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Original Article

The histamine content of dried flying fish products in Taiwan and the isolation of halotolerant histamine-forming bacteria

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ABSTRACT

Thirty dried flying fish products were purchased from fishing village stores in Taiwan and tested to detect the presence of histamine and histamine-forming bacteria. Except for histamine and cadaverine, the average content of various biogenic amines in the tested samples was less than 3.5 mg/100 g. Eight (26.6%) dried flying fish samples had histamine levels greater than the United States Food and Drug Administration guideline of 5 mg/100 g for scombroid fish and/or scombroid products, whereas four (13.3%) samples contained more than the hazard action level of 50 mg/100 g. One histamine-producing bacterial isolate was identified as *Staphylococcus xylosus* by 16S rDNA sequencing with polymerase chain reaction amplification. This isolate was capable of producing 507.8 ppm of histamine in trypticase soy broth supplemented with 1.0% L-histidine (TSBH). The *S. xylosus* isolate was a halotolerant bacterium that had a consistent ability to produce more than 300 ppm of histamine at 3% sodium chloride concentration in TSBH medium after 72 hours.

1. Introduction

Histamine, a biogenic amine, is a causative toxin of scombroid fish poisoning [1]. Scombroid poisoning is usually a mild illness with a variety of symptoms such as rash, urticaria, nausea, vomiting, diarrhea, flushing, and tingling, and itching of the skin [2]. The severity of the symptoms can vary considerably with the amount of histamine ingested and an individual's sensitivity to histamine. Scombroid fish such as tuna, mackerel, bonito, and saury that contain high levels of free histidine in their muscle tissue are often implicated in incidents of scombroid poisoning [2]. However, several species

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of nonscombroid fish such as mahi-mahi, bluefish, herring, and sardine have also been implicated in incidents of scombroid poisoning. In Taiwan, scombroid poisoning occurs occasionally and the fish implicated in these outbreaks are tuna, mackerel, milkfish, swordfish, and marlin [3–8].

Biogenic amines are formed primarily through the decarboxylation of specific free amino acids by exogenous decarboxylases released by the microbial species associated with seafood. Many bacterial species possess histidine decarboxylase and are able to produce histamine [9]. *Morganella morganii*, *Klebsiella pneumoniae*, and *Hafnia alvei* have been isolated from fish incriminated in scombroid poisoning [10], and several species of enteric histamine-producing bacteria have also been isolated from fish [11,12]. These include *Proteus vulgaris*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Serratia fonticola*, *Serratia liquefaciens*, and *Citrobacter freundii* [13,14]. Other than histamine-producing enteric bacteria, *Clostridium* spp., *Vibrio alginolyticus*, *Acinetobacter lwoffii*, *Plesiomonas shigelloides*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Aeromonas* spp., and *Photobacterium* spp. have been reported as histamine producers [11,15]. We also isolated several prolific histamine-forming bacteria such as *Enterobacter*, *Klebsiella*, *Raoultella*, and *Citrobacter* spp. from sailfish fillets, dried milkfish, tuna dumplings, and tuna sandwiches in Taiwan [16–20].

Flying fish are important traditional fisheries resources in various Caribbean, Southeast Asian, and Southern Pacific regions and countries [21]. In the past, flying fish were economically important species for coastal fisheries with the amount of catch reaching the top twenty in fisheries production in Taiwan. Darkwinged flying fish (*Cypselurus poeicilopterus*), Limpidwing flying fish (*Cheilopogon unicolor*), Spotwing flying fish (*Cypselurus poeicilopterus*), and stained flying fish (*Cheilopogon spilonotopterus*) are the main edible species harvested in Taiwan [21]. In Taiwan, most flying fish were primarily consumed as dried flying fish, and the traditional process for dried flying fish involves back-cutting, degutting, salting, and sundrying for several days. 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 [22]. However, no report exists on the presence of biogenic amines (e.g., histamine), histamine-forming bacteria, and related bacteria in dried flying fish products. Therefore, this study tested 30 dried flying fish products sold in fishing village stores in Taiwan to obtain a better understanding of the safety quality of these products.

2. Materials and methods

2.1. Samples

Thirty dried flying fish products (100–140 g each) were purchased from five fishing village stores in Taiwan from Lanyu Island (six samples), Ludao Island (nine samples), Liuqiu Island (five samples), Kaohsiung (five samples), and Hengchun (five samples). Drift gill net-caught flying fish (*Cypselurus poeicilopterus*) (120–200 g each) were commercially harvested off the Taiwan coast and delivered to fishing village stores from March 2011 to May 2011. The traditional process for drying flying fish involves back-cutting, degutting, salting, and sundrying for several days at fish village stores. In general, the dried flying fish products were not packed and were maintained at room temperature in the stores before purchase. All samples were wrapped in aseptic bags, placed in ice, and immediately transported to the laboratory for use within 8 hours.

2.2. Determination of pH value, salt content, water content, and water activity

Dried flying fish samples (10 g) were homogenized in sterile blenders with 10 mL of distilled water to make a thick slurry. The pH of this slurry was measured using a Corning 145 pH meter (Corning Glass Works, Medfield, MA). The salt content in each sample was determined in accordance with AOAC International procedures [23]. Two grams of dried flying fish sample were homogenized with 18 mL of distilled water and then titrated with 0.1M silver nitrate ($AgNO_3$) using 10% w/v potassium chromate (K_2CrO_4) solution as the indicator. The water content was analyzed by the standard gravimetric method by drying 1–3 g of the test sample at 102.0°C ± 2.0°C under atmospheric pressure for 2 hours. The consistency of the mass was tested by additional 1-hour drying steps until the difference in the mass did not exceed 0.5 mg [23]. Water activity (Aw) was determined at 27°C using an electric hygrometer (Hygrodynamics, Inc., Silver Spring, MD) [23].

2.3. Microbiological analysis and isolation of histamine-forming bacteria

A 25-g portion of the dried flying fish sample was homogenized at high speed for 2 minutes in a sterile blender with 225 mL sterile potassium phosphate buffer (0.05M, pH 7.0). Before use, the blender was sterilized by autoclaving for 15 minutes at 121°C. The homogenates were serially diluted with a sterile phosphate buffer (1:9), and 1.0-mL aliquots of the dilutes were poured onto Petri dishes (9 cm diameter). Fifteen to 20 milliliters of aerobic plate count (APC) agar (Difco, Detroit, MI, USA), which contained 0.5% NaCl, was added and gently mixed at 45–50°C. The poured plates were allowed to solidify under a biological clean bench. Bacterial colonies were counted after the plates were incubated at 35°C for 48 hours. Bacterial numbers in the dried flying fish samples were expressed as $\log_{10}$ colony-forming units (CFU)/g [23].

To isolate histamine-forming bacteria, a 0.1-mL aliquot of the sample dilute was spread on histamine-forming bacterium isolation agar fortified with L-histidine [24]. After incubating the differential agar plates for 4 days at 35°C, blue or purple colonies on the plates were removed and streaked on trypticase soy agar (Difco) to obtain pure cultures. The ability of each isolate to produce biogenic amines was determined by inoculating the isolates in trypticase soy broth (TSB) (Difco) supplemented with 1% L-histidine (TSBH), and then incubating them without shaking at 35°C for 24 hours. One milliliter of the culture broth was withdrawn for the quantitation of biogenic amines.

Analyses of total coliforms (TCs) and *Escherichia coli* in these dried flying fish samples were conducted by using the three-tube most probable number method [25]. Lauryl sulfate