Effect of Vacuum Packaging on Histamine Production in Japanese Spanish Mackerel (Scomberomorus niphonius) Stored at Various Temperatures

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ABSTRACT

The effect of polyethylene packaging (PEP) in air cushion and vacuum packaging (VP) on histamine related to the quality of Japanese Spanish mackerel (JS mackerel) was studied with samples stored at –20, 4, 15, and 25°C. The aerobic plate count (APC), total volatile basic nitrogen (TVBN), and histamine concentrations of the PEP and VP samples stored at 25°C increased as the storage time continued. The PEP and VP samples stored at temperatures below 15°C showed lower levels of APC, TVBN, and histamine, with VP samples having considerably lower levels of APC, TVBN, and histamine than PEP samples. For the frozen JS mackerel stored at –20°C for 2 months and then thawed and stored at 25°C, the VP treatment delayed the increases of TVBN and histamine longer than did the PEP treatment. Thus, the storage of VP JS mackerel at temperatures below 4°C could prevent quality deterioration and extend shelf life.

HIGHLIGHTS

• Lower APC, TVBN, and histamine were found in VP samples than PEP samples.
• VP delayed TVBN, and histamine increased at 25°C in thawed JS mackerel.
• VP JS mackerel below 4°C prevented quality deterioration and extended shelf life.

Key words: Histamine; Japanese Spanish mackerel; Storage temperature; Total volatile basic nitrogen; Vacuum packaging

Histamine, the causative agent of scombroid poisoning, is a foodborne chemical hazard. Although scombroid poisoning is usually a mild illness, with symptoms including rash, urticaria, nausea, vomiting, diarrhea, flushing, and tingling and itching of the skin (20, 23), the severity of the illness varies considerably, depending on the amounts of histamine ingested and the individual’s susceptibility to histamine. Histamine is primarily produced in fish through decarboxylation of free histidine by the activity of various bacterial species. Scombroid fish are the type of fish commonly involved in scombroid poisoning due to high levels of free histidine in the muscle tissue. These fish species include tuna, mackerel, bonito, and saury (6). In Taiwan, scombroid poisoning occurs occasionally, and it has commonly been associated with the consumption of swordfish, marlin, tuna, mackerel, and milkfish (4–6, 13).

Japanese Spanish mackerel (JS mackerel; Scomberomorus niphonius), also known as the Japanese seerfish, is a species of true mackerel in the Scombridae family. As an epipelagic predator, the fish is widely distributed in the subtropical and temperate waters of the northwestern Pacific Ocean (22). The fish has a reported maximal length of 100 cm and a maximal weight of 10.57 kg. JS mackerel is an important commercial fish in Taiwan, with an annual landing of more than 600 tonnes (600,000 kg) (22). Due to its abundance, reasonable domestic price, and delicious taste, JS mackerel has become a popular species for many producers and consumers. The fish has a free amino acid (FAA) pattern similar to that of migratory fish, such as skipjack, marlin, and tuna, which possess high levels of histidine in their white muscle. Song et al. (18) reported the histidine concentration of approximately 1,063 mg/100 g of white muscle as the most prominent free amino acid in JS mackerel, accounting for 88% of the total free amino acids.
in the fish. An incident of fishborne poisoning, due to ingestion of fried JS mackerel, recently occurred in eastern Taiwan in September 2014. The high histamine concentrations of 190 and 330 mg/100 g in the two suspected fried JS mackerel meats were the etiological factor for the incident (10).

Vacuum packaging (VP) is an effective technology for food preservation. It prolongs the shelf life of perishable fish by excluding oxygen and inhibiting the growth of aerobic spoilage bacteria (16). Vacuum-packaged fillets also have a small package volume, making it easier for international transportation. Therefore, storage under VP has been given increasing attention recently (16). Under aerobic storage conditions, several gram-negative genera, particularly *Pseudomonas, Aeromonas, Shewanella*, and *Enterobacteriaceae*, are the dominant spoilage microorganisms in freshwater and marine fish. The growth of the aerobic bacteria commonly present on the fish is, however, inhibited under VP, resulting in an increase in the number of gram-negative bacteria that can respire better than the gram-negative bacteria in this type of packaging (14).

