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Effect of Alginate-Calcium Coating Combined with Natural Antioxidants on Quality of Flying Fish (*Cheilopogon intermedius*) Fillets during Refrigerated Storage

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Edible coating is a promising food packaging technology for reducing the degree of microbial spoilage and chemical changes in highly perishable foodstuffs like fish. The effect of alginate-calcium coating with added natural antioxidants such as vitamin C, α-tocopherol, and tea polyphenol to maintain the shelf-life of flying fish (*Cheilopogon intermedius*) fillets was evaluated over a 21-day storage at refrigerated temperature (4 ± 2 °C). Fillets were left untreated (control) or were treated with alginate-calcium coating (T1), alginate-calcium coating with 5% vitamin C (T2), alginate-calcium coating with 5% α-tocopherol (T3), or alginate-calcium coating with 0.1% tea polyphenol (T4). Samples were analyzed periodically for microbiological (total viable count); chemical [pH, total volatile basic nitrogen (TVB-N), histamine, thiobarbituric acid, and *K* value]; and sensory attributes such as odor, color, flavor, and texture. The results indicated that coating treatments preserved the quality of flying fish fillets compared to the uncoated samples. Alginate-calcium coating combined with vitamin C (T2) more efficiently inhibited the growth bacteria as revealed by fewer total viable counts and reduced chemical spoilage – as reflected in pH, TVB-N, and *K* value than the other treatments ($p < 0.05$). Results of this study suggest that edible coatings could be possible alternatives to synthetic materials in maintaining or improving the quality of refrigerated fish.

Keywords: α-tocopherol, alginate-calcium coating, flying fish, tea polyphenol, vitamin C

INTRODUCTION

Small pelagic fish are considered the main source of inexpensive animal protein for lower income groups in the Philippines. Included in this group are the flying fishes (family Exocoetidae), which are an important group in the pelagic fish catch of coastal regions of many parts of the world as edible fish as well as a baitfish in many tuna fisheries (Sosis 2000). As flying fish are mainly caught in the near offshore areas, the craft engaged in this fishery function as day-boats – leaving for the fishing ground early morning and returning to the operation base late afternoon. Most of these crafts do not have facilities to preserve catch; hence, flying fish are landed mostly fresh – use of ice is still limited to the larger multi-day boats or to circumstances where ice is economically viable (Pajot 1991). The landed flying fish is rather low in quality as it does not reach the prime market, and is mostly sold in fresh or dried form for local consumption. Adequate post-harvest technologies for the utilization of this important

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fishery resource are essential because it has currently become a popular edible fish in the Philippines.

Low temperature preservation can retard the rate of spoilage in fish and also extend the shelf life to some extent, but the quality of fish muscle will still deteriorate during cold storage. Enzymes and microbial activity in fish tissues also degrade the muscle protein resulting in the quality loss of fish. Inhibiting or retarding the growth of microorganisms and reducing the rate of lipid oxidation is necessary to maintain fish quality. Coating the foods with edible materials proved to be an effective method to improve food quality as they function as solute, gas, and vapor barriers to prevent the food surface from being contaminated (Chen *et al.* 2016). Numerous studies revealed that edible coatings made of protein, polysaccharide, and oil-containing materials prolong the shelf life of fish (Feng *et al.* 2016, Feng *et al*. 2017, Li *et al*. 2016). One example of a biopolymer-based film is alginate, which is isolated from brown algae. It is a Generally Regarded as Safe (GRAS) substance and proven to keep good quality and prolong shelf life of foods by increasing water barrier, preventing microbe contamination, maintaining the flavor, reducing the degree of shrinkage distortion, and retarding fat oxidation (Yu *et al.* 2008). Further, some antimicrobial agents and antioxidants were incorporated into edible coatings like alginate to prevent quality deterioration during fish storage (Feng *et al.* 2017, Shiroodi *et al.* 2016, Song *et al.* 2011).

With greater emphasis on health and nutrition, consumer demands are now focused on fish products with natural food ingredients over artificial preservatives. Some of these are vitamin C, α-tocopherol, and tea polyphenols – all of which are well-known antioxidants. They have been demonstrated to play an important role in lipid oxidation, enzyme inhibition, metal ions chelation, and removal of original free radicals (Yi *et al.* 2011). The main objective of this study was to investigate whether the incorporation of natural antioxidants into alginate-calcium coating solutions can preserve the quality of flying fish fillets at refrigerated storage.

