

Sampling and Analysis of Histamine in Fish Products from Local Northern California Markets

Charlie Li*, Gordon Vrdoljak, Bahman Moezzi

Food and Drug Laboratory Branch, California Department of Public Health, CA, USA

***Corresponding author:** Charlie Li, Food and Drug Laboratory Branch, California Department of Public Health, 850 Marina Bay Parkway, G365, Richmond, CA 94804, USA. Tel: +15104123960; Email: charlie.li@cdph.ca.gov

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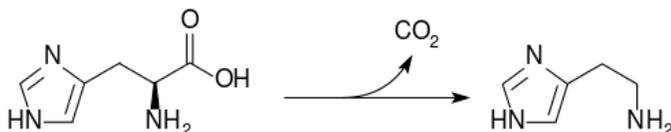
Abstract

Scombroid food poisoning is one of the most frequently reported seafood food illnesses. It is caused by histamine and it is well known that several types of fish such as tuna, King Mackerel, and Mahi are susceptible. This is due to the high levels of naturally occurring histidine and inadequate refrigeration conditions after postharvest storage. It is imperative to monitor histamine levels in seafood at various points in the supply chain and gauge the public health threat of scombroid food poisoning. The current study investigates the levels of histamine from seafood and seafood products obtained from local supermarkets in the Northern California Bay Area. Fresh, frozen, canned, fish sauce, dried fish, and pouched fish were collected through a risk-based sampling plan and analyzed. Histamine was extracted and analyzed by HPLC post-column derivatization with fluorescence detection. Rigorous Quality Assurance (QA)/Quality Control (QC) procedures were followed and the method evaluated for each batch of samples analyzed. Time and temperature storage studies for histamine generation were done for several types of seafood. Most samples collected from local supermarkets tested less than the 50ppm action limit of United States Food and Drug Administration (U.S.FDA). Some products of pouched salmon and tuna exhibited detectable levels of histamine

Keywords: Fish and Fish Products; Food Safety; Histamine; Seafood; HPLC

Introduction

Histamine fish poisoning is one of the most common seafood-related intoxications. It is also called scombroid food poisoning (scombrototoxin poisoning or scombrototoxin fish poisoning). Unlike many other types of food poisoning, this type of food poisoning is not caused by direct ingestion of a bacterium or virus. The amino acid, histidine, existing naturally in many types of fish, is enzymatically converted to histamine by histidine decarboxylase generated by bacteria at temperatures above 16°C (60°F). The following diagram shows the enzymatic reaction producing histamine from histidine:



Scombroid food poisoning is due to prolonged nonrefrigerated storage. When fresh tuna is caught from the ocean and set in a

fishing boat under the sun for a few hours without refrigeration or ice storage, the histamine level will start to build up quickly. Of importance is that once histamine is formed, it cannot be eliminated by heating, freezing, or canning. This gives the potential that frozen and canned fish could be contaminated with histamine as well [1]. Several types of fish such as tuna, Mahi Mahi, King Mackerel, and sardines have naturally high levels of histidine which could develop histamine.

In Illinois, in 1988, 8 cases of scombroid food poisoning occurred in Chicago. It was identified that those cases were traced to frozen Mahi Mahi from a suburban Chicago distributor. Six samples had histamine levels greater than or equal to 500 ppm (in the range of 500-1,600 ppm). It was indicated that there was evidence of freezer burn, a sign of thawing and refreezing. In the same year, in South Carolina, nine cases of scombroid food poisoning in Charleston were investigated. Five cases occurred after consumption of a midday meal at a restaurant, one case followed an evening meal at a seafood restaurant, and three cases occurred after an evening meal of fish prepared at home but obtained from the first restaurant. It was traced back to yellow-fin tuna steaks. U.S.FDA analyses of two samples from the yellow-fin tuna revealed histamine levels of 7,280 and 5,830 ppm [2].

An incident of foodborne poisoning from Taiwan caused illness in 3 victims due to ingestion of canned mackerel in 2001. The leftovers of the victims' canned mackerel had 1,539 ppm histamine while 3 other cans of the same brand and lot number as the suspected canned sample had histamine greater than 500 ppm [3]. From 2000 to 2007, 223 confirmed scombroid fish-poisoning outbreaks were reported from 21 States and the District of Columbia, an average of 28 annually. These outbreaks caused 865 illnesses, with Hawaii, California, and Florida reporting the majority [4]. On October 12, 2011, 1,800 cases of frozen ground tuna were recalled due to an illness outbreak related to sushi containing that tuna [5].

