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The effect of vacuum packaging on histamine changes of milkfish sticks at various storage temperatures

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ABSTRACT

The effects of polyethylene packaging (PEP) (in air) and vacuum packaging (VP) on the histamine related quality of milkfish sticks stored at different temperatures (−20°C, 4°C, 15°C, and 25°C) were studied. The results showed that the aerobic plate count (APC), pH, total volatile basic nitrogen (TVBN), and histamine contents increased as storage time increased when the PEP and VP samples were stored at 25°C. At below 15°C, the APC, TVBN, pH, and histamine levels in PEP and VP samples were retarded, but the VP samples had considerably lower levels of APC, TVBN, and histamine than PEP samples. Once the frozen fish samples stored at −20°C for 2 months were thawed and stored at 25°C, VP retarded the increase of histamine in milkfish sticks as compared to PEP. In summary, this result suggested the milkfish sticks packed with VP and stored below 4°C could prevent deterioration of product quality and extend shelf-life.

1. Introduction

Histamine is a biogenic amine responsible for scombroid poisoning. Scombroid poisoning is usually a mild illness with a variety of symptoms including rash, urticaria, nausea, vomiting, diarrhea, flushing, and tingling and itching of the skin [1]. The severity of the symptoms can vary considerably with the amount of histamine ingested and the individual's sensitivity to histamine. Scombroid fish are a type of fish commonly involved in scombroid poisoning due to the high levels of free histidine in their muscle tissue. These species include tuna, mackerel, bonito, and saury [1]. However, several species of nonscombroid fish, such as mahi-mahi, bluefish, herring, and sardine, have also often been implicated in incidents of scombroid poisoning [1,2]. In Taiwan, scombroid poisoning has occurred occasionally [2–7], and the fish implicated in these outbreaks were tuna, mackerel, swordfish, marlin, and milkfish.

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Milkfish (*Chanos chanos*) is an important aquacultured fish in the Indo-Pacific region, particularly the Philippines, Indonesia, and Taiwan [8]. Due to its abundance, reasonable domestic price, and delicious taste, milkfish has become a popular species for many producers and consumers. The fish has a free amino acid (FAA) pattern similar to migratory fish, such as skipjack, mackerel, and tuna, which possess high levels of histidine in their white muscle. A study reported that histidine at approximately 441 mg/100 g was the most prominent FAA in the white muscle of milkfish and accounted for 80% of the total FAAs in the fish [9]. Tsai et al [10] reported that milkfish was a better substrate than sailfish for histamine formation by bacterial histidine decarboxylation at elevated temperatures (> 15°C). We first reported that dried milkfish product could cause histamine intoxication (the food-borne poisoning incident occurred in southern Taiwan, in February 2006), and *Raoultella ornithinolytica* was the major histamine-producing bacterium responsible for the high content of histamine in the implicated milkfish sample [4].

Vacuum packaging (VP) is an effective packaging technology that offers a way of prolonging the shelf-life of perishable fish by excluding oxygen and inhibiting the growth of aerobic spoilage bacteria [11]. Additionally, VP fillets have a small package volume, making international transport easier. Therefore, storage under VP has been given increasing attention recently [11]. Under aerobic storage conditions, several Gram-negative genera, particularly *Pseudomonas*, *Aeromonas*, *Shewanella*, and *Enterobacteriaceae* dominate the spoilage microorganisms of freshwater and marine fish. However, VP can inhibit the growth of aerobic bacteria commonly present on fish, resulting in an increase in Gram-positive bacteria that can respire better than Gram-negative bacteria in this type of packaging [12].

Because milkfish is notorious for having many bones more than other fish, boneless milkfish portions, fish belly meat, prepared traditionally is commonly sold in stores and markets. Recently, the boneless "milkfish stick" meat separated from the side of backbone has become popular in Taiwan markets. However, an incident of foodborne poisoning due to ingestion of fried milkfish sticks occurred in southern Taiwan in September 2014. The high content of histamine (86.6 mg/100 g and 235.0 mg/100 g) detected in both suspected fried milkfish sticks could be the etiological factor for the fish borne poisoning [13]. In general, the polyethylene packaging (PEP) and transporting at ambient temperature, or storage in a refrigerator or freezer were used for milkfish sticks after process in Taiwan. However, there is no information concerning histamine formation in milkfish sticks packaged with PEP or VP during storage at different temperatures. This research aimed to investigate the effects of VP on aerobic bacterial count, histamine formation, and total volatile basic nitrogen (TVBN) under the controlled storage temperatures of −20°C, 4°C, 15°C, and 25°C.

