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Original Article

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.
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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.

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