled water. Two percent (w/v) calcium

chloride was also prepared. All solutions were cooled to room temperature prior to surface application onto fishes. Bream were immersed in the solutions for 1 min, air-dried for 1 min and then immersed in two percent (w/v) calcium chloride to gel for 1 min. They were then packed in polyethylene bags, tied off, and stored at 4 ± 1 °C for 21 days. Fish samples were taken randomly at intervals for analyzing.

2.3. Microbiological analysis

Total viable counts (TVC) were determined in plate count agar by the pour-plate method (AOAC, 2002). 25 g of fish portion was aseptically weighed and homogenized with 225 ml of sterile 0.1% peptone water for 1 min using a stomacher (FM200, fluko co., Ltd., Shanghai, China) at a speed of 6000 rpm. The homogenized samples were serially diluted (1:10) in sterile 0.1% peptone water. Samples (1 ml) of serial dilutions were plated onto plate count agar and then incubated at 35–37 °C for 48 h. Microbiological data were transformed into logarithms of the number of colony-forming units (CFU/g). All counts were performed in duplicate.

2.4. Water loss analysis

The water loss was estimated as described by Lu et al. (2009). The percentage weight loss relative to the initial weight was calculated by weighing the samples every 2 days in triplicate.

2.5. Chemical analysis

Proximate composition was determined by AOAC (2002) method.

2.5.1. Determination of pH

10 g sample of fish flesh was dispersed in 100 ml of distilled water and stirred for 30 min, and then the mixture was filtered. pH value of filtrate was measured using a digital pH meter (Mettler toledo FE20/EL20, Shanghai, China)

2.5.2. Determination of total volatile basic nitrogen (TVB-N)

The micro-titration method was employed to analyze TVB-N. 10 g sample of fish flesh was dispersed in 100 ml of distilled water and stirred for 30 min, and then the mixture was filtered. After the addition of 5 ml MgO (10 g/l) to 5 ml filtrate, the mixture was to distill through Kjeldahl Apparatus (KDY-9820, Beijing, China). The distillate was absorbed by 20 ml aqueous solution of boric acid (2%) containing a mixed indicator produced from dissolution of 0.1 g of methyl red and 0.1 g of methylene blue to 100 ml of ethanol. Afterward, the boric acid solution was titrated with a 0.01 mol/l hydrochloric acid (HCl) solution. TVB-N value was determined according to the consumption of hydrochloric acid.

2.5.3. Determination of thiobarbituric acid (TBA) value

Thiobarbituric acid reactive substances (TBARS) were determined as described by Buege and Aust (1978) with some modifications. Fish flesh (5 g) was dispersed in 20 ml of thiobarbituric acid solution (0.375% thiobarbituric acid, 15% trichloroacetic acid and 0.25 mol/l HCl). The mixture was heated in boiling water for 10 min, cooled with water and centrifuged at 3600 g for 20 min at room temperature. The absorbance of the supernatant was measured at 532 nm (UV-2600, Shanghai, China). The standard curve was prepared using malondialdehyde (MDA) and TBARS were expressed as mg MDA/kg sample.

2.5.4. Determination of K value

K value (as a percentage of the ratio between Ino + Hx to the total ATP and its degradation products) was determined as

described by Fan et al. (2008) with some modifications. 1 g sample of fish flesh was homogenized with 2 ml of cold (4 °C) 10% perchloric acid solution, and centrifuged at 1500 g for 15 min. The sediment was washed with 2 ml of cold 5% perchloric acid solution, centrifuged at 1500 g for 15 min, and the process repeated three times. All the supernatants were collected in a centrifuge tube and pH value was adjusted to 6.4–6.5 using 1 mol/L NaOH. The white crystal was removed by centrifugation (1500 g, 15 min). Then the supernatant was made up to 10 ml with deionized water, filtered through a 0.45 µm membrane filter and stored in –20 °C for further analysis. Adenosine-triphosphate (ATP) and its related compounds were analyzed by HPLC (Shimadzu LC-10AT series, Japan) equipped with SPD-10A (V) detector, VP-CDSC18 column (4.6 mm i.d. × 250 mm, 5 µm). Sample (20 µl) was injected at a flow rate at 1 ml/min, and the peak was detected at 254 nm. The amounts of ATP and its related compounds were determined and calculated based on standard ATP, adenosine diphosphate (ADP), adenosine monophosphate (AMP), inosine monophosphate (IMP), hypoxanthine riboside (HxR) and hypoxanthine (Hx). *K* value was defined by the following equation (Saito, Arai, & Matsuyoshi, 1959):

