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Determination of Histamine in Freshwater Fish Using ELISA Method: A Food Safety Concern

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Abstract: Histamine fish poisoning (HFP) is a foodborne chemical intoxication caused by consuming a bacterially contaminated fish that are capable of producing histamine. Histamine may cause food intolerance and allergic reaction. Currently, there is no information regarding the level of histamine in fish that are consumed in Bahir Dar, Ethiopia. Hence, the aim of this study is to investigate the occurrence and level of histamine in fish that are commonly consumed in Bahir Dar town, Ethiopia. A total of 52 fish samples are collected from different wholesalers and retailers. The fish include tilapia, barbus and catfish. After sample preparation and acylation, histamine quantification was performed using Enzyme-Linked Immunosorbent Assay (ELISA) following the protocol provided together with the kits (RIDASCREEN ® HISTAMIN, Art. No. R1604). Histamine was detected in all fish samples in concentration ranging from 1.3 to 290 mg/100g. Approximately, 21% of the samples exceed the tolerance limit of histamine contents (50 mg/100g) accepted by European countries. Relatively, the mean histamine level was higher in tilapia, followed by catfish and barbus at a level of 40, 34 and 30 mg/100g, respectively. The maximum level was detected in tilapia at a level of 290 mg/100g and minimum level (1.3 mg/100g) was found in catfish. The mean histamine level in all analyzed fish was below the tolerance limit of histamine contents established by EU regulation. Further studies are needed to delineate the level of other chemical contaminants such as mercury and arsenic.

Key words: Histamine · Fish · Elisa · Bahir Dar

INTRODUCTION

Fish is consumed worldwide; it contains several health beneficial nutritional components such as omega-3 fatty acids and vitamin D that can protect against various chronic diseases. Contrary, fish may also contain detrimental compounds such as mercury, arsenic and histamine as well as pathogens for instance *Listeria monocytogenes*, which could induce acute and chronic diseases.

In the seafood industry, the level of biogenic amines plays a crucial role in the safety of the final product. Biogenic amines are produced by microorganisms that are capable of decarboxylating the carboxylic group of amino acids [1]. The most commonly reported biogenic amines occurring in foods and beverages are histamine, beta-phenylethylamine, tyramine, tryptamine, putrescine, cadaverine, spermine and spermidine [2]. Among these, histamine poisoning is frequently reported linked to consumption of protein rich foods such as fish and fish products.

Histamine Fish Poisoning (HFP) is described as a food borne chemical intoxication primarily caused by intake of fish muscle containing an elevated amount of histamine [3]. Intake of high level of histamine leads to life threatening food intoxication, food intolerance and allergic reaction [4-6]. Thus, histamine is a major seafood safety concern.

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Despite the moderate level of fish production and consumption in Africa, the chemical and microbial safety related to seafood consumption is doubtful. In some regions of Ethiopia fish is consumed as a staple food. Nevertheless, the safety related to fish consumption is not well investigated. Therefore, there is a chemical and/or microbial safety concern in connection with fish consumption.

HFP is among the most common toxicities linked to fish consumption, accounting 37% of all seafood-related foodborne illnesses [7]. The level of histamine in fish that are consumed in Bahir Dar town is unknown. Consequently, the histamine concentration in fish should be determined to ensure the safety of consumers. The aim of this study is therefore to quantify the concentration of histamine in fish samples originating from the freshwater Lake Tana, Bahir Dar. The study mainly focuses on fish that are commonly consumed in Bahir Dar, Ethiopia. Determining the level of histamine is essential in order to take action to prevent histamine formation.

MATERIAL AND METHODS

A total of 52 fish samples were collected from different wholesalers and retailers in Bahir Dar town, using a commercial ice box container. Approximately equal numbers of tilapia, catfish and barbus were collected.

The samples were stored immediately at -18°C in a vertical deep freezer. Thereafter, a composite of edible tissue sample from each fish was prepared. In order to take into account the distribution of histamine in the different parts of the fish, samples have been taken from different edible parts of the fish.

