Quality Assessment of Marinated Flying Fish (Cheilopogon intermedius) Fillets During Vacuum-packed Storage at 4 °C

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Flying fish is considered a low-value fish in the Philippines but is a good source of inexpensive animal protein. The challenge is to effectively utilize this vulnerable raw material to increase its market value. The study evaluated the physicochemical changes and sensory attributes of flying fish (Cheilopogon intermedius) fillets marinated in three different marinating solutions (Marinade 1: “ham flavor”; Marinade 2: “salty-sour flavor”; Marinade 3: “spiced flavor”) and control (un-marinated) followed by vacuum-packaging then stored at 4 °C for 20 d. Chemical analyses revealed a significant reduction (p < 0.05) in the total volatile base nitrogen (TVB-N), trimethylamine nitrogen (TMA-N), histamine level, and thiobarbituric acid (TBA) value in the marinated fillets in comparison with the control. After 20 d of storage at 4 °C, mesophilic and psychrophilic bacterial counts reached 10^7–10^9 and 10^6–10^8 CFU/g, respectively. The panelists considered samples unfit for human consumption on Day 12 when the mesophilic and psychrophilic bacterial counts exceeded 10^6 CFU/g. No significant differences (p > 0.05) were detected for the overall sensory acceptability of the marinated samples; however, Marinade 1 was found to be most acceptable, followed by Marinades 2 and 3. The shelf-life of the marinated flying fish based on microbiological and sensory analyses at refrigerated storage (4 °C) was 12 d.

Keywords: flying fish, marination, physicochemical change, sensory attributes, vacuum packaging

INTRODUCTION

Flying fish (family Exocoetidae) is an important group in the pelagic fish catch of coastal regions in many parts of the world as edible fish as well as baitfish in many tuna fisheries. Flying fish or locally known as “bangsi” is among the most dominant catches in some areas in the Philippines like the western portion of the Verde Island Passages in the West Philippine Sea and around the Camotes Sea in the Visayan Seas (Dalzell 1993; Emperua et al. 2017). There was no exact production data on flying fish caught in Philippine waters because the fishing method is not as developed as tuna, scads, or sardines (Simora et al. 2016). But in the southern Philippines, flying fish constitute the second most dominant fishes caught next to round scad, and from 2005–2015, flying fish catch has a total volume of 28,596.6 MT, valued at PHP 1,041,042,350.00 (Emperua
Cheilopogon intermedius is one of the most abundant flying fish species in the Philippines but is considered to be a low-value fish. Although it has become a popular source of inexpensive animal protein for lower-income groups due to its high protein content, flying fish is a vulnerable raw material for processing. Histidine at approximately 473 mg/100 g is the most prominent free amino acid (FAA) in the white muscle of flying fish and accounts for 70% of the total FAAs in the fish (Kung et al. 2015). Histidine, through bacterial action and endogenous enzymes, can be converted to histamine which is a foodborne chemical hazard (Taylor et al. 1989). Due to this fact, existing methods of preservation for flying fish are limited to salting and drying. Adequate post-harvest technologies for effective utilization of this important fishery resource to increase its market value are essential. Marination can be an alternative way to process flying fish and extend its shelf-life, so it can reach the prime market.

One of the oldest methods used for fish preservation is marination. The term “marinades” or “marinated fish” is used to define fish products, which consist of fresh, frozen, or salted fish or portions of fish processed by treatment with an edible organic acid, usually acetic acid, and salt and put into brines, sauces, or oil (Espejo-Hermes 1998). This technique is usually practiced in order to improve the flavor and textural properties of various seafood (Hwang and Tamplin 2005). The marinades used for preservation are semi preserves in which acid, usually acetic acid, and salt is added to the fish to retard the action of bacteria and enzymes. The resulting product has a characteristic flavor and an extended but limited shelf life. Marinades stored at a cooler temperature (4–6 °C) can be kept for a longer time (Sallam et al. 2007).

Chemical, microbial, and sensory methods have been used to assess the quality and shelf life of fish and fishery products during handling and storage. Since fish generally spoil faster than other muscle foods, an adequate set of reliable and quantitative standards should be used in measuring spoilage. Deterioration of fish mainly occurs as a result of bacteriological and enzymatic activity leading to the loss of quality and subsequent spoilage (FAO 1995). Enzymatic and chemical reactions cause an initial loss of freshness in fish, whereas microbial activity is responsible for overt spoilage, which can be the basis for establishing product shelf life (Gram and Huss 1996). Spoilage of fish is a complex process in which physical, chemical, and microbiological mechanisms are implicated. Therefore, alternative means of efficient fish preservation are necessary to improve and extend the shelf-life of the product.