2. Materials and methods

2.1. Preparation of fish samples and packaging

Fresh milkfish (*Chanos chanos*) were obtained from a fish farm in southern Taiwan and delivered in an ice chest within 1 hour to the Food Safety Laboratory of National Kaohsiung Marine University. The average body weight and length of the milkfish were 600 g and 35 cm, respectively. Milkfish were deheaded, scaled, gutted, and filleted. Subsequently, the boneless "milkfish stick" meat separated from the side of the backbone was cut and rinsed immediately with distilled water. The milkfish sticks (2 × 13 cm, 18 g each) were then drained for 3 minutes and prepared for packaging. The milkfish sticks were randomly divided into two portions. Then they were packaged in well-sealed polyethylene bags (about 250 mm × 200 mm) PEP and under vacuum in pouches of polyethylene/polyamide film (about 250 mm × 200 mm, having an oxygen permeability of 40 cm^3/m^2 per 24 h/atm at 85% relative humidity, 23°C) (VP), respectively. The PEP and VP milkfish sticks samples (5 milkfish sticks/each PEP or VP bag) were stored at −20°C, 4°C, 15°C, and 25°C, respectively. The aerobic plate counts (APC), pH, and contents of TVBN and histamine were monitored for PEP and VP milkfish stick samples stored at 4°C, 15°C, and 25°C. For samples stored at 25°C, analyses were conducted every 12 hours. The samples that were stored at 4°C and 15°C were taken every 48 hours and 24 hours for analyses, respectively. The experiment was triplicate in each storage temperature and sampling time.

In another study, PEP and VP samples that had been stored at −20°C for 8 weeks were thawed and then transferred to storage at 25°C. Quadruplicate samples were analyzed every 12 hours for APC, pH, and contents of TVBN and histamine.

2.2. pH value determination

The fish samples (10 g) were homogenized in sterile blenders (Omni International, Waterbury, CT, USA) with 10 mL of distilled water to make a thick slurry. The pH of this slurry was then measured using a Corning 145 pH meter (Corning Glass Works, Medfield, MA, USA).

2.3. Microbiological analysis

A 25-g portion of the fish sample was homogenized at high speed for 2 minutes in a sterile blender with 225 mL of sterile potassium phosphate buffer (0.05 M, pH 7.0). The homogenates were serially diluted with a sterile phosphate buffer, and 1.0 mL aliquots of the dilute were poured onto Petri dishes (9 cm diameter). Then, 15–20 mL of APC agar (Difco; BD, Sparks, MD, USA) containing 0.5% NaCl at 45–50°C was added and gently mixed. The poured plates were allowed to solidify at a biologically clean bench. Bacterial colonies were counted after the plates were incubated at 35°C for 48 hours. The bacterial numbers in the milkfish stick samples were expressed as $\log_{10}$ colony forming units (CFU)/g [14].

2.4. Determination of TVBN

The TVBN content of the fish sample was measured by the method of Conway's dish for triplicate determinations [15]. The TVBN extract of the fish sample in 6% trichloroacetic acid (Sigma, St. Louis, MO, USA) was absorbed by boric acid and then titrated with 0.02N hydrogen chloride (HCl). The TVBN content was expressed in milligrams/100 g fish.

2.5. Histamine analysis

The content of histamine in the fish samples was determined in triplicate. Each fish sample was ground in a Waring Blender (Oster Co., Milwaukee, WI, USA) for 3 minutes. The ground samples (5 g) were transferred to 50 mL centrifuge tubes, 20 mL of 6% trichloroacetic acid was added, and the mixture was homogenized (Omni International) for 3 minutes. The homogenate was centrifuged (10,000*g*, 10 minutes, 4°C) and filtered through Whatman Number 2 filter paper (Whatman, Maidstone, England). The filtrate was then placed in a volumetric flask, and trichloroacetic acid was added to bring to a final volume of 50 mL. Samples of standard histamine solution and 1 mL aliquots of the fish sample extracts were derivatized with dansyl chloride according to the previously described method [6]. The dansyl derivatives were filtrated through a 0.45-μm filter, and 20 μL aliquots were used for high performance liquid chromatography injection.