$$K \text{ value (\%)} = \frac{[(\text{HxR}) + (\text{Hx})]}{[(\text{ATP}) + (\text{ADP}) + (\text{AMP}) + (\text{IMP}) + (\text{HxR}) + (\text{Hx})]} \times 100 \quad (1)$$

2.6. Sensory evaluation

Sensory of raw fish were evaluated using the quality index method (QIM) shown in Table 1 by seven member trained panelists from the laboratory staff. The QIM is based on the freshness quality grading system developed by Nielsen and Hyldig (2004). Each assessor scored for body surface, skin, body surface slime, eyes, gill and colour of belly from 0 to a maximum of 3, where 0 represented best quality and any higher score indicated poorer quality. Scores of separate characteristics were summed to give an overall sensory score. This system gave score of zero (or near zero) for very fresh fish while increasingly larger totals resulted as fish deteriorated. Minor variations in scoring individual attributes, therefore, had little influence on the overall score. Panelists were asked to state whether the fish were acceptable or not. This was used to determine the shelf life of bream.

Table 1
QIM scheme for sensory evaluation of bream modified from Nielsen and Hyldig (2004).

| Quality parameters | Description points | | | |
|---------------------|--------------------|------------------|-------------------|--------------------|
| | 0 | 1 | 2 | 3 |
| Appearance of skin | Very bright | Bright | Slightly dull | Dull |
| Skin | Firm or elastic | Soft | | |
| Slime | Absent | Slightly slimy | Slimy | Very slimy |
| Eyes | | | | |
| Clarity | Clear | Slightly cloudy | Cloudy | |
| Shape | Normal | Flat sunken | Sunken or swollen | |
| Iris | Visible | Slightly visible | Not visible | |
| Blood | No blood | Slightly bloody | Bloody | Very bloody |
| Gills | | | | |
| Colour | Dark red | Red | Brown | Dark brown or grey |
| Mucus | Absent | Slight | Moderate | Moderate or sticky |
| Smell | Neutral | Fishy | Stale | Spoiled |
| Belly discoloration | White iridescent | Some yellowish | Yellow | Excessive yellow |

2.7. Data analysis

Two independent experiments were conducted on different occasions with different fish samples. All analyses were run in triplicate (except microbiological analyses were performed in duplicate). All data were subjected to analysis of variance (ANOVA). The least significant difference (LSD) procedure was used to test for difference between means (significance was defined at $p < 0.05$) (SAS Institute, Cary, NC, USA).

3. Results and discussion

The proximate composition of bream showed 80.03% ± 0.03% moisture, 1.02% ± 0.05% crude fat, 17.67% ± 0.83% crude protein and 1.03% ± 0.03% ash on 1st day.

3.1. Total viable counts

Variations in value of TVC during storage are presented in Fig. 1. The initial number of bacteria in bream muscle was 2.90 log CFU/g which indicated good quality of fish used in this study. Generally the initial microbial load of freshwater fishes is different because of water condition and temperature. According to available literature reports, the bacterial counts of different freshwater fish species are between 2.00 and 6.00 log CFU/g (Chytiri, Chouliara, & Savvaids, 2004).