In California, there were a total of 125 cases of illness from scombroid poisoning [4]. The largest reported outbreak of scombroid food poisoning in the United States was in Marin County, California, in 2003. It was associated with a rare vehicle for scombroid food poisoning, escolar. Forty-two out of the 56 dinner attendees who ate escolar fish were sick. The California Department of Public Health, Food and Drug Laboratory Branch found high histamine levels in the fish (2,000-3,800 ppm). Trace back information indicated that the escolar was harvested, processed, and frozen in May 2003. The frozen fish was imported into California on June 5, 2003. It was not determined why those escolar fish had such high histamine levels [6]. As indicated by Kim et al. [7], many species of tuna and Mahi Mahi had been involved in the outbreaks of scombroid food poisoning according to the Centers for Disease Control and Prevention (CDC) surveillance. Temperature abuse of fish during handling, storage, and distribution could cause proliferation of histamine-producing bacteria, leading to the accumulation of toxic levels of histamine. Moreover, it is likely that some seafood processing conditions could generate histamine. Some other types of fish might produce histamine as well. Histamine is a major food safety concern for the seafood industry and consumers. Our goal was to investigate the levels of histamine in fish and fish products of concern obtained from local supermarkets in the Northern California San Francisco Bay Area.

Materials and Methods

Types of Samples and Fish

The risk-based sampling plan was recommended by the U.S.FDA Food Emergency Response Network (FERN). We systematically sampled several major types of fish products including fresh, frozen, canned, fish sauce, dried fish, and pouched fish from local supermarkets in Northern California, San Francisco Bay Area. Samples were randomly collected, and product information such as type of sample, type of product, brand name, store name, location, Country of origin, sampling date, person collecting sample, and lot number were recorded. We collected

more pouched samples than other types of samples as there is very limited histamine data for this type of product in literature. Specifically, tuna and salmon were the main types of samples that were studied. All samples are summarized in Table 1. Additional information for the names of supermarkets, brand names, sample collector, country of origin, and lot numbers, were documented (data not shown).

Type of Seafood	Commodity	Number of samples
Tuna, King Mackerel, Mahi Mahi, Milk fish, Sardine, Salmon, Tilapia, Snapper, Rainbow trout, Escolar	Fresh	24
Tuna, Salmon	Pouched	26
Tuna, King Mackerel	Canned	12
Tuna, King Mackerel, Mahi Mahi, Salmon	Frozen	4
Tuna, Cod, Salmon	Dried Fish	4
Anchovy	Fish Sauce	4

Table 1: Summary of different types of fish and products collected from May 2014 to January 2015.

Tuna, Mahi Mahi, King Mackerel, sardine, salmon, milkfish, tilapia, rainbow trout, and escolar were the main types of fresh fish for this study. Sampling periods were from May 2014 to January 2015. We also collected and analyzed other types of products as listed in Table 1, including fresh, pouched, frozen, canned, dried fish, and fish sauce. Various brands of products were collected.

Histamine Analysis

The California Department of Public Health, Food and Drug Laboratory Branch internal Standard Operation Procedure (SOP) 64 method was used for the analysis of histamine in fish and fish products. The method was developed on the modification and combination of three published methods: Association of Official Analytical Chemists (AOAC) Official Method 937.07 [8]; Veciana-Nogues et al., 1995 [9]; Walters, 1984 [10]. The method was validated before this study and periodically as needed. During validation studies, calibration standards, three unspiked samples, three 10ppm spiked samples; three method blanks, and three method standards were used in a single run sequence. The criteria for the acceptance of method performance is as follows: method blank (reagent blank) is to determine that all glassware and reagents are interference free, while method standard (spiked reagent blank) is to determine recovery without the matrix is in the range of 85 to 115%; matrix spiked recovery is in the acceptance range of 70 to 130%. Triplicate samples were used to meet the +/- 20% precision requirements of the method.