MATERIALS AND METHODS

Sample Preparation

Flying fish (*Cheilopogon intermedius*) were collected during the months of Feb–Apr 2016 at San Jose, Antique, Philippines. The average body weight and length of fish samples were 75.02 ± 9.02 g and 143.31 ± 6.26 cm, respectively. Fish were killed by cold shock (immersion in ice) and were beheaded, gutted, washed, and filleted by hand. Two skin-on fillets were obtained from each fish.

Preparation of Coating Solutions and Treatments

Sodium alginate and antioxidant formulations were based on the study of Song *et al.* (2011) with modifications. Fillet samples were divided into five coating formulations, to which the following treatments were randomly assigned: C (control, untreated); T1 (0.5% sodium alginate / 3.75% glycerin); T2 (0.5% sodium alginate / 3.75% glycerine / 5% vitamin C); T3 (0.5% sodium alginate / 3.75% glycerine / 5% α-tocopherol); and T4 (0.5% sodium alginate / 3.75% glycerine / 0.1% tea polyphenol).

Food-grade sodium alginate (Modernist Pantry, York, ME, USA) was prepared by mixing 10 g of alginate with 1000 mL distilled water and stirred at a temperature of 70–80 °C until the mixture became clear. Five hundred milliliters (500 mL) solution containing nothing or 100 g vitamin C (United Laboratories, San Juan City, Philippines) or 100 g α-tocopherol (United Laboratories, San Juan City, Philippines) or 2 g tea polyphenol (TP) (Sigma, St. Louis, MO) and 200 mL glycerin were mixed with sodium alginate solution and stirred. The fillets were dipped in the coating solutions for 1 min, air-dried for 1 min, and then immersed in 2% (w/v) food-grade calcium chloride (Modernist Pantry, York, ME, USA) to gel for 1 min. Fillet samples were then packed in polyethylene bags and kept chilled at 4 ± 2 °C in a refrigerator for 21 days. Triplicate fish samples from each treatment were sampled randomly at intervals for microbial, chemical, and sensory analyses.

Proximate Composition

The samples were analyzed for moisture, lipid, crude protein, and crude ash content using AOAC (2002) method.

Microbiological Analysis

Total viable counts (TVC) were determined in plate count agar (Difco) by the pour plate method (AOAC 2002). Fish meat (25 g) was aseptically weighed and homogenized in 225 mL of 0.1% peptone water diluent (pH 7 ± 0.1) incorporated with 2% salt. Serial dilutions, plating, and incubation were carried out for 24–48 h at 37 °C. All counts were performed in triplicate and data were transformed into logarithms of the number of colony-forming units (CFU/g).

Chemical Analyses

Determination of pH. Fish flesh (10 g) was homogenized in 50 mL distilled water and measured using a digital pH meter (C-73 pHasion, Japan).

Determination of total volatile basic nitrogen (TVB-N). The TVB-N content of the fish sample was measured by the method of Conway's dish (Cobb *et al*. 1973). The TVB-N extract of the fish sample in 6% trichloroacetic

acid (TCA) (Sigma) was absorbed by boric acid and then titrated with 0.02 N hydrogen chloride (HCl). The TVB-N content was expressed in mg/100 g fish.

Determination of histamine. Histamine content was analyzed using flourometric method (AOAC 2012) with modifications. Briefly, a 5-g sample was transferred into 50-mL polypropylene tubes and homogenized with 40 mL methanol for 1 min. The homogenates were placed in water bath at 60 °C for 15 min, cooled at room temperature, and then transferred in volumetric flasks; after which methanol was added to the final volume of 50 mL. The homogenates were then filtered using Whatman No. 1 filter paper (Whatman, Maid-stone, England). Using column chromatography, about 1 mL of sample extract was passed into a glass column containing an ion exchange resin at a flow rate of >3 mL/min. Water was added on top of the resin until the eluate reaches 50 mL. The extract was subjected to fluorometric (Trilogy® Turner) reading by pipetting 5 mL of sample into a 50-mL container. This was followed by the addition of 10 mL of 0.1 N HCl and 3 mL of 1 N sodium hydroxide (NaOH). Within 5 minutes of extraction, 1 mL of 0.1% *o*-phthalaldehyde (OPA) (Sigma) solution and 3 mL of 3.57 N phosphoric acid (H_3PO_4) were added. The fluorescence intensity (i) was recorded during the 1.5 h at an excitation wavelength of 450 nm. Histamine content was expressed as mg/100 g sample.