The objectives of the present study were to produce marinated products from flying fish and to evaluate the effects of the marination process on the physicochemical quality and sensory attributes of the marinated product with a vacuum package stored at refrigerated temperature.

**MATERIALS AND METHODS**

**Sampling, Preparation, and Marination of Fish**

One hundred kilograms (100 kg) of fresh ice-chilled flying fish (C. intermedius) was purchased, within 4 h post-harvesting, from a fishing village market in San Jose, Antique, Philippines. Fish were packed in polystyrene boxes with a sufficient amount of flake ice and transported to the laboratory within 1 h. The average length and body weight of fish samples were 49.02 ± 9.02 cm and 143.31 ± 6.26 g, respectively. Upon arrival, fish were immediately washed, headed, gutted, and filleted into two sides. Fillet samples were then divided into four batches and placed in different marinating solutions. The first batch was marinated by immersing the fish fillets in a pre-chilled (4 °C) solution depicting the “ham flavor,” referred to as Marinade 1, with a combination of 20% NaCl (w/v), 30% brown sugar (w/v), 0.02% bay leaves (w/v), 0.05% nutmeg (w/v), 0.05% black pepper (w/v), 0.5% Prague powder (6.25% sodium nitrite and 93.75% sodium chloride) (w/v), and 0.05% paprika (w/v). The second batch was marinated in a pre-chilled (4 °C) solution having the “salty-sour” taste, referred to as Marinade 2, with a combination of 15% NaCl (w/v), 35% white vinegar (v/v), 10% white sugar (w/v), 2.4% garlic powder (w/v), 2% black pepper (w/v), and 2% bay leaves (w/v). While the third batch was marinated in a pre-chilled (4 °C) solution with the “spiced flavor,” referred to as Marinade 3, with a combination of 20% NaCl (w/v), 50% white vinegar (v/v), 0.04% cayenne pepper (w/v), 2.25% white sugar (w/v), 0.08% garlic powder (w/v), 0.5% hot sauce (w/v), 0.09% white pepper (w/v), and 0.06% paprika (w/v). The fourth batch of fresh fillets was kept untreated and served as control (un-marinated).

The marinating processes were completed after 1 h at 4 °C. The fish to solution ratio was 1:1.5, and the mixture was stirred at 10-min intervals. After marination, fish fillets were removed from the marinating solution and left to drain on stainless steel wire mesh for 20 min. About 30 pieces of fillets from each treatment and control were vacuum-packed in polyamide bags (8 x 11.90 µm thick), labeled, and stored at 4 °C in a refrigerator with a digital temperature controller (Inkbird-ITC308, China) for 20 d. At 2-d predetermined time intervals, three randomly chosen packs were taken from each batch and were analyzed for physicochemical, microbiological, and sensory analyses.

**Microbiological Analysis**

Twenty-five grams (25 g) of fish meat was aseptically weighed and homogenized in 225 ml of 0.1% peptone.
water diluent (pH 7 ± 0.1). Serial dilution was prepared and 1 ml of each decimal dilution was pipetted into sterile plates. Total mesophilic aerobic bacteria were determined using plate count agar (PCA, Difco) after incubation for 48 h at 30 °C. PCA was also used for psychrophilic bacteria and incubated at 7 °C for 10 days (AOAC 2002). All counts were performed in triplicate and data were transformed into logarithms of the number of colony-forming units per gram of a sample (log CFU/g).

Compositional Analysis
Prior to vacuum-packaging and storage at refrigerated temperature, composite fresh samples of the marinated and un-marinated fish fillets were analyzed for moisture, protein, ash, and lipid based on the AOAC (2002) methods of analysis. Analyses were conducted in triplicate and all reagents were of analytical grade.

pH Measurement
Ten grams (10 g) of each sample was blended in 20 ml distilled water for 1 min and the pH value of the fish homogenate was measured by a digital pH-meter (C-73 pHasion, Japan) standardized at pH 4, 7, and 9.

