ESTIMATION OF THE HISTAMINE LEVELS OF FISHES FROM SOME SELECTED MARKETS IN ACCRA

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DECLARATION

We hereby acknowledge that the work herein presented is our own work and the results of our own research, except for the references and quotations which have been given due recognition and acknowledgement**.**

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ABSTRACT

Histamine poisoning results from the consumption of foods, typically certain types of red muscle fishes that contain high levels of histidine in their muscles. Spoiled fishes of the families, Scombridae and Scomberesocidae (e.g. tuna, mackerel, bonito), are commonly implicated in incidents of histamine poisoning. Histamine-containing foods can contribute to high histamine levels in the body. For histamine-sensitive people, this can be a problem. Recent research suggests that high levels of this essential neurotransmitter are associated with anxiety or panic attacks. Other research has shown that schizophrenia symptoms can arise from a histamine imbalance influencing brain functioning. Emotional and mental health symptoms due to abnormally high histamine could be helped simply by reducing or avoiding the high risk, high histamine, foods, and choosing low histamine foods instead. This research was therefore conducted to estimate and compare the histamine levels of fishes on some selected Ghanaian markets. 11 samples were analysed from different markets in Accra (Madina market, Tema Community one market, Makola market and Lapaz market). Three samples were analysed from Madina market and the levels were 1.96, 3.98 and 7.64ppm for tuna, smoked red fish and smoked salmon respectively. Three samples again were analysed from Tema community one market and the levels were 32.06, 73.36 and 57.53ppm for canned tuna flakes, canned tuna chunks and canned tuna solids respectively. Three more samples were analysed from the Lapaz market and the levels were 41.96, 48.52 and 9.65ppm for salmon, tuna and smoked herrings respectively. Two samples were also analysed from the Makola market and the histamine levels were 28.19 and 7.49ppm for frozen salmon and frozen red fish respectively.

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CHAPTER ONE

INTRODUCTION

Histamine poisoning also called Scombroid food poisoning is a foodborne illness that results from eating spoiled (decayed) fish. Along with ciguatera, it is listed as a common type of seafood poisoning (Clark et. al, 1999). However, it is often missed because it resembles an allergic reaction. It is most commonly reported with mackerel, tuna, bluefish, mahi-mahi, bonito, sardines, anchovies, and related species of fish that were inadequately refrigerated or preserved after being caught. The syndrome derived its name because early descriptions of the illness noted an association with Scombroidea fish (e.g., large dark meat marine tuna, albacore, mackerel); however, the Centers for Disease Control and Prevention (CDC) have identified other, nonscombroid vectors, such as mahi-mahi and amberjack. Scombroid syndrome can result from inappropriate handling of fish during storage or processing (Guss 1998). One of the toxic agents implicated in scombroid poisoning is histidine, which is broken down into histamine. Other chemicals have been found in decaying fish flesh, but their association with scombroid fish poisoning has not been clearly established. Unlike many types of food poisoning, this form is not brought about by ingestion of a bacterium or virus (Clark et. al, 1999). Histidine exists naturally in many types of fish, and at temperatures above 16°C (60°F) on air contact it is converted to the biogenic amine histamine via the enzyme histidine decarboxylase produced by enteric bacteria including *Morganella morganii* (this is one reason why fish should be stored at low temperatures). Histamine is not destroyed by normal cooking temperatures, so even properly cooked fish can be affected. Histamine is a mediator of allergic reactions, so the symptoms produced are those one would expect to see in severe allergic responses. Symptoms consist of skin flushing, throbbing headache, oral burning, abdominal cramps, nausea, diarrhea, palpitations, a sense of unease, and, rarely, collapse or loss of vision. Symptoms usually occur within 10–30 minutes of ingesting the fish and generally are self-limited (Clark et. al, 1999). Physical signs may include a diffuse blanching erythema, tachycardia, wheezing, and hypotension or hypertension. People with asthma are more vulnerable to respiratory problems such as wheezing or bronchospasms. Symptoms of poisoning can show within just minutes, and up to two hours, following consumption of a spoiled dish (Morrow et. al, 1991). Symptoms usually last for about 10 to 14 hours, and rarely exceed one to two days.

