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Original Research Article

Histamine content in local consumers demanded fishes of Tuticorin

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Abstract

Histamine is a biogenic amine, which is developed in protein rich food as a result of histidine decomposition and this decomposition is caused by growth of certain types of bacteria. The biogenic amine of histamine content is an essential quality parameter in sea food quality and strict upper limits on acceptable levels have been introduced in US and EU markets. The present work addressed histamine content of the local consumers demanded fishes from local fish market, street vendors and from the fish landing centers. The results showed higher histamine development was observed in sea foods from street vendors followed in the fishes in the local fish market. Slow histamine development observed in sea foods from the landing centers. The results revealed that, histamine development in sea foods depends upon the quality of the sea food and fishes exposed longer time to the environment is possible for the development of biogenic amines.

Key words

Commercial fishes, Histamine content, Fish spoilage.

Introduction

Seafood's are one of the most important protein sources than other foods in many parts of the world; but fish is a highly perishable food, which spoils soon after death, if not preserved properly [1]. Consumption of spoiled fish results in the outbreaks of food poisoning. Seafood may harbor a number of biological, chemical and physical hazards, the most prevalent of which are biogenic amines (BAs) and biotoxins (chemical), pathogenic bacteria and viruses (biological) and metal inclusion (physical). BAs are low molecular weight organic bases with biological activity that are formed in foods by microbial decarboxylation of the corresponding

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amino acids or by transamination of aldehydes and ketones by amino acid transaminases [2]. The most important BAs are histamine, tyramine, tryptamine, putrescine and cadaverine are formed from free amino acids namely histidine, tyrosine, tryptophane, ornithine and lysine respectively. Among that histamine poisoning is considered the most frequent food borne intoxication involving biogenic amines [3]. The consumption of high amount of BAs, above all histamine, can result in food borne poisoning which is a worldwide problem [4]. The storage temperature, handling practices, presence of microbial populations with decarboxylase activity and availability of free amino acids are considered the most important factors affecting the production of BAs in raw seafood. On the other hand, some food technological treatments such as salting, ripening, fermentation or marination can increase the levels of BAs in processed seafood. The IUPAC name of histamine is 2-(3H-imidZOL-4-y1) ethanamine dihydrochloride and the molecular weight 184 and its molecular formula of $C_5H_{11}Cl_2N_3$. Amines produced by the action of living organisms are referred as biogenic amines [5, 6]. Histidine decarboxylase and histaminases are among enzymes known to be responsible for the conversion of histidine to histamine [7, 8, 9]. Histidine is converted to histamine by microbial histidine decarboxylase enzyme. Consumption of spoiled fresh, frozen fish and tinned fish products which contain unusually high levels of histamine which results in the outbreaks of histamine fish poisoning is one such food poisoning [10]. Freshly caught fish have histamine levels of less than 2 mg/kg. Fish containing histamine levels greater than 20 mg/kg cause adverse symptoms in people. According to the US Food and Drug Administration (FDA), histamine levels between 20 and 50 mg/kg indicate that the fish has deteriorated. The FDA “action level” for histamine in raw, frozen or canned tuna is 50 mg/kg [11, 12]. The European Regulation established as maximum limits for histamine, in fishery products from fish species associated with high histidine amounts, values ranging from 100 to 200 mg/kg, while for products which have undergone enzyme maturation treatment in brine, therefore mentioned limits rise to 200 and 400 mg/kg [13].

Poisoning with histamine can cause the pseudoallergic reactions, in other words, it can produce symptoms such as: urticaria, eczema, diarrhoea or spasm of bronchi. The content of histamine is regarded as a criterion of the quality of food. Even if bacteria have been killed, the enzyme activity may continue to produce histamine. Foods rich in proteins increase the histamine production [14]. The major sources of dietary biogenic amines include several types of fish species. Histamine poisoning is one of the most common chemically induced seafood borne illnesses reported in United States. Generally, it is believed that the causative agents are biogenic amines of histamine, putrescine and cadaverine produced by Gram negative bacteria. Under the U.S. Food and Drug Administration’s HACCP program, growth of histamine producing bacteria in potentially hazardous fish is controlled primarily by limiting time and temperature conditions. The purpose of this study was to determine the potential food safety risks of histamine content of fishes in Tuticorin at different sampling such as after harvested fishes in landing center, fishes from market retailer and from street vendors.

