Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.jfda-online.com

Original Article

Determination of histamine in milkfish stick implicated in food-borne poisoning

Yi-Chen Lee [a], Hsien-Feng Kung [b], Chien-Hui Wu [c], Hui-Mei Hsu [d], Hwi-Chang Chen [e], Tzou-Chi Huang [a], Yung-Hsiang Tsai [c,*]

[a] Department of Food Science, National Pingtung University of Science and Technology, Pingtung, Taiwan
[b] Department of Biotechnology, Tajen University, Pingtung, Taiwan
[c] Department of Seafood Science, National Kaohsiung Marine University, Kaohsiung, Taiwan
[d] Department of Health, Tainan City Government, Tainan, Taiwan
[e] Southern Center for Regional Administration, Food and Drug Administration, Department of Health and Welfare, Executive Yuan, Taiwan

ARTICLE INFO

Article history:
Received 26 January 2015
Received in revised form
18 June 2015
Accepted 1 July 2015
Available online 22 July 2015

Keywords:
food poisoning
histamine
histamine-forming bacteria
milkfish stick

ABSTRACT

An incident of food-borne poisoning causing illness in 37 victims due to ingestion of fried fish sticks occurred in September 2014, in Tainan city, southern Taiwan. Leftovers of the victims' fried fish sticks and 16 other raw fish stick samples from retail stores were collected and tested to determine the occurrence of histamine and histamine-forming bacteria. Two suspected fried fish samples contained 86.6 mg/100 g and 235.0 mg/100 g histamine; levels that are greater than the potential hazard action level (50 mg/100 g) in most illness cases. Given the allergy-like symptoms of the victims and the high histamine content in the suspected fried fish samples, this food-borne poisoning was strongly suspected to be caused by histamine intoxication. Moreover, the fish species of suspected samples was identified as milkfish (*Chanos chanos*), using polymerase chain reaction direct sequence analysis. In addition, four of the 16 commercial raw milkfish stick samples (25%) had histamine levels greater than the US Food & Drug Administration guideline of 5.0 mg/100 g for scombroid fish and/or products. Ten histamine-producing bacterial strains, capable of producing 373–1261 ppm of histamine in trypticase soy broth supplemented with 1.0% L-histidine, were identified as *Enterobacter aerogenes* (4 strains), *Enterobacter cloacae* (1 strain), *Morganella morganii* (2 strains), *Serratia marcescens* (1 strain), *Hafnia alvei* (1 strain), and *Raoultella orithinolytica* (1 strain), by 16S ribosomal DNA sequencing with polymerase chain reaction amplification.

* *Corresponding author*. Department of Seafood Science, National Kaohsiung Marine University, Number 142, Hai-Chuan Road Nan-Tzu, Kaohsiung City 811, Taiwan.
E-mail address: yhtsai01@seed.net.tw (Y.-H. Tsai).

http://dx.doi.org/10.1016/j.jfda.2015.06.009

1. Introduction

Histamine is the causative agent of scombroid poisoning, a food-borne chemical hazard that usually causes a mild illness with a variety of symptoms including rash, urticaria, nausea, vomiting, diarrhea, flushing, and tingling and itching of the skin [1]. Severity of the symptoms can vary considerably with the amount of histamine ingested and individuals' sensitivity to histamine. Scombroid fish such as tuna, mackerel, bonito, and saury, which contain high levels of free histidine in their muscle tissue, are often implicated in scombroid poisoning incidents [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. In Taiwan, scombroid poisoning has occurred occasionally [2–4], and the fish implicated in these outbreaks were tuna, mackerel, milkfish, and black marlin. Recently, due to their popularity with Taiwanese consumers, swordfish and marlin fillets have become the most frequently implicated fish species in scombroid outbreaks in Taiwan [5–7].