A representative sample was obtained by homogenizing a sub-sample in a blender. A sample of 5g was accurately weighed, and extracted with 25 mL of methanol/0.1N HCl solution (800 mL methanol with 200 mL of 0.1N HCl) in a 50 mL centrifuge tube. The mixture was homogenized using a polytron for 3 minutes at 10,000 rpm, then centrifuged for 10 minutes at 5,000 rpm for 5 minutes. The supernatant liquid was passed through a 0.45 µm membrane filter, and analyzed by HPLC (Agilent 1100, Santa Clara, CA) coupled with post-column derivatization (PCX 5200, Pickering Lab, Mountain View, CA) and fluorescence detection. For fish sauce and dried fish samples, the sample preparation was slightly different from above. A sample of 1g fish sauce for extraction was used instead of 5g. For dried fish, 1g of sample was used and 1g of distilled water was added to rehydrate the sample for analysis. For escolar fish, the sample preparation was also slightly modified. Since the centrifugation time of 5 minutes was not enough for the separation of the solid from liquid layers, an additional centrifugation time of 5 minutes was needed for the separation. The other steps remained the same. For QA/QC study, every batch of 10 or less samples, a method blank, a duplicate sample, a spiked sample, a mid-calibration point (mid-cal), and a method standard were run.

Instrumental Parameters

An Agilent 1100 LC system was used. Mobile phase A consisted of 0.1 M sodium acetate/0.01 M 1-octanesulfonic acid, sodium salt and mobile phase B was 0.2M sodium acetate [in 0.01M 1-octanesulfonic acid, sodium salt]: acetonitrile {66:34}. The column used was a Metasil 5 ODS 250 x 4.6 mm, and the flow rate was 1.0 ml/min. The gradient was initially 80% A/20% B for 3 minutes, changed to 30% A/70%B for 10 minutes, continued for 25 minutes, then changed back to 80%A/20% B at 30 minutes, and kept for another 5 minutes. The total running time was 35 minutes. Post column flow rate was 0.3 ml/min, and post-column reactor temperature was 45°C. The fluorescence excitation was at 330 nm with emission monitored at 460 nm. Injection volume was 20 µl.

Calibration of the HPLC

Initial calibration of the HPLC system was performed by analyzing working standards with the lowest concentration and ending with the highest (0, 1, 5, 10, 15 and 20ppm). If the coefficient of determination of the linear regression of standard responses is at least 0.995, the system is calibrated, and the analysis of the samples will proceed. Continuing calibration verification was performed by checking the calibration of the instrument at the beginning of each analysis by analyzing the midrange calibration standard (10 ppm).

Sample Analysis

If the observed concentration of the calibration standard falls within +/- 15% of the expected value, the instrument is within

the calibration specifications and the analysis will proceed. After establishing the calibration of the instrument, analyze the extracts. If the concentration of the extract exceeds the calibration range of the system, dilute as necessary with extraction solvent.

Time and Temperature Storage Study

All of the samples were stored in separate plastic bags. The study was conducted in a storage temperature of 30°C in an incubator. A series of temperature abuse studies was conducted with various types of fish samples including canned tuna, fresh King Mackerel, sardines, albacore tuna, snapper, and salmon. Each sample was separately incubated in plastic bags for 0, 2, 4, 6 and 8 hours, and then immediately sampled for histamine analysis.

Calculations

Calculate the concentration of histamine in the sample (µg/g) as follows:

$\mu\text{g/g} = (\mu\text{g/ml}) (\text{Dilution factor})$ where µg/g is the concentration of the injected extract from the linear regression curve.

Statistical Analysis

Regression statistics, and duplicate and recovery calculations were performed using excel software.

Results and Discussion

Method Performance

The instrument was calibrated with 0, 1, 5, 10, 15, and 20 ppm histamine standards on a weekly basis. At the beginning of each batch, a mid-cal (10ppm) was run to demonstrate that the system could meet the requirement for initial calibration. The coefficient of determination is required to be at least R^2 0.995. If this condition is not met, the calibration would be re-run with fresh standards. The Limit of Quantitation (LOQ) was 1 ppm in this study; the method detection limit (MDL) was experimentally determined to be 0.35 ppm by running 5 different vials at the same level of 1 ppm histamine in extraction solvent, calculated by standard deviation multiplied by t-value (99% confidence interval) ($\text{MDL} = \text{standard deviation of 5 different run} \times \text{t-value (99\%)}$). Method blanks were free of contamination from the whole procedure of sample extraction and analysis. A method standard was analyzed in each batch and was acceptable if within the range of 85-115% recovery.