AIMS AND OBJECTIVES

This study was aimed to estimate the histamine levels of fish on some selected markets in Accra.

- This was done to identify histamine as a risk factor in food poisoning
- To develop recommendations for prevention
- To develop an integrated quality assurance system in the local markets.

CHAPTER TWO

LITERATURE REVIEW

HISTAMINE

Histamine fish poisoning is among the most common toxicities related to fish ingestion, constituting almost 40% of all seafood-related food-borne illnesses (Gould et. al, 2008). Histamine fish poisoning results from the consumption of inadequately preserved and improperly refrigerated fish. It resembles an allergic reaction but is actually caused by bacterially generated toxins in the fish's tissues (Dickinson 1982). Previous terms for histamine fish poisoning were scombroid fish poisoning, pseudoallergic fish poisoning, histamine overdose, or mahi-mahi flush. The term scombroid was used because the first fish species implicated in this poisoning were from the suborder Scombridae, which includes mackerel, tuna, marlin, swordfish, albacore, bonito and skipjack. Typical manifestations of histamine fish poisoning include skin flushing on the upper half of the body, rash, gastrointestinal (GI) complaints, and throbbing headache (Lavon 2008).

PATHOPHYSIOLOGY

Histamine poisoning directly relates to improper preservation and inadequate refrigeration. Histidine decarboxylase (HDC) found in *Escherichia coli, Morganella morganii,* and in *Proteus* and *Klebsiella* species, converts histidine, present in fish tissue, to histamine (Ricci et. al, 2010). Without adequate cooling, these bacteria multiply, increasing the histidine-to-histamine conversion rate and raising histamine levels. In fish left at room temperature, the histamine concentration rapidly increases, reaching toxic concentrations within 12 hours. In healthy fish, histamine is normally present at levels less than 0.1 mg per 100 g. In contrast, samples of fish that produce poisoning contain histamine levels of at least 20-50 mg per 100 g of fish (Taylor 1989).

A second agent in fish tissues has been theorized to play a role in histamine toxicity because attempts to recreate the symptoms by orally feeding people histamine have failed. Histamine is poorly absorbed in the GI tract, and the liver and intestinal mucosa can deactivate histamines. This second causative agent, possibly saurine (histamine hydrochloride), may enhance the activity of histamine, facilitate its absorption, or prevent its inactivation by histamine *N*-methyltransferase or diamine oxidase. Others postulate that cadaverine or putrescine may be the second agent (Prester 2011; Al Bulushi et. al, 2009).

ETIOLOGY

The fish species most commonly implicated in histamine toxicity are scombroid dark-meat fish (eg, tuna, mackerel, skipjack, bonito, marlin) and nonscombroid species, such as mahi-mahi (dolphin fish), amber jack, sardine, yellowtail, herring, and bluefish (Hungerford 2010; Lavon et. al, 2008). Although rare, cases involving whitefish also have been reported (Russel and Maretic 1986). Toxin production occurs when inadequate refrigeration allows the multiplication of bacteria that contain histidine decarboxylase, which converts amino acid histidine in the fish tissues to histamine. Subsequent cooking, smoking, or canning of the fish does not eliminate the histamine. Proper refrigeration and transport prevents histamine fish poisoning. The ambient storage temperature should be below 40°F (< 4.4°C) throughout the entire handling process (Gould et. al, 2008). Toxicity can result from the consumption of fresh fish that has been inadequately cooled and refrigerated, or of frozen fish that has been allowed to sit at room temperature for a prolonged time after thawing. Affected fish do not have a distinctive appearance or odor. After preparation, the fish may look honeycombed. Taste is a relatively insensitive measure of toxicity, since the lowest levels of histamine sufficient to cause symptoms cannot be tasted. Occasionally, fish with higher histamine concentrations may have a pungent, peppery taste. Bacterial proliferation (and thus, histamine production) occurs unevenly in the fish, depending partly on temperature discrepancies. For example, tissue closer to the surface of a previously frozen mass of fish will thaw sooner and may contain more histamine. The degree of symptoms in individuals consuming the same meal may be quite variable.