Material and methods

Sample preparation

Ten species of fish samples such as *Stolephorus commersonnii* (nethili), *Chirocentrus dorab* (Mullu vaalai), *Saurida tumbil* (Thanni panna), *Ablennes hians* (Vaalai Mural), *Epinephelus areolatus* (Kalava), *Alepes djedaba* (Manja

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paarai), *Lethrinus nebulosus* (Velameen), *Sphyraena jello* (Uuli), *Scomberomorus guttatus* (Cheela) and *Katsuwonus pelamis* (Choora) were collected from 3 sampling places such as street vendors, fish market retailer and fish landing centers. All samples were harvested from Tuticorin fishing port. Each specimen was gutted and then cut into small pieces. Since the tissues of gill and gut are considered as the major source of histamine forming bacteria in fish [15], 0.5-1 kg of muscle near the gills and stomach cavity were collected aseptically. Samples were covered with ice and immediately (<an hour) transported to the laboratory and were preserved frozen until analysis. After thawing at room temperature, samples were skinned and deboned aseptically, and the flesh were homogenized and blended, without adding any liquid.

Histamine content

10 g of homogenized sample was washed twice with 50 ml of 0.4 N Perchloric acids and centrifuged at 3000 rpm for 10 min. The volume of the supernatant was adjusted to 100 ml with 0.4 N Perchloric acids. From that, 5 ml of filtrate was taken in a separating funnel, made alkaline with 5 ml of 1 N NaOH and 10 ml of distilled water and 2.0 g NaCl were added. The filtrate was extracted 4 times with successive 25 ml portions of n-butanol. The butanolic phases were again washed with 10 ml of 1 N NaOH saturated with NaCl. The histamine was then extracted 5 times with 10 ml of 0.1 N HCl and the volume adjusted to 50 ml. The extract was finally derivatized with o-ophthaldehyde (Sigma Chemicals), and the intensity was determined using a spectrometer at wavelength of 439 nm (AOAC, 1990).

Result and Discussion

Fish spoilage may be caused by three mechanisms which are interrelated: enzymatic, chemical and microbial include water activity, stress and mechanical damage during capture, fish structure and composition rate of post mortem change/autolysis, pH and storage temperature are factors that influence the rate of fish spoilage [16]. Upon death, the defense mechanisms of the fish no longer inhibit bacterial growth in the muscle tissue and histamine-forming bacteria may start to grow, resulting in the production of BAs [17]. Evisceration and removal of the gills may reduce, but do not eliminate, the number of histamine-forming bacteria. Packing of the visceral cavity with ice may aid in chilling large fish in which internal muscle temperatures are not easily reduced. However, when done improperly, these steps may accelerate the process of histamine development in the edible portions of the fish by spreading the bacteria from the visceral cavity to the flesh of the fish. In the present study, fishes were taken from the three sampling stages such as from the landing center, market and from the street vendors. The fishes were taken from the landing centre to know the quality of freshness; second sample was taken from the market, the market range of fishes normally iced for some times. But during the auction it may not be iced until auctioning over. The third sampling was fishes bought from the street vendors, because house hold women mostly prefer the fishes from street vendor for easy to buy. But the fishes exposed to ambient temperature for longer time until afternoon. The results revealed the nature of biogenic amine and the prevention of its production. Biogenic amine formation may occur in two ways, i.e. by endogenous or exogenous decarboxylation. Endogenous decarboxylation involves production of amines by decarboxylase enzymes found in the cells of fish or other marine species while exogenous decarboxylation is caused by microorganisms which are capable of producing extracellular decarboxylases. Histamine is one of the biogenic amines that can be formed in

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food as a result of metabolic processes of microorganisms. If the concentrations of amines are above the normal level, possibly due to the bacterial contamination of food, harmful effects may occur [18]. Histamine is contained in various types of fresh and processed fishes. Histamine monitoring has now been globally accepted for safety confirmation of fish and seafood products. Storage temperature is the most important factor contributing to biogenic amine formation. Since histamine is neither volatile nor destroyed by cooking, a convenient method of detecting in seafood samples is needed, particularly where decomposition is suspected. CDC [19] and Chong [20] reported 5 - 10 mg could be a sign of histamine poisoning; 50 -100 mg will always give histamine poisoning and 20 mg is maximal in food [21].