The contents of histamine in the fish sample were determined with an Hitachi liquid chromatograph (Hitachi, Tokyo, Japan) consisting of a Model L-7100 pump, a Rheodyne model 7125 syringe loading sample injector, a Model L-4000 UV-vis detector (set at 254 nm), and a Model D-2500 Chromato-integrator. A LiChrospher 100 RP-18 reversed-phase column (5 μm, 125 mm × 4.6 mm, E. Merck, Damstadt, Germany) was used for chromatographic separation. The gradient elution program began with 50:50 (v/v) acetonitrile:water at a flow rate of 1.0 mL/min for 19 minutes, followed by a linear increase to 90:10 acetonitrile:water (1.0 mL/min) during the next 1 minute. Finally, the acetonitrile:water mix was decreased to 50:50 (1.0 mL/min) for 10 minutes.

2.6. Statistical analysis

Results were expressed as means ± standard deviation of three determinations and one-way analysis of variance. A Tukey test was carried out to assess for any significant differences between the means. All statistical analyses were performed using SPSS version 16.0 for windows (SPSS Inc., Chicago, IL, USA). A value of $p < 0.05$ was used to indicate significant deviation.

3. Results and discussion

The changes in APC of PEP or VP milkfish stick samples during storage at different temperatures are shown in Figure 1. The initial APC were approximately 5.75 log CFU/g and then APC increased rapidly in milkfish sticks during storage at 25°C. The APC reached 11.10 log CFU/g in PEP samples, and 10.21 log CFU/g in VP samples after 2.5 days at 25°C. Determination of bacterial populations in samples stored at 25°C was terminated after 2.5 days of storage owing to sample spoilage. Moreover, there was no significantly different ($p > 0.05$) between PEP and VP samples before Day 2 of storage at 25°C. At 15°C, the APC of PEP and VP samples gradually increased until they reached about 11.01 log CFU/g and 9.79 log CFU/g after 5 days, respectively. Meanwhile, the APC of PEP samples were significantly ($p < 0.05$) higher than VP samples at any times of storage. However, APC was retarded in both samples stored at 4°C during the first 2 days of storage and then increased slowly thereafter. The APC reached 8.40 log CFU/g in PEP samples, and 7.62 log CFU/g in VP samples after 12 days at 4°C. Moreover, the APC were significantly lower ($p < 0.05$) in VP samples compared to PEP samples after Day 2 of storage.

VP is an effective packaging technology that offers a way of prolonging the shelf-life of perishable fish by excluding oxygen and inhibiting the growth of aerobic spoilage bacteria [11]. In this study, significant differences ($p < 0.05$) were observed between PEP samples and VP samples at 4°C or 15°C. Based on the Taiwanese regulatory standard of 6.47 log CFU/g of APC for raw frozen fishes [16], the VP treatment extended the shelf-life of milkfish sticks from 1 to 2 days at 15°C or from 2 to 6 days at 4°C, as compared to PEP treatment (Figure 1). The result in this study is in agreement with a previous report by Ozogul et al [17] that the limit of acceptability (6–7 log CFU/g) in terms of APC was 3 days for sardines stored at 4°C in air, and 8 days for VP.

As shown in Figure 2, the pH value (5.68) in samples was detected at the initial time. The pH value increased rapidly, and reached 6.32 in PEP sample and 6.27 in VP sample after 2.5 days during storage at 25°C. At 15°C, the pH value in both PEP and VP samples slightly decreased at the first day of storage, and thereafter increased to 6.11 and 6.04 at the end of storage, respectively. However, the pH value in both samples was maintained to be constant in the range of 5.52–5.70 for 12 days of storage at 4°C. Meanwhile, there was no significant difference ($p > 0.05$) between the pH of PEP and VP samples at each sampling time in the same temperature of storage. The increase of pH values during storage at 15°C or 25°C, regardless of both samples, may be attributed to the production of basic compounds such as ammonia and trimethylamine, as well as other biogenic amines by fish spoilage bacteria [18].