Fig. 1 shows that TVC of all samples increased with storage time and the value of CK and T1 increased faster than T2 and T3. From day 0 to day 4, there were no significant differences between CK and T1 ($p > 0.05$). But there were significant differences between CK, T1 and T2, T3, which indicates that T2 and T3 strongly inhibited the growth of TVC. After 4 days of storage, the TVC of CK increased quickly and reached 7.10 log CFU/g on day 15, which exceeded the maximum acceptable level of 7.0 log CFU/g for freshwater and marine fish (ICMSF, 1986), while T1 reached about 7.00 log CFU/g on day 17. The TVC of T2 and T3 did not exceed the limit value during the entire storage and no difference was found between them ($p > 0.05$); they reached 5.54 and 5.63 log CFU/g respectively on day 21.

Compared with the control (CK), all treatments significantly inhibited the growth of bacteria in bream during the storage period.

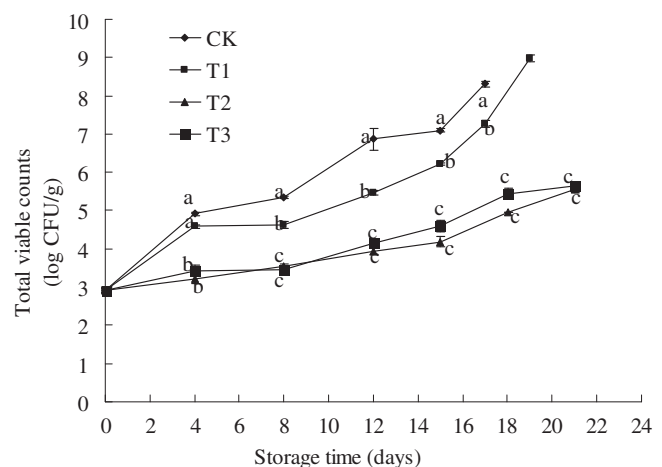


Fig. 1. Total viable counts of bream coated with sodium alginate-based material during storage at 4 °C (CK: uncoated; T1: coated with sodium alginate-based material; T2: coated with sodium alginate-based material containing Vc; T3: coated with sodium alginate-based material containing TP). Different letters (a, b, c) indicate significant difference at $p < 0.05$.

The significant reduction in TVC observed in the treated samples of bream may due to that the coating acts as a barrier against oxygen transfer and leads to inhibition of growth of the aerobic bacteria. T2 and T3 more efficiently inhibited the growth of bacteria than did the T1 treatment ($p < 0.05$). The antimicrobial activity of TP had been reported by Fan et al. (2008). Jo, Son, Sohn, and Byun (2003); Jo et al. 2003 also identified that tea catechine had antimicrobial activity. The inhibitory effect of Vc on spoilage bacteria of bream in storage agree with Chidanandaiah et al. (2009) who reported that alginate coatings along with ascorbates lowed the TVC because of its antifungal effect. There are reports about that chitosan can chelate certain ions from the lipopolysaccharide (LPS) layer of the outer membrane of bacteria and the alterations in the LPS layer may cause the outer cell surface to become more permeable, thereby releasing cellular components of bacteria (Chen, Liao, & Tsai, 1998; Chen et al., 1998; Jeon et al., 2002). Vc can also chelated metal ions. So Vc may have the same antimicrobial mechanism with chitosan. Furthermore, the pH, water activity, and composition of the food products might influence the antimicrobial packaging. Therefore, the antimicrobial activity of alginate–calcium coating containing Vc and TP requires further study.

3.2. Water loss

The water loss of alginate-coated bream stored at 4 ± 1 °C is summarized in Fig. 2. The pattern of water loss for the treatment samples was same with CK samples, but being lower in the treatment samples than the CK samples. Moisture in bream was evaporated much more rapidly and there was no statistically significant ($p > 0.05$) difference in water loss between the treatment samples and the CK samples during the first 4 days of storage, after which a significant ($p < 0.05$) effect was observed for coating samples compared to that of the uncoated bream. Pham and Willix (1984) reported that the desiccated surface layer developed during cold storage produces a further resistance to mass transfer in the case of biological substances, thus perhaps bringing about a slower increase in water loss in bream samples after a certain period of storage. No significant ($p > 0.05$) differences were observed among the T1, T2 and T3 coatings during the whole storage period. This may illustrate that the Vc and TP immobilized in the alginate–calcium coating didn't change the coating characteristics. Kester