Determination of thiobarbituric acid (TBA) value. The TBA value was determined using the method of Lemon (1975). Fifteen grams (15 g) of fish muscle was extracted using 7.5% TCA, 0.1% propyl gallate, and 0.1% ethylenediaminetraacetic acid (EDTA) (Sigma) in a ratio of 1:2. The homogenate was then centrifuged at 448 x *g* for 15 min. The clear filtrate was added with 5 mL of TBA reagent (0.02 M TBA in distilled water) and was heated to boiling for 40 min. Samples were then cooled and absorbance was measured at 530 nm (UV 7802, Sunny, China). The standard curve was prepared using malondialdehyde (MDA) and results were expressed as mg MDA/kg of tissue.

Determination of K value. *K* value was determined according to the high-performance liquid chromatography (HPLC) procedure of Yokoyama *et al.* (1992) with some modifications. Five grams (5 g) of fish muscle was homogenized for 1 min with 10 mL chilled 0.6 M perchloric acid at 4 °C. The obtained homogenate was centrifuged for 10 min with the revolving speed of 7168 x *g* at 4 °C. The supernatant was then decanted and neutralized to pH of around 6.5 with 1 M potassium hydroxide solution. The precipitated potassium perchlorate was removed by filtration after standing at 2 °C for 30 min. The filtrate was diluted to 50 mL. A 5-µL filtrate obtained from the sample extracts was injected into the HPLC system (Shimadzu LC-10VP, Japan) equipped with a UV/VIS detector and a low gradient pump (Shimadzu LC-10ATVP) with four-channel mixer (Shimadzu FVC-10ALVP) after filtration through a 0.45 µm filter membrane. The separation of the nucleotide products was obtained by a 5 µm C18 column (250 x 4.60 mm i.d.) equilibrated at 30 °C. Sample (5 µL) was injected at a flow rate of 0.5 mL/min and the peak detection was monitored at 254 nm. Correlation coefficient of peak areas against nucleotide standard concentrations and coefficients of variation for each degradation compound were calculated after injecting several replicates of each nucleotide standard solution. The peaks obtained from fish fillet muscle extracts were identified by comparing against the standard solutions. The *K* value was then calculated using the equation below:

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

Sensory Evaluation

The sensory analysis was conducted by a panel of ten semi-trained laboratory panelists according to the European Economic Council (EEC) freshness rating system (Huss 1988) for raw fish. The fillet samples were blind-coded with three-digit random numbers and the sensory attributes were based on a 10-point scale to determine: color discoloration (10 – no discoloration, 1 – extreme discoloration); odor (10 – no off-odors, 1 – unacceptable off-odors); texture (10 – firm, 1 – very soft); flavor (10 – extremely acceptable, 1 – extremely unacceptable); and overall acceptability (10 – extremely desirable; 1 – extremely unacceptable). Scores below 4 corresponded to unacceptable quality or rejection. This was used to determine the shelf life of the flying fish fillets.

Statistical Analysis

The data were subjected to one-way analysis of variance (ANOVA) and expressed as mean ± standard deviation. All statistical analyses were performed using the Statistical Package for Social Sciences, SPSS Version 16.0 for Windows (SPSS Inc., USA). The least significant difference (LSD) procedure was used to test for difference between means (value of $p < 0.05$ was used to indicate significance) (SAS Institute, Cary, NC, USA).

RESULTS

The proximate composition of flying fish revealed 75.91 ± 0.37% moisture, 21.01 ± 0.19% crude protein, 1.21 ± 0.12% lipid, and 1.50 ± 0.70% ash. Results for total viable counts (TVC) during storage are shown in Figure 1. The initial number of bacteria in flying fish muscle was 3.15 log CFU/g. TVC of all treatments increased with storage

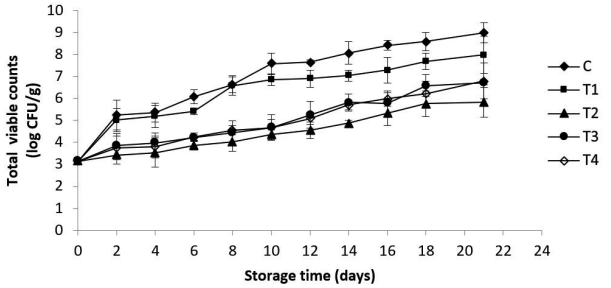

Figure 1. Total viable counts of flying fish fillets coated with alginate-based material during storage at 4 °C (C: uncoated; T1: coated with alginate-based material; T2: coated with alginate-based material with vitamin C; T3: coated with alginate-based material with α–tocopherol; T4: coated with alginate-based material with TP).