The initial TVBN content of milkfish sticks was 17.65 mg/100 g, and TVBN increased rapidly in both PEP and VP samples during storage at 25°C (Figure 3). The PEP and VP samples were found to contain TVBN at levels of 29.8 mg/100 g and 29.4 mg/100 g for 2 days, and 44.1 mg/100 g and 35.4 mg/100 g for 2.5 days after storage at 25°C, respectively. There was no significant difference ($p > 0.05$) between PEP and VP samples before Day 2 of storage at 25°C. At 15°C, the TVBN levels of PEP and VP samples gradually increased until they reached about 29.7 mg/100 g and 25.9 mg/100 g after 5 days, respectively (Figure 3). The levels of TVBN in PEP samples were higher ($p < 0.05$) than those in VP samples after storage of 2 days when stored at 15°C. The increases of TVBN in both samples stored at 15°C were much slower than in samples stored at 25°C. When samples were stored at 4°C, the levels of TVBN levels increased slightly and reached 25.8 mg/100 g and 24.3 mg/100 g in PEP and VP samples after storage of 12 days, respectively. The levels of TVBN in PEP samples were higher ($p < 0.05$) than those in VP samples after Day 4 of storage when stored at 4°C (Figure 3). However, VP cold smoked salmon presented a shelf-life of 20 days at 4°C according to TVBN analysis [19].

The production of TVBN is related closely to the metabolism of spoilage bacteria and the activity of endogenous enzyme [19]. The actions of such spoilage bacteria and enzyme result in the formation of compounds including ammonia (NH_3), trimethylamine (TMA), and dimethylamine.

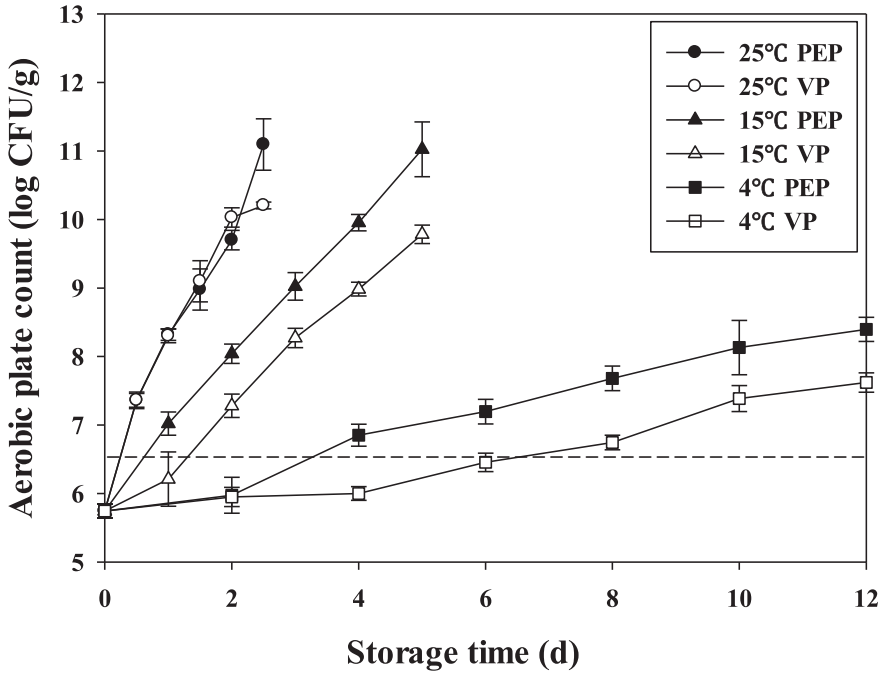

Figure 1 – Changes of the aerobic plate count (APC) of milkfish sticks stored in polyethylene package (PEP) or vacuum package (VP) during storage at 4°C, 15°C, and 25°C. Each value represents mean ± standard deviation (SD) of three replications. Dash-line represents 6.47 log colony forming units (CFU)/g of APC as the regulatory standard for raw frozen fish.

