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Determination of Histamine Content in Commonly Consumed Fish-head

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Histamine poisoning is one example of the food poisoning. The aim of the study was to determine histamine content in commonly consumed fish. A total of five different types of fish-head and five samples of each: *Clarias batrachus* (Nga-khu), *Channa stnata* (Nga-yant), *Cirrhinus marigala* (Nga-gyin), *Silonia silondia* (Nga-myin) and *Panganius hypophthalmus* (Nga-tan) were tested. Each fish-head was divided into three portions to be tested as raw, after processing and after cooking. Histamine was extracted from 75 samples (25 as raw, 25 after processing and 25 after cooking). Histamine content was tested by using simple and convenient method of visual colorimetry on a short silica-gel column cartridge. Histamine was detected in total 23 samples: 2(8.7%) from raw, 13(56.5%) from after processing and 8(34.8%) from after cooking. Out of 23 samples, histamine content were found to be more than 100 ppm in 2 samples (Nga-yant from processing, Nga-tan from after cooking), between 50-100 ppm in 4 samples (Nga-gyin from processing and Nga-myin, Nga-gyin, Nga-tan from after cooking) and less than 50 ppm in 17 samples. The maximum level of histamine content safe for consumption is 50 ppm according to the recommendation of American Food and Drug Administration and any fish with histamine content of above 50 ppm have to be discarded. In this study, 6 samples were found to contain the dangerous level of histamine.

INTRODUCTION

The safety of food is essential for effective prevention of food poisoning and other food-inducing illnesses. Histamine poisoning is an example of the food poisoning. Histamine poisoning (HA) is also known as "*scombroid fish poisoning*" although non-scombroid fish species are often implicated.[1] Free histamine is produced by means of bacteria having histidine decarboxylase act on histidine in these fishes. The usual level of histamine in fresh fish is 0.1 mg/100 g. Even before a fish smells bad, high level of histamine indicating decomposition can be detected.[2]

When fish containing high level of histamine is consumed, it can cause transient food poisoning. This food poisoning is characterized by allergy-like symptoms such as flushing, sweating, and burning of the mouth, nausea, vomiting, and diarrhea, dizziness, swelling of the tongue and face and abdominal pain.[2] The reported number of histamine poisoning cases is relatively small. A large number of unreported cases are expected, however, because of the transient tendency of the histamine poisoning.[3] Histamine is water-soluble and relatively stable to heat, thus, not decomposable for removal in the ordinary cooking process. Monitoring the histamine content in those foods will be the most effective measure for prevention of the histamine poisoning.[4]

Nowadays, fish-head is the popular cuisine in almost every restaurant. Most of the people can afford to consume it. Fish-head consumption becomes increasing. Higher bacterial contamination was associated with the gills, gut and skin than blood and meat. Histamine content had been tested in fish

meat [5, 6, 7, 8] but not in fish-head. Thus, this study was conducted to determine the histamine content in commonly consumed fish-heads.

MATERIALS AND METHODS

Collection of samples

Heads of five different types of fish: *Clarias batrachus* (Nga-khu), *Channa stnata* (Nga-yant), *Cirrhinus marigala* (Nga-gyin), *Silonia silondia* (Nga-myin) and *Panganius hypophthalmus* (Nga-tan), five samples of each, were collected in plastic bags and delivered to the laboratory of Biological Toxicology Research Division, DMR(LM). Each fish-head was divided into three portions immediately to be tested as: raw, after processing and after cooking. After that, the samples were processed at the laboratory.[4]

Extraction of histamine

The extraction of histamie from fish samples was carried out as follows:

Five grams of samples were homogenized with 40 ml of 5% trichloroacetic acid (TCA), then diluted to 50 ml as the final volume with the same solvent. Subsequently, the homogenate was transferred into a test tube and centrifuged (11,200 g) at 4°C for 10 minutes. The supernatant was stored in a 50 ml-plastic bottle in a refrigerator until testing.[4]

Fabrication of HA cartridge

First, a small amount of cotton was stuffed inside the bottom of 1 ml disposable syringe to retain packing materials. Fifty milligrams of silica-gel 60 were put on top of the cotton. The silica-gel was "sandwiched" by inserting another layer of cotton. Finally, the material inside the syringe was compacted by tamping with a thin rod.[4]

Assay with HA cartridge

One milliliter of TCA extract was mixed with 250 µl of 1 M sodium hydroxide. Then, the whole mixture was passed through the HA cartridge; subsequently, the cartridge was washed with 200 µl of 0.1M phosphate buffer (pH 6), followed by 1000 µl of distilled water. Finally, 200 µl of 1.0 mM 2, 3-Naphthalenedicarboxaldehyde (NDA) solution was passed through the HA cartridge slowly, and the color inside the HA cartridge was observed after 3 minutes and the concentration of histamine present was estimated by conforming the color tone with those of the standards. [4]

Preparation of histamine standard solutions with HA cartridges

To be used as the control for this experiment, histamine standard catridges were separately prepared to make concentrations of 0, 25, 50, 100, 500 and 1000 mg kg^{-1}. Then, 1mM NDA solution was passed through each HA cartridge and the color changes inside the HA cartridges were observed after 3 minutes.[4]

RESULTS

Five different types of fish-head: *Clarias batrachus* (Nga-khu); *Channa stnata* (Nga-yant)*; Cirrhinus marigala* (Nga-gyin); *Silonia silondia (*Nga-myin) and *Panganius hypophthalmus* (Nga-tan) were tested. Each fish-head was divided into three portions to be tested as raw, after processing and after cooking. Out of total 75 samples (25 samples as raw, 25 samples after processing and 25 samples after cooking), histamine was detected in total 23 samples. Out of 23 samples, 2(8.7%) were from raw, 13(56.5%) were from after processing and 8(34.8%) were from after cooking (Table 1).