Water Holding Capacity (WHC)
WHC of the samples was determined by low-speed centrifugation, as described by Eide et al. (1982) with slight modifications. Briefly, 2 g of homogenized fish sample was weighed into a 50-ml conical tube inserted with Whatman No. 1 filter paper and immediately centrifuged at 750 X g for 5 min. The liquid loss is expressed as the percentage of weight lost during centrifugation. The weight loss was divided by the water content of the sample and expressed as % WHC.

TVB-N and TMA-N
The TVB-N and TMA-N of the fish sample were measured, in triplicate, by Conway microdiffusion method (Woyewoda et al. 1986). The 10-ml TVB-N extract of the fish sample in 10-ml 6% trichloroacetic acid (TCA) (Sigma) was absorbed by boric acid, then added with 1 ml saturated potassium carbonate solution and titrated with 0.02 N hydrogen chloride. The TVB-N content was expressed in mg/ 100 g fish.

The same experimental procedure as for TVB-N was used for the TMA-N measurement but, prior to the addition of potassium carbonate, 1 ml of 10% neutralized formalin was pipetted to the extract to react with ammonia and, thus, allow only the TMA-N to diffuse over the unit. The TMA-N content was expressed in mg/ 100 g fish.

Histamine Determination
Histamine content was analyzed using the fluorometric 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 a water bath at 60 °C for 15 min, cooled at room temperature, then transferred in volumetric flasks and methanol was added to the final volume of 50 ml. The homogenates were then filtered using Whatman No. 1 filter paper. 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. The extract was then subjected to fluorometric (Trilogy®, Turner, USA) reading by pipetting 5 ml of sample into a 50-ml container. The fluorescence intensity (i) was recorded during the 1.5 h at an excitation wavelength of 450 nm. Histamine content was expressed as mg/ 100g sample.

TBA Determination for Rancidity
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% EDTA (ethylenediaminetetraacetic acid) in a ratio of 1:2. The homogenate was then centrifuged at 1000 X g for 15 min. The clear filtrate (5 ml) was added with 5 ml of TBA reagent (0.02M 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.

Sensory Evaluation
The sensory analysis was conducted according to the European Economic Council freshness rating system (FAO 1995) for fish. Ten semi-trained laboratory panelists, who are familiar with the raw material as well as with marinated fish products, evaluated the sensory attributes at each sampling point. The fillet samples were steamed and 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 – characteristic fresh fish flavor; 1 – unacceptable off-flavors); and overall acceptability (10 – extremely desirable; 1 – extremely unacceptable). Scores below 4 corresponded to unacceptable quality or rejection.

Statistical Analysis
The data were subjected to one-way analysis of variance 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 procedure was used to test for the difference between means (value of $p < 0.05$ was used to indicate significance) (SAS Institute, Cary, NC, USA).

RESULTS AND DISCUSSION

The additive and/or synergistic effects of combining marination and refrigerated storage contributed to the preservation of flying fish fillets and, thus, extending the shelf-life of the product. The major ingredients used in the marination are vinegar, salt, and spices such as garlic and pepper. The application of vinegar as a food preservative is a traditional method of preventing spoilage because it is an effective acidulant, causing depression of pH below the growth range of many bacteria (Jay 2000). Salt is added to foods for its effects on sensory, functional, and preservation properties. It inhibits microbial growth by restriction of the available water (i.e. lowers $a_w$) in the meat and fish products (Sallam et al. 2007). The presence of natural antimicrobial substances in spices (garlic and pepper) also prevented the proliferation of food spoilage bacteria. Vacuum-packaging also extended the shelf-life of the product as it is effective in reducing oxidative reactions in the product at relatively lower costs. The complete removal of oxygen ensures longer preservation against microbial deterioration and provides some increase in shelf life (Kumar and Ganguly 2014).

Microbiological Changes in Marinated Flying Fish Fillets

Changes in mesophilic and psychrophilic bacterial counts of flying fish marinades during refrigerated storage are shown in Figure 1. Low initial bacterial counts of the vacuum-packed marinated flying fish, with levels below $10^2$–$10^3$ CFU/g for both mesophilic and psychrophilic bacteria, indicate high fish quality and good manufacturing practices during product preparation. Generally, the number of mesophilic bacteria increased throughout storage time reaching to a maximum of 9.83 log CFU/g, 8.66 log CFU/g, 7.34 log CFU/g, and 7.66 log CFU/g for control, Marinades 1, 2, and 3, respectively, at the end of the storage period (Figure 1A). The mesophilic bacterial count was significantly lower in Marinade 2 as compared to control ($p < 0.05$), but no significant differences ($p > 0.05$) were observed among the marinade treatments. On the other hand, the number of psychrophilic bacteria also increased throughout storage time reaching to a maximum of 8.33 log CFU/g, 7.66 log CFU/g, 6.74 log CFU/g, and 7.35 log CFU/g for control, Marinades 1, 2, and 3, respectively, at the end of the storage period (Figure 1B). A significant difference was observed between the psychrophilic bacterial count of Marinade 2 and control ($p < 0.05$), but no significant differences ($p > 0.05$) were observed among the marinade treatments.