In general, all recovery data were within the range of 70-130%, though it is skewed to the lower range of 70-100%. That might be due to a matrix effect. Overall, spiked recovery data were acceptable throughout the whole study. All of the mid-cal 10 ppm quality control samples have a range between 8.0 to 12.0 ppm. The differences between the measured and known values appear

to be randomly distributed. Mid-cal standards were run in the beginning and end of sequence regardless of numbers of samples. Table 2 summarizes the method performance, mid-cal, and method standards had excellent recoveries and matrix spike was also acceptable. The HPLC post column derivatization method was validated successfully in our laboratory.

	Average recovery (%)	Standard deviation	Number (n)
Mid-cal	101	7.31	52
Method standard	102	0.84	25
Matrix spike	91.0	13.5	25

Table 2: Summary of recoveries of mid-cal, method standard, and matrix spike.

Analytical Results of Fish and Fish products

Figure 1 shows that most canned salmon and mackerel have detectable histamine levels, which are far below the U.S. FDA regulatory limit of 50ppm. We should note that although the histamine levels in those canned foods are low, histamine will go up quickly if the canned tuna is not handled properly after it is opened. Histamine starts to build up when the free amino acid histidine and bacteria inherent in the tuna interact. The canning process kills bacteria, but once the can is opened, the tuna must be kept in a refrigerator if not consumed in a reasonable time to reduce spoilage from bacteria reintroduced from the environment [11]. The inhibition of histamine formation in canned and pouched samples could be due to the effect of phosphate and sodium chloride. Phosphate acts as an inhibitor for histamine formation [12].

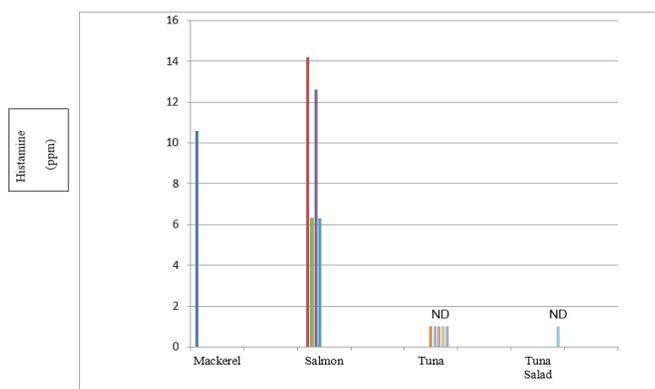


Figure 1: Histamine levels of various types of canned fish samples.

Figure 2 shows very high histamine levels in 3 fish sauce samples. All of the samples were imported from Asian countries.

It has been reported that a high amount of histamine could be attributed to the fermentation process [13]. The fermentation process provides conditions conducive to the production of histamine due to the presence of bacterial enzyme histidine decarboxylase and free amino acid histidine coupled with favorable environmental growth conditions for histamine forming bacteria. The formation of histamine is significantly influenced by salt concentration. Studies suggest that tuna viscera fermented at a low salt concentration (10%) have higher histamine formation in tuna viscera than at a higher salt concentration of 17.5%. Higher concentration of salt may inhibit the enzyme histidine decarboxylase [13].

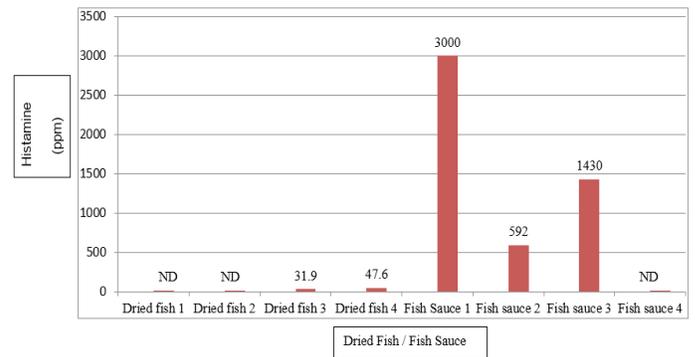


Figure 2: Histamine levels of various types of dried fish and fish sauce samples.