Biogenic amines are formed mainly through decarboxylation of specific free amino acids by exogenous decarboxylases released by the microbial species associated with seafood. Many bacterial species are known to possess histidine decarboxylase and have the ability to produce histamine [8]. In addition to *Morganella morganii*, *Klebsiella pneumoniae*, and *Hafnia alvei*, which have been isolated from the fish incriminated in scombroid poisoning [9], several species of the enteric bacteria capable of producing histamine have also been isolated from fish [10,11]. These include *Proteus vulgaris*, *Proteus mirabilis*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Serratia fonticola*, *Serratia liquefaciens*, and *Citrobacter freundii* [12,13]. Other than the enteric bacteria, *Clostridium* spp., *Vibrio alginolyticus*, *Acinetobacter lowffi*, *Plesiomonas shigelloides*, *Pseudomonas putida*, *Pseudomonas fluorescens*, *Aeromonas* spp., and *Photobacterium* spp. have also been reported as histamine producers [10,14]. Our research group also isolated several prolific histamine-forming bacteria, including *Enterobacter*, *Klebsiella*, *Raoultella*, and *Citrobacter* spp. from sailfish fillets, dried milkfish, tuna dumpling, and tuna sandwich in Taiwan [2,15–18].

Milkfish (*Chanos chanos*) is an important aquacultured fish in the Indo-Pacific region, particularly the Philippines, Indonesia, and Taiwan [19]. Histidine at approximately 441 mg/100 g is the most prominent free amino acid found in the white muscle of milkfish, accounting for 80% of the total free amino acids in the fish [20]. Tsai et al [21] reported that milkfish was a better substrate than sailfish for histamine formation by bacterial histidine decarboxylation at elevated temperatures (>15°C). Our research group first reported that dried milkfish products could cause histamine intoxication (the food-borne poisoning incident occurred in February 2006, in southern Taiwan), and *Raoultella ornithinolytica* was the major histamine-producing bacterium responsible for the high content of histamine in the implicated milkfish sample [4].

An incident of food-borne poisoning due to ingestion of fried fish sticks occurred in Tainan city, southern Taiwan, in September 2014. The incident caused 37 victims to fall ill. They all experienced allergy-like symptoms, including rash, nausea, diarrhea, and flushing, however, all recovered within 24 hours. To elucidate the causative agent, two suspected fried fish sticks collected from the suspected kitchen and 16 other raw fish stick samples purchased from retail stores in southern Taiwan were analyzed for levels of biogenic amines, aerobic plate count (APC), total coliforms (TC), *Escherichia coli*, total volatile basic nitrogen (TVBN), and histamine-forming bacteria. Polymerase chain reaction (PCR) amplification of mitochondrial DNA sequence analysis was used to identify the species of the suspected fish sample.

2. Materials and methods

2.1. Samples

Two leftover fried fish sticks, which were associated with the September 2014 poisoning incident, were collected from the elementary school kitchen in Tainan City. In addition, 16 raw fish stick samples of the same fish species as that of the leftover fish sticks were obtained from 16 retail stores (including the suspected supplying store) in Tainan city, in order to determine the overall quality of fish stick products in Tainan city. Prior to purchase, the raw fish stick samples were all processed by processing factories, kept at frozen or refrigeration temperature, and brought to retail markets for sale (300 g each sample/bag). All samples were collected in aseptic bags, placed on ice, and immediately transported to the laboratory for analysis.

2.2. Determination of pH value and salt content

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). The salt content in each sample was determined by homogenizing 2 g of fish sample 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.

2.3. Microbiological analysis and isolation of histamine-forming bacteria

A 25-g portion of the fish sample was homogenized at a high speed for 2 minutes in a sterile blender with 225 mL of sterile potassium phosphate buffer (0.05M, pH 7.0). The homogenates were serially diluted with a sterile phosphate buffer, and 1.0 mL aliquots of the dilutes 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 under a biological clean bench. Bacterial colonies were counted after the plates were incubated at 35°C for 48 hours. Bacterial numbers in the tuna dumpling samples were expressed as $\log_{10}$ colony-forming units (CFU)/g [22].

To isolate histamine-forming bacteria, a 0.1-mL aliquot of the sample dilute was spread on histamine-forming