It has been reported that bacteria are not completely killed by marinating and live cells are still able to grow in marinated samples. In this condition, they are able to continue their activity more or less rapidly according to their ability to adapt to the medium during storage (Fuselli et al. 1994). Another factor contributing to the increase in the microbial count would be the growth of specific spoilage organisms such as Aeromonas spp. and Shewanella putrefaciens aside from the Gram-negative psychrotrophic rods under vacuum-packed conditions (FAO 1995). Lyhs et al. (2001) showed that H$_2$S-producing bacteria occur in high numbers and represent an important part of the spoilage flora of vacuum-packaged “gravid” rainbow trout.

However, the increase in the bacterial count for the control samples was almost 2.0 log higher than the marinated samples. The results obtained in this study is in agreement with the findings of Sallam et al. (2007) in which reduction rates of 1.55- and 1.7- logs were achieved for marinated Pacific saury (Cololabis saira) fillets in 2% and 3% acetic acid combined with 12% NaCl, respectively, in comparison with the control samples. The growth of microorganisms is decelerated in brines with a salt content of 6.5% and if acetic acid is present, the bacteria
are more sensitive to the acidic condition (Jay 2000). Salt inhibits microbial growth by restriction of the available water in the fish meat. Acetic acid, on the other hand, can penetrate through the cell membrane of microorganisms where it can acidify the cytoplasm and denature proteins (Eklund 1983). The application of vinegar is an effective acidulant that causes the depression of pH below the growth range of many bacteria (Jay 2000). Similar results were also reported by Kilinc and Cakli (2005) in which microbial count decreased from 4.88 log 10 CFU/g to < 10 log CFU/g in raw sardines (Sardinia pilchardus) after marination using 7% acetic acid and 14% NaCl solution. Furthermore, the presence of antimicrobial compounds such as allicin in garlic and piperine in pepper, which are both effective against a wide range of Gram-positive and Gram-negative bacteria (Lai and Roy 2004), also contributed to the positive effect of marination in control of bacterial growth. This accords with previous observations, which showed a positive effect on reducing the number of bacteria in the marinated fish samples with the incorporation of spices such as garlic and black pepper (Pakawatchai et al. 2009; Maktabi et al. 2016).

### Physicochemical Changes

**Proximate composition.** Variations in the chemical composition of the fish muscle depend on age, sex, environment, and season (FAO 1995). It is important to determine the chemical composition of fish for processors to know the nature of the raw material before adequate processing techniques can be correctly applied. The proximate composition, on a dry matter basis, of the control and marinades is shown in Table 1. Significant differences were detected in the moisture and protein contents of the control and marinated samples ($p < 0.05$), while no significant differences were observed for lipid and ash contents ($p > 0.05$). It can be observed that the moisture content significantly decreased ($p < 0.05$) when the raw fillets were processed into different marinades. The decrease in moisture content might be attributed to the presence of organic salts in the marinades, which may have replaced some of the water present in the raw material (Espejo-Hermes 1998; Adepoju et al. 2018). Protein was found significantly highest ($p < 0.05$) in Marinade 2 while lowest in the control samples. Processing methods such as marination can cause fish fillets to lose water with a consequent increase in protein content (Colakoglu et al. 2011). This may be due to the considerable proteolysis that may have occurred as a result of enzymatic activity during the “ripening” process and high proteinaceous compounds were produced in the meat (Espejo-Hermes 1998; Tokur 2007). Marinades 1 and 2 have significantly high ($p < 0.05$) crude protein contents because these treatments contained vinegar, which may have contributed to a faster “ripening” process, thus increasing the production of proteinaceous compounds in fish meat. Results obtained in this study are in agreement with Sallam et al. (2007) who observed that the brining and marination processes in Pacific saury (Cololabis saira) fillets decreased the moisture content and increased the other components analyzed in comparison with the control raw samples.