In Taiwan, the processed JS mackerel is usually packaged in polyethylene packaging (PEP), transported at ambient temperature, and stored in the refrigerator or freezer. Because there existed no information on histamine formation in JS mackerel meats that were packaged in PEP and stored at different temperatures, we undertook this research to investigate the effect of storage at −20, 4, 15, and 25°C on the changes in aerobic bacterial count, histamine formation, and total volatile basic nitrogen (TVBN) in the test samples. Similar experiments were conducted with VP JS mackerel meats to determine if such a storage technology would provide a better way for quality control of JS mackerel.

**MATERIALS AND METHODS**

**Preparation of fish samples and packaging.** Troll-caught JS mackerel (3 to 5 kg each) were commercially harvested off the Taiwan Strait coast. Fresh JS mackerel (*Scomberomorus niphonius*) was obtained from a fishing port in southern Taiwan and delivered in ice chests within 1 h to the Food Safety Laboratory of the National Kaohsiung University of Science and Technology. Subsequently, JS mackerel were deheaded, skinned, gutted, and filleted. The meat cubes (5 by 5 cm and 20 g each) were rinsed immediately with distilled water, drained for 3 min, and then packaged randomly into two portions: one portion in well-sealed polyethylene bags (about 250 by 200 mm; PEP) and the other portion under vacuum in pouches of polyethylene-polyamide film (about 250 by 200 mm, having an oxygen permeability of 40 cm²/ cm² · per 24 h · 0.1 MPa at 85% relative humidity, 23°C; VP). The PEP and VP JS mackerel meats were then stored at −20, 4, 15, or 25°C for a designated time and then analyzed for aerobic plate counts (APC), pH, and concentrations of TVBN and histamine.

For the PEP and VP JS mackerel samples stored at 25°C, analyses were conducted every 8 h for the first day and, thereafter, every 12 h. The samples that were stored at 15°C were taken for analyses every 12 h for the first 2 days and, thereafter, every day. For samples stored at 4°C, analyses were performed every 2 days. In all these studies, triplicate JS mackerel samples were used for analyses for each storage temperature and sampling time. For the group of mackerel samples that were stored at −20°C, the PEP and VP samples were thawed first after 8 weeks of storage and then transferred to storage at 25°C. Triplicate samples were then analyzed every 12 h for APC, pH, and concentrations of TVBN and histamine.

**Determination of pH values.** The fish samples (10 g) were homogenized in sterile blenders (Omni International, Waterbury, CT) with 20 mL of distilled water to make thick slurries. The pH of the slurry was then measured by using a Corning 145 pH Meter (Corning Glass Works, Medfield, MA).

**Microbiological analysis.** A 25-g portion of the fish sample was homogenized at high speed for 2 min in a sterile blender with 225 mL of sterile sodium phosphate buffer (0.05 M and pH 7.0). The homogenates were serially diluted with a sterile phosphate buffer, and 1.0 mL of the diluents were poured onto petri dishes (diameter, 9 cm). Then, 15 to 20 mL of APC agar (Difco, BD, Sparks, MD) containing 0.5% NaCl and at 45 to 50°C was added and gently mixed. The poured plates were allowed to solidify on a clean bench. Bacterial colonies were counted after the plates were incubated at 35°C for 48 h. The bacterial numbers in the fish samples were expressed as log CFU per gram (2).

**Determination of TVBN.** The concentration of TVBN in the fish sample was measured by the method of Conway’s dish for triplicate determinations (3, 7). Five grams of the fish sample was mixed with 20 mL of 6% trichloroacetic acid (Sigma, St. Louis, MO) in a 50-mL centrifuge tube and then vortexed for 10 min. The TVBN extract was centrifuged at 1,350 × g for 5 min. The supernatant was filtered through a filter paper (Whatman no.1, Whatman, Maidstone, UK) for analysis. TVBN was released with the addition of saturated K₂CO₃ and was absorbed by a borax acid solution and then titrated with 0.02 N HCl. The TVBN concentration was expressed in milligrams of N/100 g of fish (3).

**Histamine analysis.** The concentration of histamine in the fish samples was determined in triplicate. Each fish sample was ground in a Waring Blender (Oster Co., Milwaukee, WI) for 3 min, and 5-g ground samples were transferred to 50-mL centrifuge tubes. After 20 mL of 6% trichloroacetic acid was added to each tube, the mixture was homogenized (Omni International) for 3 min. The homogenates were centrifuged (10,000 × g, 4°C) for 10 min, and the supernatants filtered through Whatman no. 2 filter paper (Whatman). The filtrate was then placed in a volumetric flask and 6% trichloroacetic acid added to bring to a final volume of 50 mL. Samples of standard histamine solutions and 1-mL aliquots of the fish sample extracts were each derivatized with dansyl chloride according to the previously described method (5). The dansyl derivatives were filtered through a 0.45-μm-pore-size filter, and 20-μL aliquots were used for high-performance liquid chromatography injection.