Most fish sauce samples tested had very high amounts of histamine, while half of the dried fish samples studied did not contain detectable amounts of histamine. The fish sauce samples from other studies were often found to have high levels of histamine as well. However, the intake of fish sauce contributes very little compared to the intake of other types of scombroid fish. About 75% of the Thai population eats fish sauce. In the United States, fish sauce is not considered a major spice for the general population. Nevertheless, 3 of the 4 fish sauce samples we collected from local supermarkets exceeded other countries' guidance maximum levels of either 200 ppm from Thailand or 400 ppm from Commission Regulation EU. Thus, it is recommended that more systematic sampling and analysis of histamine in various fish sauce brands sold in the United States be part of future research. Very limited information on histamine in fermented fish products is available. The data gathered may help to promote U.S. FDA regulation in the future and protect the public from scombroid poisoning. In general, dried fish shows very low or non-detectable histamine. These dried fish products were originally imported from Taiwan. Huang et al., [14] reported that 30.4% of the tested dried fish products contained histamine level above the 50 ppm FDA action limit of the United States..

A study from Taiwan indicated that cod steak and escolar steak had low histamine [15]. The most number of illnesses due to histamine in the United States was an outbreak from escolar in California in 2003 [4]. Prior to this, reports of histamine in escolar were not common. It was difficult for us to obtain escolar samples from local supermarkets due to advisory warnings from the FDA. Thus, there were only a couple of escolar samples available to be analyzed from local restaurants for this study.

All of the sardine samples had moderate to high histamine levels with one sample containing 179 ppm and the rest of samples from 10-50 ppm as shown in Figure 3. Other non-scombroid fish such as sardines and Mahi Mahi have also been involved in the illness outbreaks. There was an additional report that scombrototoxin could occur in salmon. Our result shows that salmon fillets had more than 20ppm of histamine. It is interesting to note that snapper, a type of rockfish, had a histamine level over 40ppm. Histamine contamination could be present in red flesh fish [16], and red snapper belongs to this type of fish category. Several types of tuna fillets such as tuna Maguro, tuna Hamachi and tuna loin had non-detectable histamine as well as tilapia and milk fish samples.

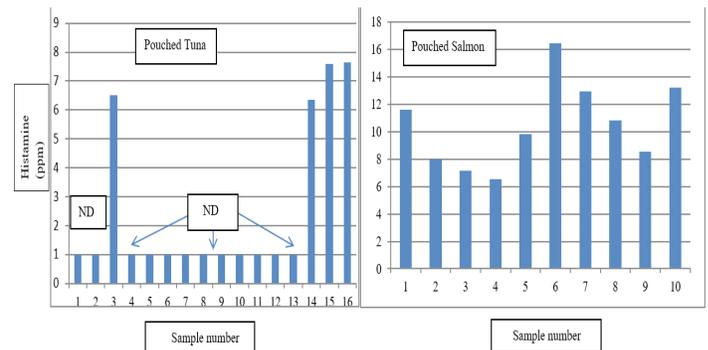


Figure 4: Histamine levels of various types of pouched fish samples.

Temperature Abuse Study

Histamine-forming bacteria are capable of growing and producing histamine over a wide temperature range [17]. Growth of histamine is more rapid at high-abuse temperatures (e.g., 70°F (21.1°C) or higher) than at moderate-abuse temperatures (e.g., 45°F (7.2°C)). Growth is particularly rapid at temperatures near 90°F (32.2°C). Our experimental design was set up for an incubation temperature of 30°C. Histamine is more commonly the result of high temperature spoilage than of long-term, relatively low-temperature spoilage, which is commonly associated with organoleptically detectable decomposition. A sardine sample shows a fast-increasing rate of histamine from 179 ppm up to over 800 ppm within 8 hours incubation time, a King Mackerel also shows a significant increase from around 100 to close to 400 ppm after 8 hours incubation (Figure 5). These fresh fish samples reached a very dangerous level of histamine after only a few hours incubation (Figure 5). When temperature is high in summertime, if not refrigerated, fresh fish could develop a high histamine level, and become a major public health and food safety concern to consumers.