Most of the samples of the present study contained little histamine, but the sample from an up-market retailer had a moderate histamine level of 3.1-12.6 mg/kg and, the level was raised to 12.1- 38 mg/kg in fishes purchased from street vendors. Among the others, sample taken from the street vendors had higher level of histamine above 15 mg/kg due to insufficient preservation and longer time exposed to ambient temperature. In our present study, samples of different scombroid family such as (*Scomberomorus guttatus* and *Katsuwonus pelamis*) non-scombroid fishes (*Stolephorus commersonnii, Chirocentrus dorab, Saurdia tumbil, Ablennes hians, Epinephelus areolatus, Alepes djedaba, Lethrinus nebulosus,* and *Sphyraena jello*) **(Photo – 1 to Photo – 10)** were collected as per **Tabel - 1**. The result indicates, compared to scombroids fish's histamine production was low in non scombroids fishes in all the sampling area. Fishes taken from the landing center in the midnight after landing had very less amount of histamine and the fishes like *Stolephorus commersonnii, Saurdia tumbil, Lethrinus nebulosus* were completely unspoiled. These samples were collected from the market and street vendors should have histamine. It is also interesting to note that, samples taken from a street vendor, they bought the fishes from the landing center and it was partially iced or kept in ice water for some times. After that it was taken for the sale and during sale time it was without ice and it was exposed to temperature above 30°C until all are getting over. During the market hours it was exposed to ambient temperature until all auctioning is over. Storage is at ambient temperature until unloaded at the processing plant, with the first-caught fish being already stored for up to 10 hours. Such a long period may cause histamine-producers to undergo in doublings, an increase of 1000 times (three log scales) over the assumed initial level of 10/g or cm reaching a level of 10000/cm^2 at fish surfaces or 10000/g in the gut [22]. During the exposure time the fishes undergo to development of biogenic amines because after death, the defense mechanisms of the fish which protect its tissue from bacterial infection are no longer functional [23]. Bacteria may invade through the skin, body cavity and intestinal tract and by vascular penetration through the gills and kidney [24, 25]. The bacteria may produce histamine and other amines during growth. Evisceration and removal of the gills in a sanitary manner may reduce the number of histamine producing bacteria, resulting in higher quality products [26]. However such measures will not completely remove bacterial growth. As well as, the practice may turn into a blackish if not performed in a sanitary way, as the evisceration may help in the spreading of the bacteria to the flesh of the fish [27].

Auerswald, et al. [28], reported histamine levels in freshly caught fish are generally low, usually below 0.1 mg/100g and it was agreed with our study. At anytime, exposure of certain fish to elevated temperatures after the catch and before consumption can cause formation of

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histamine from histidine by bacterial histidine decarboxylases. In the recent study also exposes of elevated temperature was limited in fishes taken from landing center and it was longer in market and street vendors. While most of the studies agree that histamine formation is negligible in fish stored at 0˚C or below, data concerning storage conditions at higher temperatures are variable and do not allow for the establishment of standard procedures for avoiding potential negative effects of transport/storage conditions on fish safety [29]. However, fish is more likely to form BAs when decomposition occurs at harvest or in the first stages of handling in the fishing vessels, rather than later in the distribution chain [30]. The histamine poisoning otherwise called as scombroids fish poisoning. The term "scombroid" is derived from the name of the family *Scombridae* which includes the fish species that were first implicated in histamine intoxication (i.e. in tuna and mackerel). These species of fish share in common high levels of free histidine in their muscle tissues. It is known that other non-scombroid fish species are also implicated in scombroid poisoning, such as mahi-mahi (*Coryphaena* spp.), sardines (*Sardinella* spp.), pilchards (*Sardina pilchardus*), anchovies (*Engraulis* spp.), herring (*Clupea* spp.), marlin (*Makaira* spp.), blue fish (*Pomatomus* spp.), Western Australian salmon (*Arripis truttaceus*), sock eye salmon (*Oncorhynchus nerka*), amber jack (*Seriola* spp.), Cape yellow tail (*Seriola lalandii*), and swordfish (*Xiphias gladius*). In the present study also both scombroid and non scombroid family of the fishes were taken and the results showed biogenic amines was high in scomberoid fishes of *Scomberomorus guttatus* and *Katsuwonus pelamis.* Histamine levels in live seafood are generally low, as shown by several authors who measured histamine concentrations in freshly caught fish: values below 1ppm were found in scombroid species, such as the skip jack (*Katsuwonus pelamis*) [10, 31], black skip jack (*Euthynnus lineatus*) [32] whereas, in non-scombroid fish, such as in hake, (*Merluccius merluccius*), no histamine was found at all [33, 34]. For instance, while 50 mg/kg of histamine may be found in one fish section, its level may exceed 500 mg/kg in another [17]. Thus, even if the same histamine - containing fish is ingested, some consumers may be poisoned and some may not [35, 36].