The concentrations of histamine in the fish samples 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 by 4.6 mm; Merck, Darmstadt, 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 min, followed by a linear increase to 90:10 acetonitrile-water (1.0 mL/min) during the next minute. Finally, the acetonitrile-water mix decreased to 50:50 (1.0 mL/min) for 10 min.
The microflora in the VP of fish becomes dominated by various gram-positive organisms, mainly lactic acid bacteria (14, 15). Therefore, this occurred concomitantly to the growth of lactic acid bacteria, which led to the increase in lactic acid levels and the pH decrease during storage (14, 15). In addition, the lower values of pH in VP samples compared with PEP samples at the end of storage of the growth of aerobic spoilage bacteria (16). On the basis of the Taiwanese regulatory standard of 6.47 log CFU/g for APC in raw frozen fishes (19), VP would extend the shelf life of JS mackerel from 1 to 1.5 days at 15°C or from 3 to 5 days at 4°C, compared with PEP treatment (1.5 days at 15°C and 3 days at 4°C) (Fig. 1). The results of this study are similar to the previous report that the limit of acceptability (6.47 log CFU/g) of APC for milkfish stored at 4°C was 2 days in air cushion packaging and 6 days with VP (12). Nevertheless, Ozogul et al. (17) demonstrated that the limit of acceptability (6 to 7 log CFU/g) of APC for sardines stored at 4°C was 3 days in air packaging and 8 days for VP. The difference in storage periods in these studies could be due to the use of different fish species.

**RESULTS AND DISCUSSION**

**Changes in APC in JS mackerel meats stored in PEP or VP during storage.** The changes in APC numbers in the PEP and VP JS mackerel samples during storage at different temperatures are shown in Figure 1. The APC numbers increased rapidly from the initial reading of ca. 2.54 to 9.78 log CFU/g in PEP samples and 9.72 log CFU/g in VP samples after 3 days of storage at 25°C. No significant difference ($P > 0.05$) in APC numbers occurred between the PEP and VP samples at each sampling time. Determination of bacterial populations in these samples stored at 25°C was terminated after 3 days because of sample spoilage. For samples stored at 15°C, the APC numbers increased gradually until they reached about 9.48 log CFU/g for PEP samples and 9.24 log CFU/g for VP samples after 5 days. The PEP samples had significantly higher ($P < 0.05$) APC numbers than the VP samples after 1 day of storage at 15°C. For both the PEP and VP mackerel samples stored at 4°C, the APC numbers were retarded for the first 3 days of storage. They then increased to 9.60 log CFU/g in the PEP sample and 8.82 log CFU/g in the VP sample after 13 days of storage. The APC numbers in the VP samples were significantly lower ($P < 0.05$) than those of the PEP samples after 3 days of storage.

The results of this study to show the occurrence of significant differences ($P < 0.05$) in APC numbers between the PEP and VP samples stored at 4 and 15°C supported the belief that VP is an effective packaging technology to prolong the shelf life of perishable fish by inhibiting the

**FIGURE 1.** Changes in the aerobic plate count (APC) in JS mackerel meats stored in PEP or VP during storage at 4, 15, and 25°C. Each value represents the mean $\pm$ SD of three replications. Dashed line represents 6.47 log CFU/g APC as the regulatory standard for raw frozen fish.

**FIGURE 2.** Time-related changes in the pH values of JS mackerel meats packed in PEP or VP and stored at 4, 15, and 25°C. Each value represents the mean $\pm$ SD of three replications.
same temperature were observed. This indicated the VP treatment delayed the increase of pH longer than the PEP treatment did at the same temperature of storage.