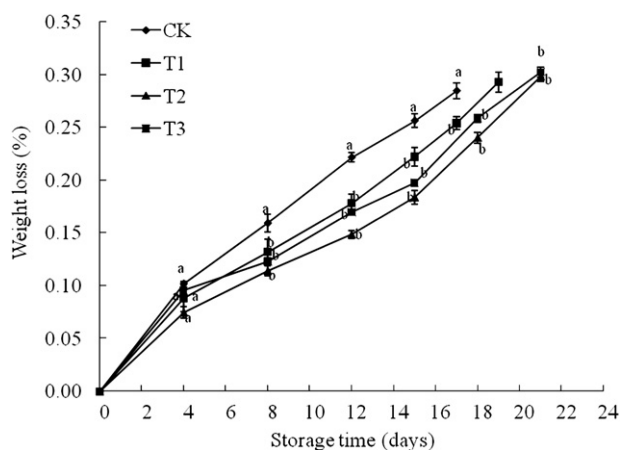


Fig. 2. Weight loss of bream coated with sodium alginate-based material during storage at 4 °C (CK: uncoated; T1: coated with sodium alginate-based material; T2: coated with sodium alginate-based material containing Vc; T3: coated with sodium alginate-based material containing TP). Different letters (a, b, c) indicate significant difference at $p < 0.05$. NS indicate not significant difference.

and Fennema (1986) reported that the reduction in dehydration of alginate-coated products is attributed mainly to the fact that the gel coating acts as a sacrificing agent, i.e., moisture in the gel evaporates prior to any significant desiccation of the enrobed food. Lu et al. (2009) demonstrated that the alginate–calcium coatings on the fish fillets acted effectively as water vapor barriers during the entire storage period.

3.3. pH value

Change in pH value of different treatments showed the same trend in which the values decreased initially and then increased (Fig. 3). The initial decrease of pH value may be attributed to the decomposition of glycogen in whole fish, but many researchers attribute it to the dissolution of CO₂ in the fish samples for fish fillets (Manju, Srinivasa Gopal, & Jose, 2007; Fan, et al, 2008). The increase of pH value is due to the production of volatile basic components, such as ammonia and trim ethylamine by fish spoilage bacteria (Ruiz-Capillas & Moral, 2001). From 1st day to 4th day of the storage period, there were no significant differences ($p > 0.05$) among all fish samples, but it began to increase at different speed after four days. Compared to the control (CK), all treatments delayed the increase speed. It can be concluded that the lower pH value of T1, T2 and T3 might restrain microbial growth and inhibit the activity of the endogenous proteases at different degree, leading to the extension of preservation of bream (Fan et al., 2009).

3.4. Total volatile basic nitrogen

Fig. 4 shows the effects of different treatments on TVB-N production in the fish samples stored at 4 ± 1 °C. TVB-N value was found to increase in all samples during storage. This increase is related to the activity of spoilage bacteria and endogenous enzymes (Kyrana, Lougovois, & Valsamis, 1997; Vareltzis, Koufidis, & Gavriilidou, 1997). TVB-N value of all samples showed a slow increase in the early storage, but a marked increase was observed after day 12. This trend agrees with previous related research concerning other fish species (Aubourg, 2001; Rodríguez, Losada, & Aubourg, 2005; Quitral, Donoso, & Ortiz, 2009). In this study, the