EPIDEMIOLOGY

The fish species most commonly implicated in histamine toxicity live in temperate or tropical waters, making populations on adjacent land areas more likely to experience outbreaks. Nevertheless, histamine fish toxicity is worldwide in scope, affecting people of all races, both sexes, and all ages. Histamine toxicity from fish makes up 5% of food-borne disease outbreaks reported to the CDC, but is likely highly underreported. During 1998-2008, 262 confirmed and 71 suspected outbreaks of histamine fish poisoning were reported to the CDC. Taken together, these affected 1,383 people, causing a total of 59 hospitalizations. In the great majority of cases, the fish that caused the outbreak was prepared in a restaurant (Wilson et. al, 2012).

PROGNOSIS

Patients with histamine fish toxicity have a good prognosis. The clinical course may be prolonged and of greater severity in patients with a history of atopy. Reported complications include severe bronchospasm, angioedema, hypotension, pulmonary edema, and cardiogenic shock (Feldman et. al, 2005). Patients with comorbid illnesses such as coronary artery disease are at risk for acute coronary syndromes caused by the tachycardia and hypotension associated with severe cases. However, no known fatalities have been linked directly to histamine fish poisoning (Grinda et. al, 2004).

CHAPTER THREE

METHODOLOGY

Principle

The sample is extracted with 2.5% Trichloroacetic acid and neutrilised to pH 7. Colorimetric determination of histamine relies on the reaction of histamine with some reagent to produce a colour complex. The method usually involves a coupling reaction with diazotized aromatic compound followed a clean up to exclude interfering compounds.

Reagents

All reagents used for these analyses were of the analytical grade.

P- bromoaniline, Hydrochloric acid, sodium nitrite, Trichloroacetic acid, sodium acetate, acetic acid, sodium bicarbonate, amberlite CG-50 resin, potassium hydroxide and distilled water.

Apparatus

Blender, Funnel, Balance, Measuring cylinders, beakers, Filter papers, Petri dish, pH meter, magnetic stirrer, volumetric flask, test tubes, chromatographic tubes and pipettes.

Procedure

The scales and skin were carefully removed from the fish and as much flesh cut as possible. The flesh was minced thoroughly to attain homogeneity. 10g of the minced flesh was weighed into a waring blender jar. 100mls of freshly prepared 2.5% trichloroacetic acid (TCA) was measured and added to the jar. It was blended at high speed for 2minutes. The TCA sample solution was then filtered and the volume of filtrate recorded. The TCA sample solution was neutralized to attain a pH of 7 using 1N KOH and the new volume after neutralization recorded. 1g of Amberlite resin was packed into a narrow chromatography column. 150ml of acetate buffer was used to wash the column and eluent drained out to make the surface of the liquid align the surface of the resin. 75ml of the neutralized TCA sample solution was then applied to the prepared column and the flow rate adjusted to 1.5ml/min. The column was washed with 100ml of acetate buffer after the entire 75ml TCA neutralized sample had eluted through the column to remove interfering substances. 25ml of 0.2N HCL was used the histamine and collected into a 50ml beaker. A blank determination was also performed alongside the samples using same procedure. 1ml of the HCL eluate was added to 15ml 5% Na_2CO_3 in a stoppered test tube previously chilled in an ice water bath. 2ml of chilled diazo compound was added to the mixture and allowed to stand at 0^{oC} for 10minutes prior to absorbance measurement. The absorbance was the measured at 495nm using distilled water as a reference.

Standard Preparation

A histamine standard powder was purchased from sigma with the certificate of analyses and expiry date stated. A stock solution was prepared from this Histamine powder using 0.2N HCL and 5 different concentrations (5ug/m, 10ug/ml, 15ug/ml, 20ug/ml and 25ug/ml) were prepared from the stock. Their individual absorbance was read at 495nm using distilled water as a control. A calibration curve was then constructed using concentration against absorbance.