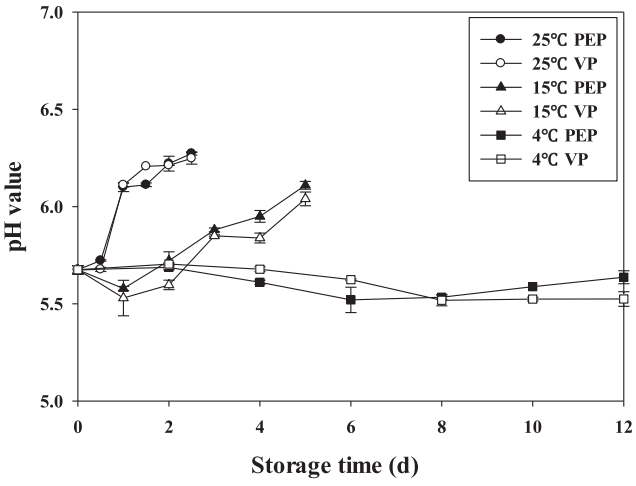

Figure 2 – Changes of the pH values of milkfish sticks stored in polyethylene package (PEP) or vacuum package (VP) during storage at 4°C, 15°C, and 25°C. Each value represents mean ± standard deviation (SD) of three replications.

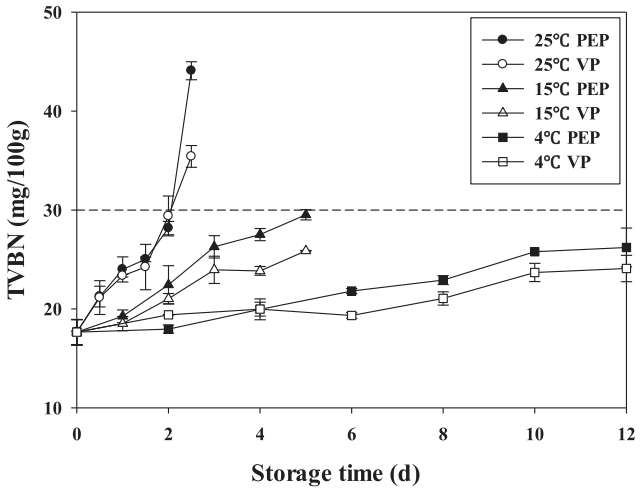

Figure 3 – Changes of the total volatile basic nitrogen (TVBN) of milkfish sticks stored in polyethylene package (PEP) or vacuum package (VP) during storage at 4°C, 15°C, and 25°C. Each value represents mean ± standard deviation (SD) of three replications. Dash-line represents 30 mg/100 g of TVBN as a reference of fish decomposition.

In addition, the increase in TVBN was also caused by a combination of microbiological and autolytic deamination of amino acids and the complete microbial reduction of trimethylamine oxide (TMAO) to TMA [20]. The higher bacterial producers of TVBN in fish were *Enterobacteriaceae*, *Photobacterium* spp., and *Lactobacillus* spp. [20]. Therefore, TVBN, including TMA, dimethylamine, and NH_3, is one of the most widely used indicators for fish quality and spoilage [19]. Although these TVBN levels before storage of 2 days were not higher than the 30 mg/100 g level commonly used as a reference for fish decomposition, results of microbiological analysis (> 10^8 CFU/g) indicated that the samples were spoiled already after being stored at 25°C for 1 day (Figure 1). Chiou et al [9] found low levels of TMAO and TMA at 2 mg/100 g and < 0.4 mg/100 g, respectively, in the white muscle of milkfish.

Hebard et al [20] demonstrated that marine fish, such as mackerel, sardines and swordfish, had 75–225 mg/100 g of TMAO and 2.1–16.8 mg/100 g of TMA. In this study, the lower levels of TVBN observed with both samples during storage could be related to lower amounts of TMAO and TMA. Moreover, the levels of TVBN in VP samples were lower ($p < 0.05$) than those in PEP samples during the second half of storage when stored at 4°C or 15°C (Figure 3). The inhibition of TVBN production in VP samples may be attributed to the effect of vacuum by excluding oxygen and inhibiting the growth of aerobic spoilage bacteria [11,12].