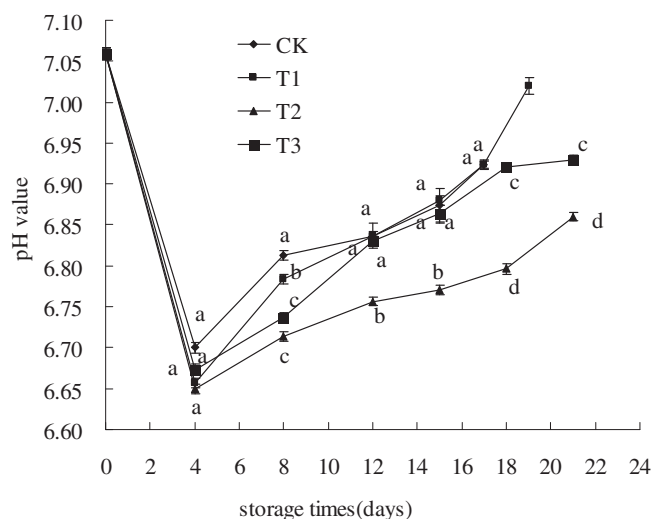


Fig. 3. The pH value of bream coated with sodium alginate-based material during storage at 4 °C (CK: uncoated; T1: coated with sodium alginate-based material; T2: coated with sodium alginate-based material containing Vc; T3: coated with sodium alginate-based material containing TP). Different letters (a, b, c) indicate significant difference at $p < 0.05$. NS indicate not significant difference.

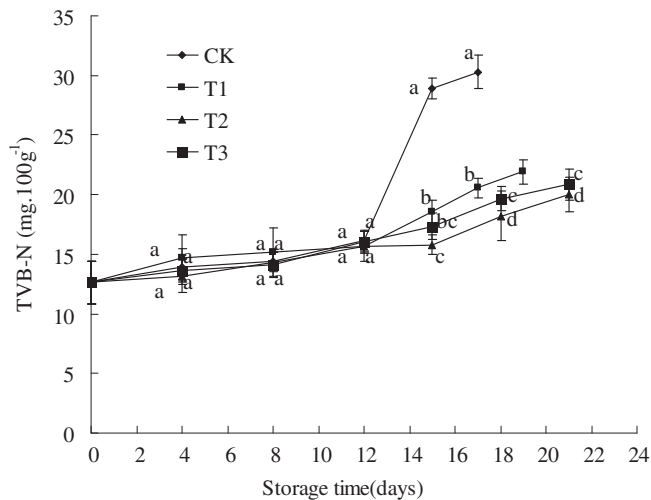


Fig. 4. TVB-N value of bream coated with sodium alginate-based material during storage at 4 °C (CK: uncoated; T1: coated with sodium alginate-based material; T2: coated with sodium alginate-based material containing Vc; T3: coated with sodium alginate-based material containing TP). Different letters (a, b, c) indicate significant difference at $p < 0.05$. NS indicate not significant difference.

initial TVB-N value of bream was $12.62 \text{ mg} \cdot 100 \text{ g}^{-1}$ which is similar to the other reports, in which the initial TVB-N value of tilapia was $10.00 \text{ mg} \cdot 100 \text{ g}^{-1}$ and $9.50 \text{ mg} \cdot 100 \text{ g}^{-1}$ for northern snakehead (Chan, Shwu, & Chieh, 2002; Lu et al., 2009). The TVB-N value of T1, T2, and T3 were lower than that of CK during the storage period and the differences became marked in the latest stage of the experiment (days 12–21) ($p < 0.05$). On day 15 of storage, the TVB-N values of CK, T1, T2 and T3 was $28.93 \text{ mg} \cdot 100 \text{ g}^{-1}$, $18.54 \text{ mg} \cdot 100 \text{ g}^{-1}$, $15.79 \text{ mg} \cdot 100 \text{ g}^{-1}$ and $17.35 \text{ mg} \cdot 100 \text{ g}^{-1}$ respectively. On day 17 of storage, the TVB-N value of T1 increased to $20.55 \text{ mg} \cdot 100 \text{ g}^{-1}$; however, that of T2 and T3 were still below $20.00 \text{ mg} \cdot 100 \text{ g}^{-1}$. On day 21 of storage, the TVB-N value of T2 and T3 increased to 20.03 and $20.84 \text{ mg} \cdot 100 \text{ g}^{-1}$ and the difference between them was significant ($p < 0.05$). Kirk and Sawyer (1991, pp. 509–511) reported that a level of $20 \text{ mg} \cdot 100 \text{ g}^{-1}$ of TVB-N in fish muscle is usually regarded as fresh. According to this freshness standard, bream of CK could only maintain their freshness for about 12 days. T1, T3 and T2 could maintain the bream quality up to 17 days, 19 days and 21 days respectively under 4 ± 1 °C. The application of antioxidant alginate coating could extend the shelf life, and the sodium alginate coating with Vc was more effective compared to TP-containing coating. This can be attributed to either a more rapidly reduced bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds or both (Banks, Nickelson, & Finne, 1980), which was due to the effect of Vc or TP in fish samples. The sodium alginate coating without antioxidant could extend the shelf life of bream by reducing water loss. These results are in agreement with that of Lu et al. (2009) who observed that alginate–calcium coating slowed the development of TVB-N relative to untreated northern snake head fillets, and Fan et al. (2008) who found that TP inhibited the increase of TVB-N in silver carp.