time, but the values significantly increased faster for C and T1 ($p < 0.05$). The TVC of C and T1 increased quickly and reached 7.58 and 7.04 log CFU/g, respectively on the 10th and 14th day of refrigerated storage. There were significant differences ($p < 0.05$) between the control (C1) and the treatments (T1, T2, T3, T4), with T2 having significantly lowest ($p < 0.05$) microbial load at the end of the storage period at 5.84 log CFU/g. T2 more efficiently inhibited the growth of bacteria than other antioxidant treatments ($p < 0.05$). The TVC of T3 and T4 did not exceed the limit value of 7.0 log CFU/g (ICMSF 1986) during the entire storage time and no differences were found between them ($p > 0.05$); they reached 6.71 and 6.79 log CFU/g, respectively on Day 21.

For the chemical analyses, pH values decreased initially and then increased as storage progressed (Figure 2A). From Day 8 to the end of storage, the pH values increased but lower pH values were found in T2 and T4 – although not significant ($p > 0.05$). On the other hand, a slow increase in the TVB-N values of all samples was observed in the early stages of storage, but a marked increase was noted after Day 8 (Figure 2B). The initial TVB-N value of the fillets was 13.14 mg/100g, and this increased significantly ($p < 0.05$) with storage for control and treated samples. Based on the results, control samples could maintain their freshness based on TVB-N results for about 10 days, whereas T1, T2, T3, and T4 could maintain fillet quality up to 12 days at refrigerated storage. In addition, TVB-N contents of T1, T2, and T3 were significantly lower than the control at the end of the storage period ($p < 0.05$).

Changes in TBA value of various treatments are shown in Figure 2C. An increasing trend was seen in the TBA values of all treatments throughout the storage period. The present study showed that TBA values of flying fish fillets were low having less than 1 mg MDA/kg in all treatments until the end of the 21-day storage period.

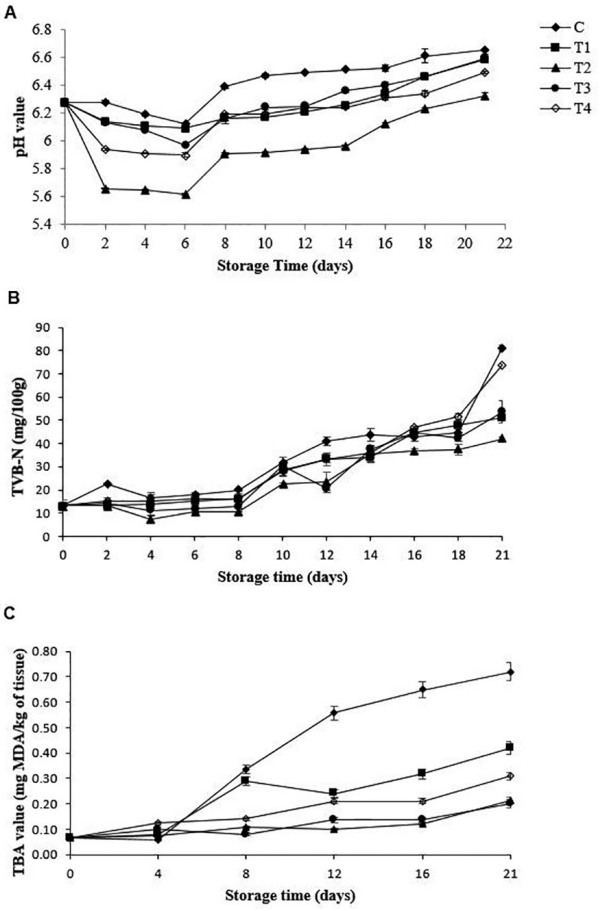

Figure 2. The pH (**A**), total volatile base nitrogen (TVB-N) (**B**), and thiobarbituric acid (TBA) (**C**) values of flying fish fillets coated with alginate-based material during storage at 4 °C (C – uncoated; T1 – coated with alginate-based material; T2 – coated with alginate-based material with vitamin C; T3 – coated with alginate-based material with α–tocopherol; T4 – coated with alginate-based material with TP).