Histamine producing bacteria are commonly present in the marine environment. They usually exist on the gills and in the gut of live marine fish species and cause no harm to the fish themselves. According to Lerke [37], histamine contents in spoiled tuna can be as high as 919 mg/dl (9190 ppm). The contents in the anterior of the fish are generally higher than in the posterior parts. Therefore the anterior portion is preferred for histamine analysis [22]. In the flesh near the stomach cavity and gill, the contents may be much higher than in other parts. So in the present study samples were taken from the near gill and stomach in the anterior portion. The quantity of developed histamine depends on the bacterial species; temperature and duration of exposure to the bacteria effect, and it can be as high as 1000 mg/ kg. Good quality fish should not contain more than 10 mg/kg of histamine. In the present study fishes from the landing center had histamine content was less than 3mg/kg, but from the market except the fishes (*Katsuwonus pelamis* and *Scomberomorus guttatus*) remaining fishes had the histamine content less than 10 mg/kg. The fishes taken from the fish vendors had histamine content more than 10 mg/kg. Nausea, vomiting, headache and other symptoms are induced by consumption of foods containing high levels of histamine. According to the Regulation (EC) No 2073/2005 [38], histamine limit values for fish in the countries of the European Union are 100 mg/kg. Rapid cooling of fish immediately after

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fishing is a crucial element in the strategy of prevention of spoiled fish food, and consequently, of the formation of biogenic amines in fish [39].

It is noteworthy to mention that the caught fishes cannot immediately be collected after entangling and they remain inside the water for a while with considerable duration for further transferring on board to be cooled before being frozen and stored. If these delays are prolonged, some post mortem decomposition and accumulation of histamine may occur in the fish [40] and it was agreed with our study. Other factors such as unsuitable handling, post-catching contamination, inadequate chill-storage procedures, inadequate freezing and thawing procedures also affect the probability of histamine accumulation. Histamine accumulation is a result of time/temperature abuse that leads to spoilage during storage and processing. To minimize the risk, the FDA [27] recommends that the internal temperature of the harvested fish to be lowered and maintained below 4°C as soon as possible (within 6 hours of fish death at the maximum). Temperatures of 4°C or below are considered sufficient for minimizing the growth of pathogens.

A histamine level of 20 mg/kg is an indicator of decomposition [41] and several countries have set legal limits of histamine concentrations that are regarded as safe for human consumption: Australia, 200 mg/kg [42], Europe, 100 mg/kg [43], USA, 50 mg/kg [11] and South Africa, 100 mg/kg [44]. In this study, apply the stricter FDA level. Salting and canning may remove bacterial contamination [14, 45] but they cannot destroy the causative toxin (histamine) of scombroid seafood poisoning [46]. Any histamine found in such products should be, therefore, an indication of the conditions to which the seafood was exposed before processing.