3.5. Thiobarbituric acid value

TBA value has been widely used to estimate the extent of lipid oxidation (Shahidi, 1994) and the presence of TBA reactive substances is due to the second stage auto-oxidation during which peroxides are oxidized to aldehyde and ketone (Lindsay, 1991). TBA value of various samples during storage at 4 ± 1 °C is depicted in

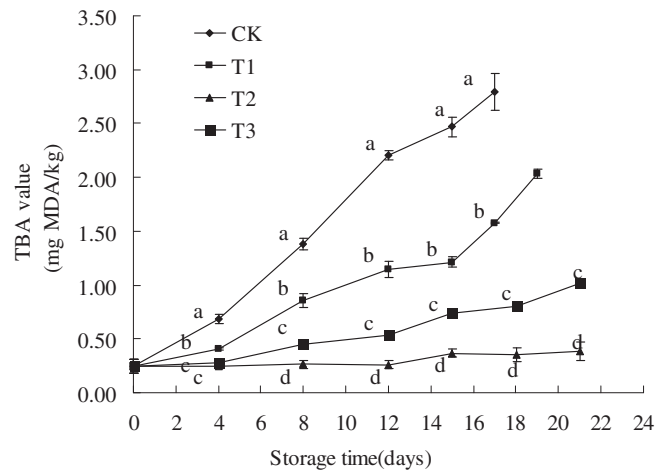


Fig. 5. Thiobarbituric acid value (TBA; mg malondialdehyde (MDA)/kg sample) of bream coated with sodium alginate-based material during storage at 4 °C (CK: uncoated; T1: coated with sodium alginate-based material; T2: coated with sodium alginate-based material containing Vc; T3: coated with sodium alginate-based material containing TP). Different letters (a, b, c) indicate significant difference at $p < 0.05$.

Fig. 5. The results showed that TBA value of all treatments increased continuously during storage. This observation was similar to the results from Lu et al. (2009), Manju et al. (2007), Jeon et al. (2002) and Fan et al. (2008). Increase in TBA value may be attributed to the partial dehydration of fish and the increased oxidation of unsaturated fatty acids. According to Connell (1990), a TBA value of 2 mg MDA/kg was regarded as the limit beyond which the fish will normally develop an objectionable odour and taste. In this study, the initial TBA value of bream was 0.25 mg MDA/kg and the value exceeded 2 mg MDA/kg on day 12 and 19 of storage for CK and T1 respectively. However, the TBA values of T2 and T3 samples did not greatly alter during the whole storage period and they reached 1.02 and 0.38 respectively on day 21. The value of T1, T2 and T3 were significantly ($p < 0.05$) lower than that of the uncoated samples throughout the storage period, indicating that the sodium alginate-based coating effectively inhibited lipid oxidation. Similar observations have been made by Wang et al. (1994), Wanstedt et al. (1981) and Zeng and Xu (1997), who coated fish, ground pork patties, shrimps and scallops with sodium alginate and found it could control lipid oxidation effectively. Lipid oxidation can be initiated and accelerated by various mechanisms including the production of singlet oxygen, enzymatic and non-enzymatic generation of free radicals and active oxygen (Kubow, 1992). The alginate-based film layer on the fish surface may have been resistant to oxygen diffusion, thus may have retarded lipid oxidation. Some reports showed that the gas barrier properties of chitosan and protein-based films were crucial for prolonging the shelf life of foods (Butler, Vergano, & Testin, 1996; Gennadios, Brandenburg, & Weller, 1993; Jeon et al., 2002). Compared to T1, the TBA values of T2 and T3 were lower due to the presence of antioxidant (Vc and TP) and T2 had the best effect.