CHAPTER FOUR

RESULTS

11 samples were analysed from different markets in Accra (Madina market, Tema Community one market, Makola market and Lapaz market). Three samples were analysed from Madina market and the levels were 1.96, 3.98 and 7.64ppm for tuna, smoked red fish and smoked salmon respectively. Three samples again were analysed from Tema community one market and the levels were 32.06, 73.36 and 57.53ppm for canned tuna flakes, canned tuna chunks and canned tuna solids respectively. Three more samples were analysed from the Lapaz market and the levels were 41.96, 48.52 and 9.65ppm for salmon, tuna and smoked herrings respectively. Two samples were also analysed from the Makola market and the histamine levels were 28.19 and 7.49ppm for frozen salmon and frozen red fish respectively. A summary of the results is shown in the tables below.

Table 1.0: Histamine levels of fish from Madina market in Accra.

TYPE OF FISH	**HISTAMINE LEVELS (ppm)**
SMOKED SALMON	7.64
SMOKED RED FISH	3.98
TUNA	1.96

Table 2.0: Histamine levels of fish from Tema community one market in Accra.

TYPE OF FISH	**HISTAMINE LEVELS (ppm)**
CANNED TUNA FLAKES	32.06
CANNED TUNA CHUNKS	73.36
CANNED TUNA SOLID	57.53

Table 3.0: Histamine levels of fish from Lapaz market in Accra.

TYPE OF FISH	**HISTAMINE LEVELS (ppm)**
SALMON	41.96
TUNA	48.52
SMOKED HERRINGS	9.65

Table 4.0: Histamine levels of fish from Makola Market in Accra.

TYPE OF FISH	**HISTAMINE LEVELS (ppm)**
FROZEN SALMON	28.19
FROZEN RED FISH	7.49

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMMENDATIONS

DISCUSSION

Eleven samples were analysed from four different markets across Accra. Canned Tuna flakes, canned tuna chunks and canned tuna solids were analysed from the Tema community one market and the results were 32.06ppm, 73.36ppm and 57.53ppm respectively. When these results are compared to the histamine regulatory limits according to the Food and Drugs Administration (100ppm) for canned fish products, they were within the acceptable limits. Frozen fresh fishes were also analysed from the Makola market in Accra and the histamine levels were 28.19ppm and 7.49ppm for frozen Salmon and frozen red fish respectively. According to the Food and Drugs Administration, the regulatory limit for fresh fish should not exceed 20ppm. When these results from Makola Market are compared to this limit, the fresh frozen Salmon exceeded this regulatory limit but the fresh frozen red fish was below the limit. This may results from the fact that the freezing of the Salmon was not properly done and hence histamine levels accumulated to exceed the regulatory limit. Again smoked Salmon, smoked Red fish and smoked Tuna were also anlysed from the Madina market and the histamine levels were 7.64ppm, 3.98ppm and 1.96ppm respectively. The regulatory limits for processed fish according to the FDA should not exceed 30ppm and when these results are compared to the limits they were all within the regulatory limits. From the Lapaz market in Accra, none frozen fresh Salmon, none frozen fresh Tuna and smoked Herrings were also analysed and the results were 41.96ppm, 48.52ppm and 9.65ppm respectively. When this is compared to fresh fish regulatory limit for histamine according to the FDA (20ppm), only the smoked herrings was within the acceptable limits with the Salmon and Tuna exceeding this limit. The high levels of histamine in these none frozen fishes were due to temperature differences since histamine formation is highly favored at temperatures above 0^{oC}

CONCLUSION

Based on the results obtained, more than half of the fish samples analysed for histamine levels were safe for human consumption based on the Food and Drug Administration's regulatory limits for human consumption with the exception of fresh none frozen Salmon, Frozen fresh Salmon and none frozen fresh Tuna with values of 41.96ppm, 28.19 and 48.52 respectively.

RECOMMENDATIONS

It is recommended that more research should be conducted on canned fish products on the Ghanaian markets to ascertain its histamine levels.

It is also recommended that fish mongers be educated on histamine contamination and how to properly store fish products to prevent histamine contamination.

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