Similar to TVBN, formation of histamine in samples was significantly faster in samples stored at 25°C than at 15°C and 4°C ($p < 0.05$) (Figure 4). In the PEP and VP samples, histamine contents increased to 325.1 mg/100 g and 64.4 mg/100 g after 2.5 days of storage at 25°C, respectively. The histamine contents were significantly lower ($p < 0.05$) in VP samples as compared to PEP samples after Day 1.5 of storage (Figure 4). At 15°C, although no histamine was detected in any of the PEP and VP samples before 3 days of storage, it began to accumulate after Day 3 of storage, reaching 31.8 mg/100g and 17.9 mg/100 g at the end of storage (Day 5), respectively. Meanwhile, histamine contents of VP samples were significantly ($p < 0.05$) lower than those of PEP samples after 3 days of storage. Histamine accumulation in both samples stored at 4°C for 12 days was negligible (< 2.0 mg/100 g). Similarly, the result in this study is in agreement with a report by Ababouch et al [21] that the rate of histamine development was much greater at an ambient temperature, and icing significantly reduced or totally inhibited histamine accumulation in sardine (*Sardina pilchardus*) muscles. Generally, histamine accumulation in sardines stored in VP is significantly lower than in those stored in air, since bacterial growth is inhibited in VP. Aerobic flora cannot grow in this type of packaging due to the exclusion of O_2 [17].

In this study, the PEP and VP samples when stored at 25°C for 1 day (12.3 mg/100 g and 11.2 mg/100 g, respectively) and at 15°C for 4 days (17.2 mg/100 g and 7.3 mg/100g, respectively) had histamine contents greater than the 5.0 mg/100 g allowable limit suggested by the US Food and Drug Administration (FDA) for scombroid fish and/or products [22]. A fish with obvious spoilage detectable by the consumer will most likely not be consumed, whereas a fish with a good appearance and no detectable spoilage odors but a high histamine level may be consumed. By the time the samples started to show decomposition based on the > 30 mg/100 g content of TVBN, the histamine level had already reached more than 50 mg/100 g at temperatures of 25°C for 2.5 days storage (Figures 3 and 4). The US FDA has indicated that fish containing histamine at levels above 50 mg/100 g (500 ppm) should be considered a potential hazard for human health [22]. Therefore, use of TVBN value as an indicator to predict histamine contents and risk of scombroid poisoning should be avoided. In fact, Baranowski [23] recommended that urocanic acid should be used as an alternative to predict histamine as a quality index of fish during storage at low temperature, since urocanic acid has been detected as a predominant metabolite of histidine during incubation of different bacteria at low temperature. Ozogul et al [17] reported that lower histamine-forming bacteria (HFB) were obtained from sardines stored under VP compared to air packaging, indicating that the exclusion of O_2 in the pack inhibited the growth of HFB. Therefore, since HFB cannot grow in this type of packaging due to the exclusion of O_2, histamine accumulation in VP samples is significantly ($p < 0.05$) lower