3.6. K value

During postmortem fish storage, nucleotides in the muscle tissue degrade in a series of stages as a result of endogenous biochemical changes. The level of major adenine nucleotides and their related compounds (K value assessment) have been utilized extensively as an index of freshness of fish muscle before bacterial spoilage commences (Olafsdóttir, Martinsdóttir, & Oehlenschler, 1997). Variations in K value during the 4 ± 1 °C storage are

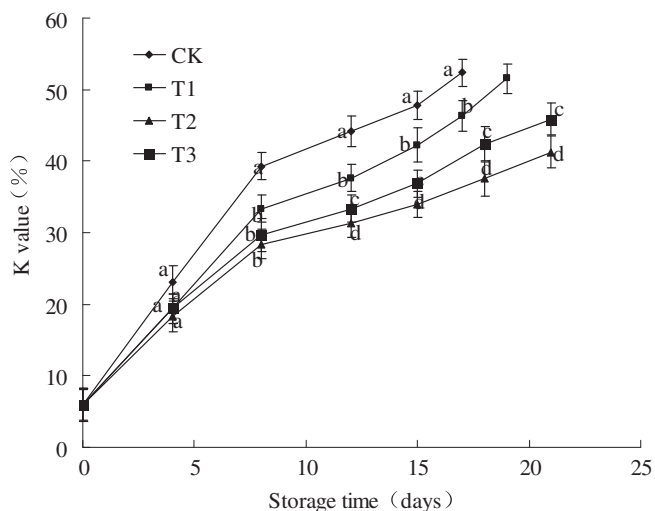


Fig. 6. K value of bream coated with sodium alginate-based material during storage at 4 °C (CK: uncoated; T1: coated with sodium alginate-based material; T2: coated with sodium alginate-based material containing Vc; T3: coated with sodium alginate-based material containing TP). Different letters (a, b, c) indicate significant difference at $p < 0.05$. NS indicate not significant difference.

shown in Fig. 6. Aleman, Kakuda, and Uchiyama (1982) reported that the initial K value was around 5% for freshly caught fish. In this study, the initial K value of bream was 5.9%. The result showed that K values of bream increased with storage time and fast increase was observed in the period of day 0–8, then the rate of increase became lower. Although Surette, Gill, and LeBlanc (1988) have demonstrated that the degradation enzymes may be endogenous or microbial, a substantial amount of microbial enzymes seemed not to be a prerequisite for nucleotide catabolism. This resulted in the immediate rise in K value during earlier storage (0–8 day). The evolution of bream muscle K value with time was similar to that of the Sea Bass (Liang, Chang, & Shiau, 1998), the Atlantic Herring (Ozogul, Taylor, & Quantick, 2000), sea bream (Huidobro, Mendes, & Nunes, 2001), and silver carp (Fan et al., 2008). However, other fish species, such as the black skipjack (Mazorra-Manzano, Pacheco-Aguilar & Díaz-Rojas, 2000) showed a gradually