Changes in TVBN in JS mackerel meats stored in PEP or VP during storage. Figure 3 shows the changes in TVBN concentrations in mackerel samples stored at various temperatures. TVBN concentrations in the PEP and VP samples stored at 25°C increased rapidly from the initial level of 8.36 mg/100 g to 27.0 and 20.1 mg/100 g, respectively, in 2 days and 41.2 and 35.8 mg/100 g, respectively, in 3 days. Significant difference (P < 0.05) in TVBN concentrations occurred between the PEP and VP samples following storage at 25°C for 1.5 days. For the PEP and VP samples stored at 15°C, the TVBN concentrations gradually increased to about 36.9 and 31.0 mg/100 g, respectively, after 5 days (Fig. 3). The PEP samples were found to have significantly higher (P < 0.05) concentrations of TVBN than the VP samples following storage at 15°C for 1.5 days. The increases of TVBN concentrations in both samples stored at 15°C were much slower than those in samples stored at 25°C. When samples were stored at 4°C, the levels of TVBN in samples increased slightly and reached 17.9 and 15.4 mg/100 g in PEP and VP samples, respectively, after storage for 13 days. The levels of TVBN in PEP samples were significantly higher (P < 0.05) than those in VP samples after 9 days of storage at 4°C (Fig. 3). On the basis of the Taiwanese regulatory standard of 25 mg/100 g TVBN for raw frozen fish (19), the VP treatment extended the shelf life of JS mackerel from 1.5 to 2 days at 25°C, compared with the PEP treatment (1.5 days) (Fig. 3).

TVBN production in seafood is closely affected by the metabolism of the spoilage bacteria and the activity of endogenous enzymes (8). The actions of such spoilage bacteria and enzymes result in the formation of compounds, such as ammonia (NH₃), trimethylamine, and dimethyl-

FIGURE 3. Changes in the total volatile basic nitrogen (TVBN) of JS mackerel stored in PEP or VP during storage at 4, 15, and 25°C. Each value represents the mean ± SD of three replications. Dashed line represents 25 mg/100 g TVBN as the regulatory standard for raw frozen fish.

Changes in histamine in JS mackerel meats stored in PEP or VP during storage. Similar to TVBN production, the formation of histamine in mackerel samples was significantly faster in those stored at 25°C than at 15 or 4°C (P < 0.05; Fig. 4). The histamine concentrations in the PEP and VP samples increased to 8.14 and 7.51 mg/100 g, respectively, after 3 days of storage at 25°C with the VP samples, showing significantly lower (P < 0.05) levels of histamine than the PEP samples after 1.5 days of storage (Fig. 4). Histamine was not detected in any of the PEP and VP samples stored at 15°C for 1 day. It began to accumulate after 1.5 days of storage, reaching 6.06 and 4.65 mg/100 g, respectively, at the end of 5 days of storage. For every storage period after day 1, the histamine concentrations in the VP samples were always significantly (P < 0.05) lower

FIGURE 4. Changes in the histamine concentration of JS mackerel stored in PEP or VP during storage at 4, 15, and 25°C. Each value represents the mean ± SD of three replications. Dashed line represents 5.0 mg/100 g histamine as the allowable limit by the FDA.
than those of the PEP samples. Although histamine accumulation in both the PEP and VP samples stored at 4°C for 7 days was negligible (<0.1 mg/100 g), it reached 4.98 mg/100 g for the PEP sample and 3.01 mg/100 g for the VP sample after 13 days of storage. The VP samples were found to have significantly $(P < 0.05)$ lower histamine concentrations than the PEP samples after 9 days of storage. Ozugul et al. (17) reported that lower levels of histamine-forming bacteria (HFB) were obtained from sardines stored under VP compared with air packaging, indicating that the exclusion of O$_2$ in the package inhibited the growth of HFB and thus the significantly lower levels of histamine accumulation in the VP samples than those stored in air packaging.

The results of this study are in agreement with the report by Ababouch et al. (1) that the rate of histamine development in sardine (Sardina pilchardus) muscles was greater at ambient temperatures, and icing significantly reduced or totally inhibited histamine accumulation. In this study, the JS mackerel samples stored at 25°C for 2.5 days (histamine at 6.39 mg/100 g in PEP and 6.01 mg/100 g in VP) or at 15°C for 5 days (histamine at 6.06 mg/100 g in PEP) all had histamine concentrations greater than the 5.0 mg/100 g allowable limit suggested by the U.S. Food and Drug Administration (FDA) for scombroid fish or products or both (21). Kung et al. (12) reported that the PEP and VP milkfish stick samples, when stored at 25°C for 1 day or at 15°C for 4 days, had histamine concentrations greater than the FDA guideline level of 5.0 mg/100 g (21). The difference could be due to the fish species used in the various studies. Under aerobic storage conditions, several gram-negative genera, particularly Pseudomonas, Aeromonas, Shewanella, Enterobacteriaceae, are the dominant spoilage microorganisms in freshwater and marine fish. The growth of the aerobic bacteria commonly present on the fish is, however, inhibited under VP (14). Recently, Enterobacter aerogenes, Raoultella ornithinolytica, and Morganella morganii that were isolated from JS mackerel meats were also identified as prolific histamine formers (10). These histamine-forming isolates belonged to Enterobacteriaceae, which are generally thought to be the primary cause of histamine development in scombroid fish (10). Therefore, it was speculated that the lower level of histamine in the VP samples compared with the PEP samples at the same time of storage in this study was caused by VP inhibiting the growth of prolific HFB in JS mackerel meat.