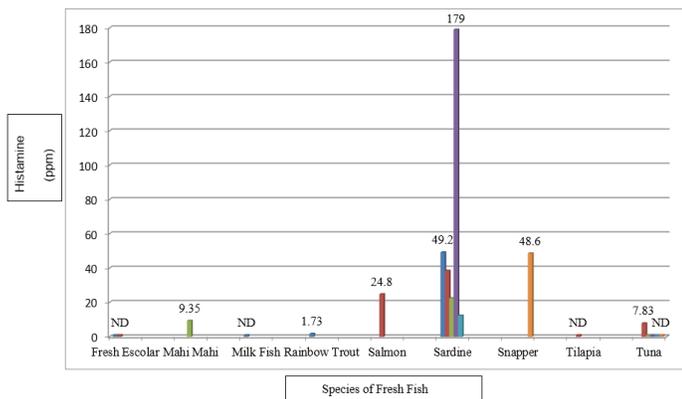


Figure 3: Histamine levels of various types of fresh fish samples.

Figure 4 illustrates the distribution of histamine levels for different types of pouched tuna and pouched salmon samples. There is concern about this type of sample due to its prevalence in the market, and if the type of packaging materials used could possibly cause the generation of histamine during storage. Pouched seafood has not been tested rigorously before. Overall, all samples show that the levels of histamine were below the regulatory limit of 50ppm. However, many types of pouched salmon samples had measureable histamine. It was noted that pouched tuna and salmon could contain phosphate that inhibits the formation of histamine. Initial levels of histamine can become elevated when the storage temperatures go up, especially in hot areas, summertime, and without refrigeration.

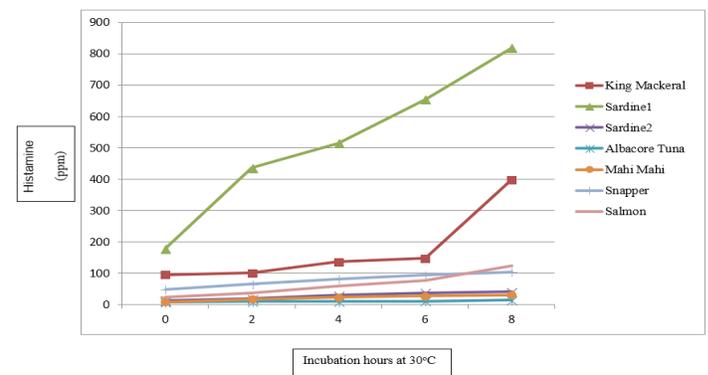


Figure 5: Change of histamine levels over the incubation time for various fresh fish samples.

Conclusions

A HPLC post-column derivatization method for the analysis of histamine in seafood products based on three published AOAC methods was validated in our laboratory. All of the QA/QC parameters were within the established control limits. Different types of fish and fish products were collected from local supermarkets and analyzed for histamine levels. Most fresh and frozen samples tested had non-detectable or low levels of histamine except for a few sardine samples. However, time and temperature storage studies indicate that histamine in fish and fish products could go up within a few hours at 30°C. If an initial level is high, then histamine will accelerate to very high levels with temperature abuse.

Pouched salmon, tuna products, canned salmon and mackerel products showed detectable histamine, but all below the regulatory limit of 50 ppm. Since all the samples tested were well below the regulatory limit, these commodities have only minor risk from histamine. Continued surveillance is recommended in case seafoods are used in processing that may have been abused by temperature and may contain histamine. It is recommended that future surveillance should continue to be focused on fresh and frozen seafood with high histidine and at risk for scombroid food poisoning. Tuna, salmon, King Mackerel, and sardine are examples of seafood that can suffer high histamine levels due to temperature abuse during processing and shipping.

It is recommended that more systematic sampling and analysis of histamine in various fish sauce brands sold in the United States be part of future research. The data gathered may help to promote U.S. FDA regulation in the future and protect the public from scombroid poisoning.

Conflicts of Interest Statement

Authors have no conflicts of interest.

Acknowledgements

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