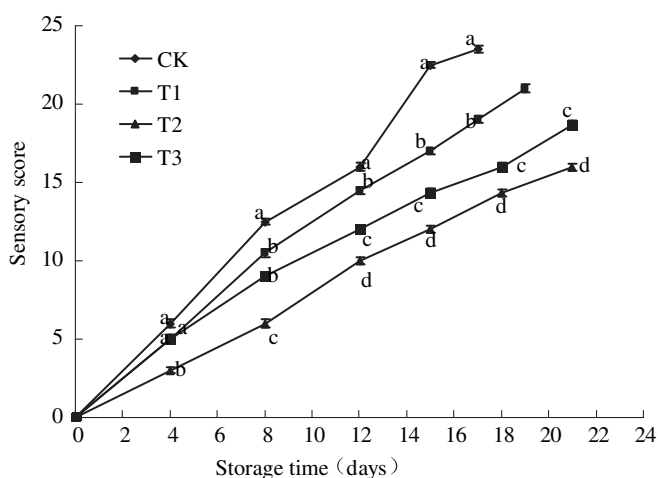


Fig. 7. Sensory score of bream coated with sodium alginate-based material during storage at 4 °C (CK: uncoated; T1: coated with sodium alginate-based material; T2: coated with sodium alginate-based material containing Vc; T3: coated with sodium alginate-based material containing TP). Different letters (a, b, c) indicate significant difference at $p < 0.05$.

increasing K value during the early storage and no changes were observed later. In this study, the changes in the K value of the control and all treatments had the same trend except that treatments exhibited lower K value during the storage. In the 0–8 days of storage period no significant difference were obtained between CK and treatments, however, in the 8–17 days the K value of CK was higher than that of all treatments ($p < 0.05$). There was no significant difference in the period of day 0–8 among various treatments. And after 8 days significant difference were obtained between each other of T1, T2 and T3. The best treatment for prevention of nucleotide degradation was T2, and then was T3. The decomposition of IMP results from the activity of 5-nucleotidase. Therefore, it can be concluded that the lower K value in T1, T2 and T3 could be due to reduced activity of 5-nucleotidase as a result of the bioactive alginate-based edible coating (Fan, et al., 2009; Aubourg, Pineiro, & Gallardo, 2005; Losada, Pineiro, & Aubourg, 2005; Nejib, Moza Abdallah & Ismail Mohammed, 2005). According to Ehira (1976) and Ehira and Uchiyama (1974) the rejection level of the K value is close to 60%. However, the K values were lower than 45% in all fish samples when they were reject in our study. This result coincided with the reports of Fan et al. (2008) and Tejada and Heras (2007).

3.7. Sensory

Changes in the sensory score of raw bream over the entire storage are shown in Fig. 7. Zero represents absolutely fresh fish and 33 represents completely spoiled fish. The sensory scores of both the untreated samples and treated samples increased with storage time. The observed shelf life of bream, as determined by panelists who indicated that the fish were not acceptable, was 12 days for CK (sensory score: 16), 15 days for T1 (sensory score: 17), 21 days for T2 (sensory score: 15.97) and 18 days for T3 storage (sensory score: 16). The panelists rejected the fish of both the untreated samples and treated samples during storage even though the microbial load did not exceeded the limit of 7 log CFU/g (ICMSF, 1986) throughout the storage period. This early rejection indicated that not only bacterial number plays a role in the shelf life of fish, but also other factors such as bacterial types (Ryder, Buisson, & Scott, 1984), autolytic activity (Scott, Fletcher, & Hogg, 1986), bio-physicochemical properties of fish and storage conditions (Hanna, 1992) should be considered. In this study, the result showed similarity with that of Ryder et al. (1984) and Nejib et al. (2005). The sensory scores of CK, T1, T2 and T3 differed significantly between each other when stored for same times. CK fish scored higher than the treatment fish ($p < 0.05$). T1 and T3 fish scored higher than T2 fish ($p < 0.05$) during the whole storage period. From the data, we can learn that all treatments maintained better sensory quality for the fish than those of the untreated samples. T2 exhibited the longest shelf life, followed by T3, and shelf life of T1 was longer than that of CK. These conclusions were supported by the results from chemical quality analyses. The results were in accordance with Lu et al. (2009) who found that the shelf life of untreated northern snakehead fillets was 7 days according to sensory score, and the fish with alginate–calcium coating were still considered to be acceptable during this storage period.

4. Conclusion

This study showed that coating treatments maintained better quality of bream than that of the control. T2 and T3 inhibited the growth of bacteria more efficiently than T1. And they also more efficiently reduced the degree of chemical spoilage, retarded water loss and enhanced the overall sensory values of bream than T1 and CK. T2 had the best effect.

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