Changes in APC, pH, TVBN, and histamine in JS mackerel meats stored in PEP or VP at −20°C and then thawed at 25°C. The frozen PEP and VP mackerel samples showed no changes in APC readings, pH values, and TVBN concentrations during the 8-week storage at −20°C. These samples also contained no histamine (data not shown). However, once these frozen samples were thawed and held at 25°C, they started to show gradual increases in APC numbers, reaching 9.70 log CFU/g in PEP samples and 9.66 log CFU/g in VP samples in 72 h (Fig. 5A). These two groups of thawed mackerel samples showed no difference $(P > 0.05)$ in pH values of 6.10 to 6.30 during the storage at 25°C for 72 h (Fig. 5B).

The TVBN values in both groups of thawed samples increased slightly from 10.20 to 15.50 mg/100 g before 36 h of storage at 25°C. The readings then increased to 55.65 mg/100 g for the PEP samples and 35.65 mg/100 g for the VP samples at the end of 72-h storage (Fig. 5C). TVBN was detected at 28.10 mg/100 g in the PEP sample and 19.50 mg/100 g in the VP sample at 48 h of storage at 25°C. A significant difference $(P < 0.05)$ in the TVBN concentration was found between the PEP and VP samples at 48 and 72 h of storage (Fig. 5C). Therefore, on the basis of the Taiwanese regulatory standard of 25 mg/100 g for TVBN for raw frozen fish, the VP treatment would extend the quality and shelf life of the thawed JS mackerel that had been held at 25°C longer than would the PEP treatment (19).

Although no histamine was detected in any of the frozen JS mackerel samples right after thawing, it began to accumulate rapidly after 12 h of storage at 25°C (Fig. 5D). Histamine levels reached 5.03 and 9.97 mg/100 g for the PEP samples that were stored at 25°C for 2 and 3 days, respectively, and 5.25 mg/100 g for the VP samples that were stored for 3 days. Although the thawed PEP and VP samples showed no difference $(P > 0.05)$ in histamine concentration before 24 h of storage at 25°C, the thawed PEP samples had significantly higher $(P < 0.05)$ levels of histamine accumulation than the VP samples after 24 h of storage. Therefore, on the basis of the FDA guideline of 5 mg of histamine per 100 g for scombroid fish or products or both (21), the VP treatment could extend the shelf life of the thawed JS mackerel from 36 to 60 h, compared with the PEP treatment (36 h).

In this study, histamine formation was followed by bacterial proliferation in JS mackerel meat, when previously frozen fish was placed at 25°C. Histamine levels in the previously frozen samples were always higher than those that had not been previously frozen at the same storage time, regardless of packaging (Figs. 4 and 5D). The accumulation of histamine in thawed fish arises from the release of histidine decarboxylase from the autolyzed HFB, which might occur when fish is frozen just before the level of bacterial growth reaches the concentration of formed histamine (11). Therefore, it was speculated that the higher level of histamine in previously frozen fish placed at 25°C in this study is caused by histidine decarboxylase itself from HFB cells autolyzing during frozen storage, even when HFB survive frozen storage. The histidine decarboxylase released converts histidine into histamine in thawed fish fillets stored at an improper temperature (11).

We demonstrated in this study that JS mackerel samples, when stored at 25°C, would rapidly accumulate histamine at levels above the FDA guideline of 5 mg/100 g, regardless of whether they were previously frozen or not, or packaged in PEP or VP. Although the VP mackerel samples stored at 15°C showed a retarded rate in bacterial growth, reduced formation of histamine and TVBN, and extended the shelf life, as compared with the PEP samples, they still produced histamine at levels above the FDA
guideline of 5 mg/100 g over an extended storage time. It is suggested that low temperature storage at below 4°C or freezing of VP JS mackerel is the optimal control method in maintaining fish quality and preventing rapid histamine production.

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