Further, the TBA value of the uncoated sample at 0.72 mg MDA/kg was significantly higher ($p < 0.05$) at the end of the storage period than the coated samples. Notably, very minimal increase in TBA value was observed in T2 and T3 samples.

Low levels of histamine were detected until Day 4 of storage for all the fillet samples (Figure 3A). However, histamine levels rose significantly from day 8 until the end of the storage period for all the treatments. The data show that control samples reached the defect action level of >5 mg/100 g (FDA 1995) at Day 8 with histamine content of 11.95 mg/100g. On the other hand, coated samples T1, T2, T3, and T4 reached the unsafe limit of at days 10, 16, 10, and 14, respectively – with histamine levels of 5.68, 5.10, 7.06 and 6.60 mg/100g, respectively. While variations in *K* value during refrigerated storage are shown in Figure

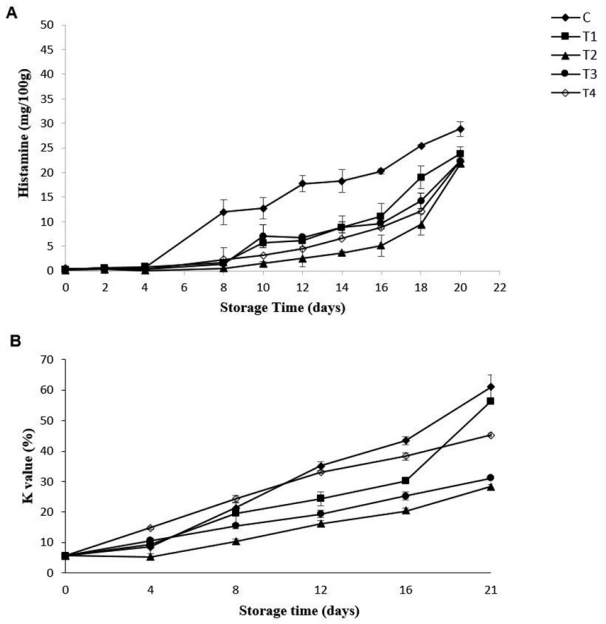

Figure 3. The histamine (**A**) and *K* value (**B**) of flying fish fillets coated with alginate-based material during storage at 4 °C (C – uncoated; T1 – coated with alginate-based material; T2 – coated with alginate-based material with vitamin C; T3 – coated with alginate-based material with α–tocopherol; T4 – coated with alginate-based material with TP).

3B with the initial *K* value at 5.7%. There is a significant increase ($p < 0.05$) in *K* values of uncoated and coated samples from Day 0 to Day 21. According to Ehira (1976), the rejection level of the *K* value is close to 60%. In this study, only the control (uncoated samples) reached the rejection limit at 61.1% at the end of the storage period, whereas T1, T2, T3, and T4 have *K* values of 56.4, 28.3, 31.1, and 45.3%, respectively at Day 21.

The results of the sensory assessment are presented in Table 1. All treatments in terms of color, odor, texture, flavor, and overall acceptability reached "unacceptable" scores of below 4 at Day 16. Generally, samples coated with alginate-based material with antioxidants especially T1 and T2 have higher overall acceptability scores at the end of the storage period than uncoated samples.

DISCUSSION

Variation in proximate components of fish is related to their feeding habits, size, catching season, habitat, and sexual variations – as well as other environmental factors. Based on the proximate components of flying fish, it is a good raw material for processing because of the low lipid content, which makes it less prone to oxidation during storage. A high protein content of *C. intermedius* also makes it an ideal species to process and convert into nutritious fish-based products.

Results of microbial analysis revealed that at the end of storage period, sodium alginate treatments with antioxidants did not exceed the microbial load limit of 7.0 log CFU/g for fresh and frozen fish set by both ICMSF (1986) and Philippine National Standard (2011). This connotes that treatments with combined sodium alginate and antioxidants were effective in retarding microbial growth as compared to control and sodium alginate alone. The significant reduction in TVC of the fillet samples with alginate and antioxidants may be due to the fact that the edible coating acts as a barrier against oxygen transfer and leads to inhibition of aerobic bacterial growth (Song *et al.* 2011). The antimicrobial effect of vitamin C can be due to cell membrane alterations that can cause structural damage to the bacteria. It was observed that such damage has caused irregularly constricted bacterial cells, as observed by phase contrast microscopy upon the addition of vitamin C (Verghese *et al*. 2018). On the other hand, tea polyphenols were demonstrated to have antimicrobial and antioxidant effects by altering bacterial cell membrane mechanisms and as a barrier against oxygen (Feng *et al*. 2017). Scant reports were found on the antimicrobial activity of α-tocopherol as most studies focused on its antioxidant effectiveness. Exceptionally, Ulusoy *et al*. (2009) found out that tocopherol – along with other phenolics contained in rose (*Rosa damascena*) essential oil – demonstrated strong antibacterial activities against some bacterial strains.