Moreover, mishandling coupled with high temperature abuse is likely when handling fish significantly enhance histamine formation. The amount of post-harvest time at elevated temperatures to which a fish can be exposed (e.g., during processing, storage and distribution) without adverse effects depends primarily on whether the fish was previously frozen or heat-processed sufficiently to destroy histamine forming bacteria [17]. Rossano, et al. [29], studied the influence of storage temperature and time of freezing on histamine formation in anchovies, showing the ability of freezing to inhibit or slow down its formation. It is recommended as follows, fish should be put on ice, in cooled seawater or brine at the temperature of 4.4 ˚C or lower for 12 hours after death or at 10 ˚C or lower for 9 hours after death. Fish exposed to air or water temperature exceeding 28.3 ˚C, should be put on ice, in cooled sea water or brine at the temperature of 4.4 ˚C or lower for 6 hours after death [27]. This will prevent rapid development of the enzyme histidine decarboxylase, because the hazard control is no more possible after the formation of this enzyme [39].

Conclusion

Our results demonstrated that few sea food samples in this study had above the legal limit for histamine. It also clearly notes that the type of seafood outlet is not indicative of a possible histamine contamination but rather the freshness of the sample. Seafood is susceptible to contaminated by biogenic amines at different points of the food chain. High levels of BAs can be prevented through the application of good hygiene practices and proper temperatures during handling, delivery and storage. Although BAs formation is the result of bacterial growth, the presence of these undesirable compounds, especially histamine, is not always noticed by consumers. In fact, while a fish with obvious spoilage will most likely not to be consumed a fish with a good appearance and no detectable spoilage odors may be consumed even if it contains a high histamine level. Thus, the application of appropriate control measures is fundamental for assuring seafood safety and such a responsibility is shared among the seafood catchers, processors, distributors, retailers and merchants.

The FDA has issued guidelines aiming at establishing procedures for the safe processing and importing of fish and fishery products based on the hazard analysis and critical control points (HACCP) approach [17]. According to the most recent HACCP guidelines for the control of histamine production, a core temperature of 4.4 ˚C or less should be achieved and maintained throughout handling, processing and distribution of potentially hazardous fish. The primary goal of these guidelines is the growth inhibition of spoilage bacteria capable of producing histamine through proper handling and chilling of fish. In order to achieve this objective, all the fishes should be iced immediately after landing aboard the vessel so that the temperature at sites of microbiological concern is reduced at levels capable of controlling the growth of histamine-producing bacteria. Permanent control of the histamine presence in food rich in proteins should be introduced, because of the possibility of histamine development in such foodstuffs, detrimental to human health. Since the "screening" method for quantitative determination of histamine is easy to perform, the control of histamine presence should be legally regulated for the protection of human health.

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Table – 1: Histamine content of fishes collected from various sampling stages.

Fish samples	Sampling from street vendors			Market retailers			Landing center		
	Histamine content (mg/kg)	Temp (°C)	Time (h) After catch	Histamine content (mg/kg)	Temp (°C)	Time (h) After catch	Histamine content (mg/kg)	Temp (°C)	Time (h) After catch
Stolephorus commersonnii	17.2	32°C	12	3.1	32°C	8	0.0	21°C	1
Chirocentrus dorab	19.4	32°C	12	6.3	32°C	8	0.6	21°C	1
Saurida tumbil	20.0	32°C	12	4.9	32°C	8	0.1	21°C	1
Ablennes hians	17.7	32°C	12	6.6	32°C	8	0.13	21°C	1
Epinephelus areolatus	23.4	32°C	12	8.8	32°C	8	1.42	21°C	1
Alepes djedaba	26.9	32°C	12	7.5	32°C	8	0.75	21°C	1
Lethrinus nebulosus	12.1	32°C	12	9.1	32°C	8	0.2	21°C	1
Sphyraena jello	18.3	32°C	12	3.5	32°C	8	0.26	21°C	1
Scomberomorus guttatus	25.3	32°C	12	11.1	32°C	8	0.83	21°C	1
Katsuwonus pelamis	38	32°C	12	12.6	32°C	8	0.6	21°C	1

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Photo – 1: *Scomberomorus guttatus*

Photo – 2: *Katsuwonus pelamis*

Photo – 3: *Stolephorus commersonnii*

Photo – 4: *Chirocentrus dorab*

Photo – 5: *Saurida tumbil*

Photo – 6: *Ablennes hians*

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Photo – 7: *Epinephelus areolatus*

Photo – 8: *Alepes djedaba*

Photo – 9: *Lethrinus nebulosus*

Photo – 10: *Sphyraena jello*

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