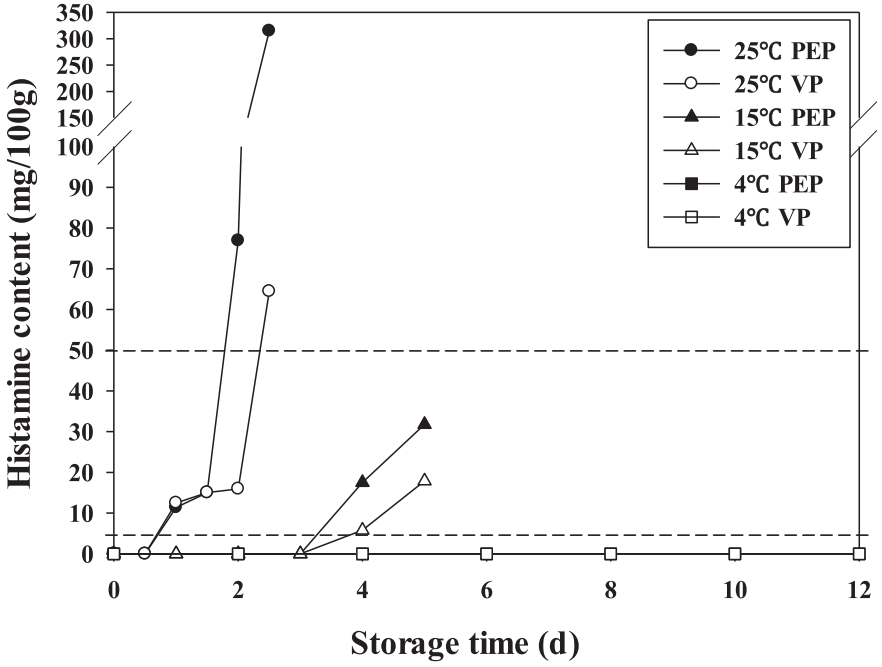

Figure 4 – Changes of the histamine content of milkfish sticks stored in polyethylene package (PEP) or vacuum package (VP) during storage at 4°C, 15°C, and 25°C. Each value represents mean ± standard deviation (SD) of three replications. Both dash-lines represent 5.0 mg/100 g of histamine as the allowable limit of the US Food and Drug Administration (FDA) and 50 mg/100 g of histamine as potential hazard level for humans, respectively.