Changes in pH can affect the quality of the product during storage. In this study, an observed decrease in pH until Day 6 of storage may be due to the decomposition of glycogen in whole fish, but other studies attribute it to the dissolution of carbon dioxide in the fillet samples (Fan *et al*. 2009, Manju *et al*. 2007). From Day 8 to the end of storage, the pH values increased, which could be attributed to the production of volatile basic compounds such as ammonia and trimethylamine by fish spoilage microbial action. Similar trends were observed by Cai *et al.* (2015), Lu *et al.* (2010), and Song *et al*. (2011) who found an increase after 8 days in pH value of refrigerated Japanese sea bass, after 4 days in refrigerated bream, and after 6 days in snakehead fish fillets at 4 °C, respectively. It can also be observed that at the end of the storage period, lower pH values were found in T2 and T4, which might restrain microbial growth and inhibit the activity of endogenous proteases – thereby preserving the quality of the flying fish fillets.

TVB-N is widely used as an indicator of deterioration in fish muscle and measures the compounds composed of ammonia and amines. A level of 35–40 mg TVB-N/100 g fish muscle is usually regarded as spoiled (Lakshmanan

Table 1. Sensory scores for the different attributes of fillets with different coating treatments during refrigerated storage (C – uncoated; T1 – coated with alginate-based material; T2 – coated with alginate-based material with vitamin C; T3 – coated with alginate-based material with α-tocopherol; T4 – coated with alginate-based material with TP).