**Changes in pH and WHC.** Table 2 shows the changes in pH and WHC of flying fish marinades during refrigerated (4°C) storage. A slight increase in pH was observed at the end of the storage period for both control and marinated samples; however, such an increase in pH was higher in the control samples than in marinated samples. This finding is consistent with those reported for marinated anchovies (Poligne and Collignan 2000), marinated sardine (Kilinc and Cakli 2005), and marinated saury (Sallam et al. 2007) during storage at refrigerated temperature. During the storage of marinades, heterofermentative lactic acid bacteria can grow and degrade the amino acids with the formation of carbon dioxide and other decarboxylation products, which bind acetic acid and raise the pH of marinades (Shenderyuk and Bykowski 1989).

On the other hand, WHC of muscle is regarded as an essential quality parameter and a high WHC is of great importance to the industry and to the consumer. It influences the appearance of the muscle before cooking, its behavior during cooking, and its juiciness when consumed (Olsson et al. 2003). Although WHC decreased throughout the storage period, still all treatments had more than 80% WHC at the end of the 20-d storage. These values were higher compared to those obtained by Varghese and Mathew (2017) in which the WHC of climbing perch was 54.90% at the end of the 18-d storage in ice. The decrease in WHC could be caused by the water-protein

### Table 1. Proximate composition (% dry basis) of marinades from flying fish at Day 0 of refrigerated storage (4°C).

<table>
<thead>
<tr>
<th>Product</th>
<th>Moisture (%)</th>
<th>Lipid (%)</th>
<th>Ash (%)</th>
<th>Crude protein (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control, un-marinated</td>
<td>75.91 ± 0.37a</td>
<td>1.21 ± 0.12a</td>
<td>1.50 ± 0.07a</td>
<td>21.01 ± 0.19b</td>
</tr>
<tr>
<td>Marinade 1</td>
<td>72.06 ± 0.16a</td>
<td>0.92 ± 0.07a</td>
<td>1.29 ± 0.05a</td>
<td>20.38 ± 0.11a</td>
</tr>
<tr>
<td>Marinade 2</td>
<td>71.44 ± 0.12a</td>
<td>1.84 ± 1.13a</td>
<td>0.93 ± 0.95a</td>
<td>25.54 ± 0.12a</td>
</tr>
<tr>
<td>Marinade 3</td>
<td>73.80 ± 0.10a</td>
<td>1.28 ± 0.18a</td>
<td>1.00 ± 0.07a</td>
<td>23.63 ± 0.11a</td>
</tr>
</tbody>
</table>

*a,b,c,d* Means ± SD in the same column followed by different superscripts are significantly different ($p < 0.05$).
associations in fresh raw fish muscle, which were partly replaced by protein-protein interactions during storage. The muscle cell shrinks, causing the liquid to leak out of the cells to the intercellular space of the fish meat (Ofstad and Hermansson 1997). The minimal decrease in WHC observed in the samples can be attributed to the action of salt and marinades, which increases the strength of water-binding besides expanding the myofibrils. Refrigerated temperature also contributed to the minimal water loss as the myofibril becomes firmer and the ability of the salt solution to depolymerize the muscle filaments might be increased (Ofstad and Hermansson 1997).

### Changes in TVB-N and TMA-N

The initial TVB-N in the control sample was 5.55 mg/100g while for Marinades 1, 2, and 3, initial TVB-N values were 4.62, 4.67, and 5.05 mg/100 g, respectively (Figure 2A). Marinating processes did produce a reduction in the TVB-N values, especially for Marinades 2 and 3 at Days 2 and 4 of refrigerated storage. Similarly, Kilinc and Cakli (2005) determined a considerable decrease in TVB-N from 10.3 to 6.5 mg/100 g after the marinating process of sardine fillets in a solution containing 7% acetic acid and 14% salt. However, during storage, a significant increase (p < 0.05) was observed in the TVB-N values of all the treatments and at the end of the 20th day of storage, TVB-N values increased to a maximum of 24.51, 13.86, 10.26, and 24.66 mg/100 g for control, Marinades 1, 2 and 3, respectively. A maximum permissible level of 35 mg/100 g TVB-N in fish flesh was specified by European Commission guidelines (Commission Decision 95/149/EC 1995) but the marinated flying fish fillets were still below this limit by > 10 mg/100 g TVB-N values, which indicates that the marinating solutions effectively reduced the chemical changes in the marinated fillets. A gradual increase in TVB-N during the initial stages of storage may be initiated by autolytic degradation of nucleotides and free amino acids, while an abrupt increase at the late stages of storage is most likely caused by a combination of microbial and autolytic activities, as well as the complete microbial reduction of trimethylamine oxide (TMAO) to TMA (Sallam et al. 2007). Higher TVB-N values for the control and Marinade 3 at Day 20 can be explained by higher microbial counts of these samples since TVB-N is produced mainly by bacterial decomposition of fish flesh. TMAO-reducing microorganisms involved the genera of bacteria typical of the marine environment (Alteromonas,