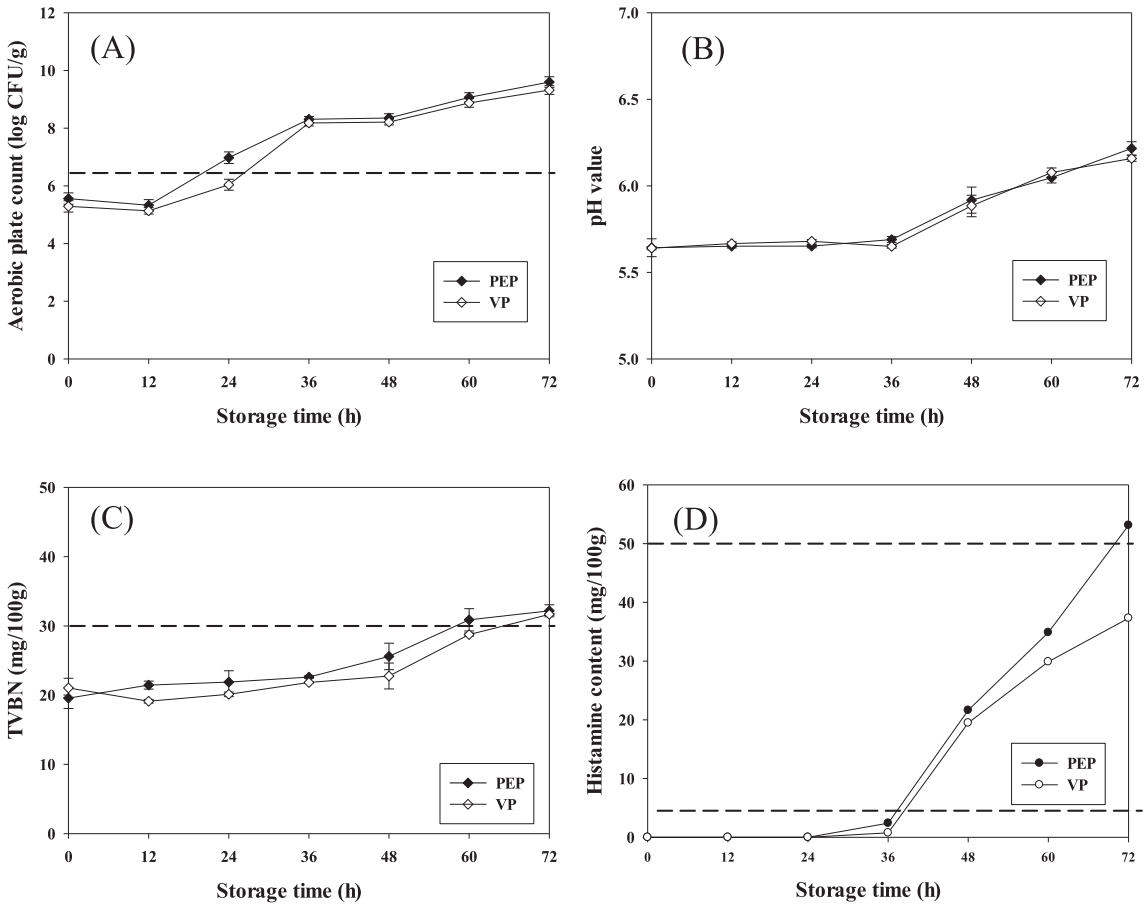

Figure 5 – Changes of (A) aerobic plate count (APC), (B) pH value, (C) total volatile basic nitrogen (TVBN), and (D) histamine of milkfish sticks stored in polyethylene package (PEP) or vacuum package (VP) at −20 °C for 8 weeks, and then thawed and stored at 25°C. Each value represents mean ± standard deviation (SD) of three replications. Dash-line in (A) represents 6.47 log CFU/g of APC as the regulatory standard for raw frozen fish. Dash-line in (C) represents 30 mg/100 g of TVBN as a reference of fish decomposition. Both dash-lines in (D) represent 5.0 mg/100 g of histamine as the allowable limit of the US Food and Drug Administration (FDA) and 50 mg/100 g of histamine as potential hazard level for human, respectively.

than in PEP samples after the second half storage at 25°C and 15°C.

The tested milkfish sticks in VP or PEP did not change the APC, pH, and TVBN level during the 8-week storage at −20°C (data not shown). No histamine was detected in any of the samples during the 8-week storage at −20°C (data not shown). Once the frozen PEP and VP samples at −20°C for 8 weeks were thawed and then held at 25°C, they started to show gradual increase of APC, reaching levels of 9.60 log CFU/g in PEP samples and 9.22 log CFU/g in VP samples for 72 hours, respectively (Figure 5A). The pH value in both samples remained constant before 36 hours of storage, and thereafter, the pH in PEP and VP samples, increased to 6.23 and 6.19 at the end of storage (72 hours), respectively. No significant different ($p > 0.05$) of pH was found between PEP and VP samples at each sampling time (Figure 5B). Similarly, the TVBN value in both samples ranged from 19.0 mg/100 g to 22.5 mg/100 g and remained constant before 36 hours of storage, and thereafter, the TVBN contents in PEP and VP samples increased to 32.2 mg/100 g and 31.9 mg/100 g at the end of storage (72 hours), respectively. No significant difference ($p > 0.05$) of TVBN was found between PEP and VP samples at each sampling time (Figure 5C). Although no histamine was detected in any of the frozen milkfish stick samples right after thawing, it began to accumulate after 24 hours of storage at 25°C (Figure 5D). Because the bacteria grew rapidly in these samples, the increases in histamine levels were also rapid. In this study, the PEP and VP samples when stored at 25°C for 2 days (21.6 mg/100 g and 19.4 mg/100 g, respectively) had histamine contents greater than the 5.0 mg/100 g allowable limit suggested by the US FDA for scombroid fish and/or products [22]. Histamine was detected at 53.13 mg/100 g in the PEP sample and 37.51 mg/100 g in the VP sample following 72 hours of storage at 25°C (Figure 5D). Although no significant difference ($p > 0.05$) was found between the histamine contents of PEP and VP samples before 48 hours of storage, histamine accumulation in VP samples was significantly ($p < 0.05$) lower than in PEP samples after 48 hours of storage.

4. Conclusion

In this study, we demonstrated that hazardous levels of histamine could be accumulated in milkfish sticks when stored

at temperatures of 25°C, regardless of whether they were frozen or not. At this storage time in a refrigerator, the milkfish sticks will not produce hazardous levels of histamine. At below 15°C, storage of milkfish sticks under VP conditions inhibited bacterial growth, reduced the formation of histamine and TVBN, and extended the shelf-life, as compared to PEP samples. It is suggested that the milkfish sticks on controlling histamine production should be packaged with VP and maintained below 4°C during storage.

Conflicts of interest

All authors declare no conflicts of interest.

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