Attributes	Treatment	Days of storage					
		0	4	8	12	16	21
Color	C	6.80 ± 0.74^{a}	5.56 ± 0.91^{ab}	6.40 ± 0.63^{ab}	3.64 ± 0.53^{a}	3.96 ± 0.68^{a}	3.84 ± 0.55^{a}
	T1	7.37 ± 0.50^{b}	8.02 ± 0.33^{c}	6.46 ± 0.83^{ab}	5.39 ± 0.80^{b}	4.19 ± 0.77^{a}	4.90 ± 0.47^{b}
	T2	5.64 ± 0.81^{a}	4.47 ± 0.90^{a}	4.93 ± 0.52^{a}	3.43 ± 0.49^{a}	4.29 ± 0.77^{a}	3.74 ± 0.59^{a}
	T3	5.37 ± 0.67^{a}	5.06 ± 0.55^{a}	6.87 ± 0.55^{b}	6.25 ± 0.36^{b}	4.03 ± 0.82^{a}	3.89 ± 0.53^{a}
	T4	6.73 ± 0.47^{a}	7.31 ± 0.50^{bc}	7.33 ± 0.48^{b}	4.79 ± 0.50^{ab}	4.51 ± 0.82^{a}	4.05 ± 0.74^{b}
Odor	C	7.34 ± 0.49^{b}	6.46 ± 0.70^{a}	5.67 ± 0.49^{a}	3.10 ± 0.34^{a}	3.35 ± 0.70^{a}	3.94 ± 0.52^{b}
	T1	7.06 ± 0.73^{b}	7.61 ± 0.52^{a}	6.98 ± 0.76^{ab}	3.98 ± 0.64^{a}	4.82 ± 0.82^{a}	4.88 ± 0.63^{b}
	T2	5.32 ± 0.78^{a}	5.54 ± 0.83^{a}	5.23 ± 0.91^{a}	4.67 ± 0.60^{a}	4.32 ± 0.77^{a}	4.94 ± 0.64^{b}
	T3	6.93 ± 0.53^{b}	5.37 ± 0.64^{a}	7.11 ± 0.44^{ab}	4.63 ± 0.74^{a}	3.41 ± 0.82^{a}	2.66 ± 0.74^{a}
	T4	6.21 ± 0.70^{a}	5.65 ± 0.84^{a}	7.98 ± 0.39^{b}	4.11 ± 0.50^{a}	2.69 ± 0.60^{a}	4.27 ± 0.73^{ab}
Texture	C	7.24 ± 0.69^{b}	7.78 ± 0.34^{b}	5.09 ± 0.72^{a}	5.10 ± 0.66^{ab}	3.87 ± 0.76^{a}	3.37 ± 0.60^{a}
	T1	5.30 ± 0.84^{ab}	6.23 ± 0.61^{ab}	6.15 ± 1.08^{a}	3.04 ± 0.74^{a}	4.05 ± 0.89^{a}	3.71 ± 0.71^{a}
	T2	5.02 ± 0.94^{ab}	5.19 ± 0.71^{a}	6.62 ± 0.50^{a}	5.75 ± 0.65^{b}	4.35 ± 0.99^{a}	4.39 ± 0.64^{b}
	T3	4.88 ± 0.59^{a}	4.82 ± 0.69^{a}	7.48 ± 0.50^{a}	4.79 ± 0.80^{ab}	4.82 ± 0.91^{a}	3.26 ± 0.51^{a}
	T4	4.55 ± 0.78^{a}	6.16 ± 0.71^{ab}	6.38 ± 0.95^{a}	5.44 ± 0.64^{b}	4.26 ± 0.99^{a}	4.20 ± 0.90^{a}
Flavor	C	7.14 ± 0.61^{b}	7.58 ± 0.42^{b}	6.39 ± 0.62^{a}	5.40 ± 0.24^{b}	3.67 ± 0.34^{a}	3.37 ± 0.45^{b}
	T1	5.03 ± 0.14^{ab}	6.20 ± 0.61^{ab}	6.05 ± 0.08^{a}	4.04 ± 0.73^{a}	3.85 ± 0.90^{a}	3.76 ± 0.76^{a}
	T2	5.22 ± 0.24^{ab}	5.29 ± 0.11^{a}	6.12 ± 0.51^{a}	5.15 ± 0.25^{b}	3.55 ± 0.92^{a}	3.38 ± 0.54^{b}
	T3	4.78 ± 0.19^{a}	4.80 ± 0.69^{a}	4.38 ± 0.23^{a}	4.79 ± 0.83^{a}	3.82 ± 0.92^{a}	3.26 ± 0.67^{b}
	T4	4.25 ± 0.88^{a}	4.86 ± 0.71^{a}	4.33 ± 0.45^{a}	4.44 ± 0.44^{b}	3.16 ± 0.90^{a}	3.20 ± 0.09^{b}
Overall	C	7.31 ± 0.50^{a}	7.36 ± 0.72^{a}	6.17 ± 0.71^{a}	4.60 ± 0.64^{ab}	3.08 ± 0.68^{a}	3.35 ± 0.77^{b}
	T1	6.58 ± 0.55^{a}	6.94 ± 0.57^{a}	6.46 ± 1.03^{a}	3.46 ± 0.78^{a}	4.23 ± 0.88^{a}	4.35 ± 0.47^{a}
	T2	5.43 ± 0.49^{a}	6.11 ± 0.66^{a}	6.34 ± 0.63^{a}	5.55 ± 0.35^{b}	4.31 ± 0.83^{a}	4.08 ± 0.56^{a}
	T3	5.57 ± 0.66^{a}	5.62 ± 0.63^{a}	7.98 ± 0.42^{a}	5.59 ± 0.68^{b}	4.17 ± 0.84^{a}	2.72 ± 0.59^{c}
	T4	6.30 ± 0.79^{a}	6.11 ± 0.76^{a}	6.32 ± 0.80^{a}	5.05 ± 0.69^{ab}	3.51 ± 0.86^{a}	4.01 ± 0.67^{a}

Note: ^{a,b,c}Means with different superscript letters in the same row indicate significant difference at $p < 0.05$.

2000). A slow increase in TVB-N observed in this study is in agreement with previous studies concerning other fish species (Hamzeh and Rezaei 2012, Song *et al*. 2011). Increase in TVB-N values during refrigerated storage may be related to microbial activities or several enzymatic processes – including the deamination of free amino acids, degradation of nucleotides, and oxidation of amines (Lu *et al.* 2009). The alginate-calcium coating with antioxidants prolonged the shelf-life of the fillets, which could be attributed to either a more rapidly reduced bacterial population reduction, decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds, or both (Song *et al.* 2011). Moreover, since TVB-N is related to fish muscle deterioration, the use of edible coating with antioxidants might have preserved the structure of the myofibril – the major component of fish muscle. Li *et al.* (2016) showed that addition of 1.2% gelatin and 0.4% tea polyphenol revealed the most intact nanostructure of myofibril after 17 days of cold storage – as analyzed by atomic force spectrometry.