### Table 2. Changes in pH and WHC of flying fish marinades during storage at 4 °C.

<table>
<thead>
<tr>
<th>Storage time (d)</th>
<th>Control</th>
<th>Marinade 1</th>
<th>Marinade 2</th>
<th>Marinade 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>WHC (%)</td>
<td>pH</td>
<td>WHC (%)</td>
<td>pH</td>
</tr>
<tr>
<td>0</td>
<td>6.20±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>96.18±4.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>5.48±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>82.56±0.48&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>6.80±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>93.98±1.52&lt;sup&gt;d&lt;/sup&gt;</td>
<td>6.25±0.05&lt;sup&gt;c&lt;/sup&gt;</td>
<td>83.33±1.02&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>6.55±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>92.91±0.91&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.37±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>80.49±2.2&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>6.58±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>93.43±1.34&lt;sup&gt;b&lt;/sup&gt;</td>
<td>6.37±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
<td>83.30±0.01&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>8</td>
<td>6.41±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.96±2.58&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.13±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.30±2.72&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>10</td>
<td>6.40±0.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.98±2.45&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.14±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>91.13±1.31&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>12</td>
<td>6.39±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>87.87±2.59&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.22±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>91.55±0.32&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>16</td>
<td>6.40±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.67±0.91&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.28±0.00&lt;sup&gt;b&lt;/sup&gt;</td>
<td>88.17±1.35&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>20</td>
<td>6.36±0.06&lt;sup&gt;b&lt;/sup&gt;</td>
<td>83.44±0.46&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.29±0.02&lt;sup&gt;c&lt;/sup&gt;</td>
<td>80.72±0.81&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

* Means ± SD in the same column followed by different superscripts are significantly different.
* Means ± SD in the same row followed by different superscripts are significantly different.
Photobacterium, Vibrio, and S. putrefaciens) but are also carried out by Aeromonas and intestinal bacteria of the Enterobacteriaceae. During anaerobic conditions, these bacteria can use a variety of carbon sources as the substrate in its TMAO-dependent anaerobic respiration, including formate and lactate (DiChristina and DeLong 1994) and still can cause spoilage in vacuum-packed products.

In seafood spoilage, TMA-N particularly contributes to the characteristic ammonia-like off-odor and “fishy” off-flavors (Gram and Huss 1996). The initial TMA-N level in raw fillets was 0.53 mg/100 g. As shown in Figure 2B, there was a slight increase in TMA-N values from Days 2–6 of refrigerated storage, although TMA-N levels for Marinade 2 remain low until the end of the storage period at 1.48 mg/100 g. TMA-N levels of control plus Marinades 1 and 2 increased significantly (p < 0.05) from Day 8 onwards with TMA-N values of 10.68, 7.10, and 7.02 mg/100 g, respectively at the 20th day of storage. The lower TMA-N contents in the marinated fillets than in control samples can be attributed to the inhibitory effects of acetic acid, sugar, and spices present in the marinades on microbial growth, including the TMAO-reducing microorganisms. Moreover, a similar pattern of increase in the TMA-N level during refrigerated storage was also observed in marinated Pacific saury (Sallam et al. 2007), marinated sardine (Kilinc and Cakli 2005), and vacuum-packed, salted sea bream (Chouliara et al. 2004). FAO (1995) indicated that a good quality fish contains < 1.5 mg/100 g TMA-N and the limit of acceptability is 10–15 mg/100 g TMA-N. In this study, the control samples reached the TMA-N acceptability limit on the 20th day of storage at 10.68 mg/100 g, while the marinated fillets did not exceed such value at the end of the storage period.