Table 1. Distribution of histamine in different types of fish-head samples

Types of fish-head	Raw No.	Processing No.	After cooking No.
Clarias batrachus	-	-	-
Channa stnata	1	3	2
Cirrhinus marigala	1	4	3
Silonia silondia	-	3	1
Panganius hypophthalmus	-	3	2
Total (%)	2(8.7)	13(56.5)	8(34.8)

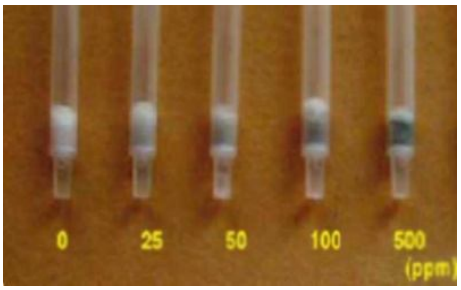


Fig. 1. The color development control of histamine standard solution with HA cartridge

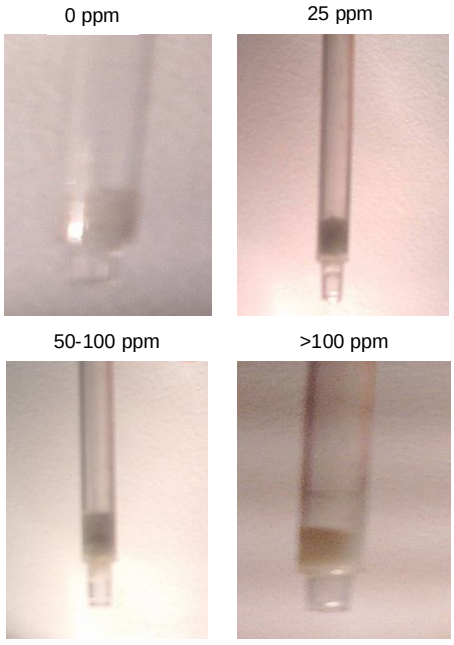
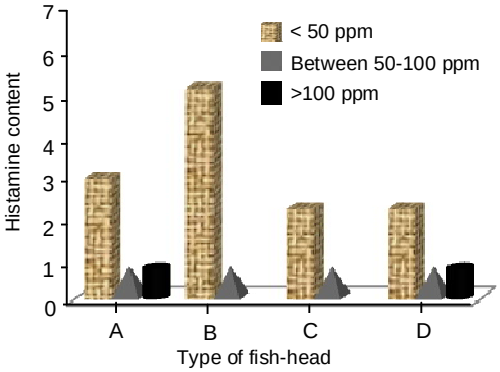


Fig. 2. The color development of histamine content solution from fish samples



A =*Channa stnata*
B =*Cirrhinus marigala*
C =*Silonia silondia*
D =*Panganius hypophthalmus*

Fig. 3. Histamine content in different types of fish-head

The resulting color tones of histamine standard solution and samples of histamine content solution in fish (Fig. 1 & 2). Regarding histamine content, 2 samples had more than 100 ppm (*Channa stnata* from processing, *Panganius hypophthalmus* from after cooking), 4 samples had between 50-100 ppm (*Cirrhinus marigala* from processing and *Silonia silondia*, *Cirrhinus marigala*, *Panganius hypophthalmus* from after cooking) and 17 samples had less than 50 ppm (Fig. 3).

In this study, histamine was not detected in all three portions of the fish head of *Channa stnata* (Nga-khu). The maximum level of histamine safe for consumption recommended by American FDA is 5 mg/100 g (50 ppm), and any fish containing histamine above this level have to be discarded.

DISCUSSION

The bacteria contamination promotes the development of histamine in fish. The possibility of cross-contamination during handling and processing of fish in the fish industry has been demonstrated. It is well-known that the most susceptible part of fish to bacterial colonization is the gill, followed by the outer skin and the slime of fish. The gill was identified as the source of *M. morganii* in mackerel and sardine, and its presence was found in most of the fish tested.

The reported number of histamine poisoning cases is relatively small. A large number of unreported cases are expected, however, because of the transient tendency of the histamine poisoning. The maximum level of histamine safe for consumption recommended by American FDA is 5 mg/ 100 g (50 ppm), and any fish containing histamine above this level has to be discarded. Histamine levels causing illness in fish are mostly above 20 mg/100 g (200 ppm). Levels above 50 mg/100 g (500 ppm) are hazardous for consumption.[3] Freezing may inactivate the enzyme-forming bacteria. Both the enzyme and the bacteria can be inactivated by cooking.

However, once histamine is formed, it cannot be eliminated by heat or freezing. After cooking, recontamination of the fish with the enzyme-forming bacteria is necessary for additional histamine to form. For these reasons, histamine development is more likely in raw, unfrozen fish.[9] After forming histamine, it cannot be destroyed by freezing, cooking, smoking and canning. Therefore, histamine content in fishes should be tested and HA method is quick and accurate method for detection of histamine.[10] The types of fish tested in this study are commonly consumed by people. Histamine was detected in 23 samples, among them, 6 samples contained the dangerous level of histamine even after processing and cooking.

Conclusion

Histamine content in fish is responsible for allergy-like food poisoning and this study revealed the presence of histamine in fish-head samples and showed the need for proper handling of fish to prevent intro-duction and cross-contamination of prolific histamine formers in fish and proper processing methods.

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