The extent of lipid oxidation in flying fish fillets was measured by variation in TBA values. The presence of TBA reactive substances is due to the second stage auto-oxidation in which peroxides are oxidized to aldehydes and ketones. This may be due to the partial dehydration of fish and the increased oxidation of unsaturated fatty acids (Song *et al*. 2011). A TBA value of 2 mg MDA/kg was regarded as the level beyond which fish will normally develop an objectionable odor and taste (Connell 1990). Lipid oxidation contributes to the formation of unpleasant

odors, rancid taste, and discoloration; it can likewise modify proteins through cross-linking reactions, affecting amino acids and decreasing functionality as a result of protein denaturation (Li *et al.* 2016). However, in this study, low values of TBA were observed in the duration of the storage period; the reason for this may be the low lipid content of flying fish (1.21 ± 0.12%). Also, the alginate-calcium coating with the addition of antioxidants created a film layer on the fish surface that may have been resistant to oxygen diffusion, thereby preventing lipid oxidation (Song *et al.* 2011). Storage at low temperature also minimized lipid oxidation, as revealed by low TBA values for the coated samples. This is in accordance with previous studies that storage of fish at refrigerated and frozen temperatures can have a positive effect on lipid oxidation (Aydin and Gokoglu 2014).

Histamine content as an index of quality was determined in flying fish fillets because of the high free histidine at approximately 473 mg/100 g in the white muscle of flying fish – equivalent to 70% of the total free amino acids in the fish (Tsai *et al.* 2006). Histidine is converted to histamine by decarboxylase enzymes produced by certain bacteria during spoilage (Lehane and Olley 2000). Histamine is potentially hazardous and is believed to be the causative agent in scombroid poisoning. The maximum allowable limit for histamine varies from 10–50 mg/100 g sample depending on the country (Ababouch *et al.* 1991); a US Food and Drug Administration (USFDA) level of >5 mg/100 g indicates decomposition for scombroid fish and/or products and, hence, unsuitability for human consumption (FDA 1995). In this study, the rate of histamine development in flying fish fillets was slowed down due to refrigeration temperature (4 ± 2 °C). In addition, alginate-calcium coating with antioxidants proved to be an effective barrier against bacterial decarboxylases capable of rapidly converting free histidine in the flying fish muscle to histamine.

K value, as an index adenosine triphosphate (ATP) degradation, is widely used as an effective indicator of fish freshness. During post-mortem 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 as an index of freshness before bacterial spoilage commences in the fish muscle (Huss 1988). The *K* value of freshly caught fish is around 5% as reported by Huss (1988), and this is study reported an initial *K* value of 5.7%. The low *K* values observed in the alginate-coated samples with antioxidants may be due to the reduced activity of 5-nucleotidase as a result of the bioactive alginate-calcium coating (Fan *et al.* 2009) and slower ATP degradation (Sugimoto and Fujiata 1986).

The acceptability of fish and fishery products upon storage is dependent on their sensory attributes. The fillet samples were considered acceptable for human consumption until sensory scores dropped to 4. These findings seem to correlate well with microbial and chemical quality analyses. Good quality characteristics in terms of sensory assessment was retained in coated samples because edible coatings serve as good oxygen barriers – retarding lipid oxidation and improving sensory characteristics (Li *et al.* 2016).

CONCLUSION

The combined effect of alginate-calcium coating and natural antioxidants maintained better quality of flying fish fillets than uncoated samples. Samples treated with alginate-calcium coating with added vitamin C (T2) reduced the degree of microbial deterioration more efficiently than other treatments. Further, the same treatment also slowed down chemical changes such as lower TVB-N, histamine, TBA, and *K* values compared with other coated samples and control. Therefore, these edible coatings could be possible alternatives to synthetic materials in improving the quality of refrigerated fish. Standardization of the methods such as shortening fillet process time can be done to improve the quality of the product. Further optimization experiments on the levels of edible coating that is acceptable on a wider range of consumer and a pilot scale study should also be considered in the future for this product to be commercially feasible.

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