Changes in Histamine Content

The present study used very fresh raw material as reflected in the initial histamine content of 0.21 mg/100 g of flying fish on Day 0. As shown in Figure 3A, an increase (p < 0.05) in the value of histamine was observed during the late stages of refrigerated storage. Histamine levels for control samples increased significantly throughout storage with 7.68 mg/100 g histamine on the 20th day of storage. According to Philippine National Standard (2011) guidelines for quick-frozen fish fillets, histamine values of > 20 mg/100 g is indicative of a decomposed sample. In this study, none of the samples exceeded the histamine limit but the control samples had a significant increase (p < 0.05) in histamine levels at the end of the storage period as compared to the treatments. Meanwhile, histamine values of Marinades 1, 2, and 3 were 1.53, 0.13, and 0.19 mg/100 g, respectively, at the end of the storage period. The low histamine values only suggest that the marinating solutions were effective in preventing the development of histamine in the fish muscle.

Changes in TBA

TBA values is a widely used indicator for the assessment of the degree of lipid oxidation in fish and fish products. It has been suggested that the maximum TBA value, indicating good quality fish, is 5 mg malonaldehyde (MA)/kg while fish may be consumed safely up to a TBA value of 8 mg MA/kg (FAO 1995). In the present study, the TBA values of marinades at Day 0 were 0.08, 0.34, 0.12, and 0.32 mg MA/kg for control, Marinades 1, 2, and 3, respectively (Figure 3B). After Day 4 of refrigerated storage, a sharp increase in the initial TBA values to high levels of 0.41, 0.73, 0.24, and 0.58 mg MA/kg were observed in control, Marinades 1, 2, and 3, respectively. During storage, there was a tendency towards an increase in TBA values up to a maximal point (8 d of storage), followed by a gradual decrease to lower values (12 d of storage) and a gradual decrease until Day 20. The decrease in TBA content after the peak point has been attributed to the interaction between MA and decomposition products of protein to give tertiary degradation products of proteins, which could cause instability and easy reaction with the carbonyl compounds (Fernandez et al. 1997). However, statistical analysis revealed that there are no significant differences (p > 0.05) in the TBA value of all samples during storage. The present results indicated that oxidative rancidity in flying fish fillets remained relatively low throughout the entire period of vacuum-packed storage at 4 °C and its level was within the acceptability limits.
for fish consumption. This is due to the low lipid content of flying fish being considered as a lean fish species. The level of TBA and the pattern of its increase in this study was similar to that reported for various fish species brined in different concentrations of NaCl solution during vacuum-packaged refrigerated storage, including threadfin bream (Jeevanandam et al. 2001), sea bream (Chouliara et al. 2004), and club mackerel (Goulas and Kontominas 2005). The TBA values in these studies also increased to a maximal level at a certain period during storage, and thereafter it decreased gradually.

Sensory Evaluation
The overall sensory acceptability of the flying fish fillets (pooled data over the storage period) is presented in Figure 3C. Although no significant differences (p > 0.05) were found between the samples, Marinade 1 was found to be the most acceptable (9.54), followed by Marinade 2 (9.46), Marinade 3 (9.17), and control (9.16) at the initial day of storage. On Day 12, the panelists considered the control samples to be unfit for human consumption with a score of 4.0 as the samples were described as soft, slightly dark in color with a slight bitter taste, lack of typical product odor, and presence of slight ammonia off-odor. However, no off-odor could be detected by any of the panelists in the marinated samples, which are organoleptically evaluated until the end of the storage period.

CONCLUSION
The quality and acceptability of marinated products are mainly, if not solely, dependent on how the maturation process proceeded. Apart from that, the raw material used also determines the quality of the final product and the technological process of marinating, given that it is a process without heat treatment and is dependent on the degree of ripening. The present study revealed that the different marinating solutions used in flying fish fillets can delay the physicochemical changes as compared to control samples. The combined effects of vacuum-packed storage at 4 °C and marinating in the presence of natural antimicrobial substances (in garlic and spices) as well as brining and addition of an acidulant (vinegar) decelerated the growth of microorganisms. The shelf-life of the marinated flying fish based on microbiological and sensory analyses at refrigerated storage was 12 d. Results of this study suggest that flying fish as a low-value species can be utilized in the marinade formulation, which can be an alternative means of cost-effective preservation yet a high market potential for flying fish.

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CONFLICT OF INTEREST
The authors declare no conflict of interest.

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