Sample preparation was performed according to the procedures specified in the histamine test kit (RIDASCREEN ® HISTAMIN, Art. No. R1604) obtained from R-Biophram AG, German. After sample preparation, acylation and Enzyme-Linked Immunosorbent Assay (ELISA) was conducted using the reagents following the protocol provided together with the kits. The details of the analysis can be obtained from the protocol or explained by [8].

RESULTS AND DISCUSSION

Histamine was detected in all samples analyzed in this study. The level of histamine detected range from 1.3 to 290 mg/100g of fish. The maximum level of histamine is found in tilapia at a level of 290 mg/100g of fish. Table 1 presents the overall statistics of histamine level in the analyzed fish samples.

Table 1: Overview of histamine levels in fish samples

	Histamine level (mg/100g)		
Fish samples	max	min	No. samples exceeding EU tolerance limit
Tilapia	290	3.8	5
Barbus	86	1.7	3
Catfish	148	1.3	3

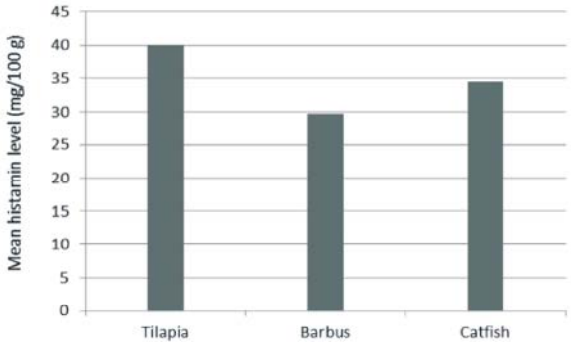

Fig. 1: Mean level of histamine per fish type

Relatively, low level of histamine is detected in catfish. The level of histamine in 11 out of the 52 analyzed samples was higher than the tolerance limit of histamine contents (50mg/100g) established by EU regulation. Comparatively, the detected histamine level in tilapia seems to contribute significantly to surpass the EU tolerance limit.

Figure 1 illustrates the mean histamine level (mg/100g) per fish samples analyzed. It can be seen that tilapia contains a relatively higher concentration of histamine, followed by catfish and barbus.

Literature search showed that the level of histamine especially to tilapia, barbus and catfish is not well investigated. However, the detected levels in this study are within the range of literature values in general fish products.

Even though the mean level of histamine (per fish type) detected in this study is below the tolerance limit (50 mg/100g) established by EU regulation, 21 % of the samples analyzed were above the limit, mostly contributed by tilapia.

Histamine formation is associated with the growth of bacteria that possess the enzyme histidine decarboxylase. In fish, numerous bacteria are considered as the principal contributors to histamine formation. These include *Morganella morganii, Proteus vulgaris, Klebsiella pneumoniae, Hafnia alvei, Enterobacter aerogenes, Citrobacter freundii, Serratia* spp. and *Escherichia coli* [9-11]. Hence, control of the growth of these bacteria would help to reduce the level of histamine in fish and fishery products.

All foods that are rich in protein are susceptible for histamine formation, if desirable conditions are present for the microorganisms and the enzyme [12]. In addition to fish and fishery products, fermented products, such as salami, cheese and canned sauerkraut could contain histamine [13].

According to Lehane and Olley [12] study, <5mg/100g is safe to eat, 5-20mg/100g is possibly toxic, 20-100 mg/100g is probably toxic and >100 mg/100g is toxic and unsafe for human consumption. Based on this category of toxicity level of histamine, approximately 6% of the analyzed samples in this study can be regarded as unsafe for human consumption.

In order to prevent or reduce the formation of histamine on fish and fishery products, the rapid cooling of fish after catching and the maintenance of adequate refrigeration during handling and storage is recommended [12]. Optimal temperature for histamine formation is variable due to different microorganism involved in the formation of histamine in fish [14]. The control of histamine is basically targeted on the control of its initial formation; this can be achieved by control of microbial population that can form histamine [14, 15].

To ensure the quality and safety of fish products, the application of international standards of food hygiene together with the application of risk analysis and hazard analysis and critical control point (HACCP) principles are imperative.

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