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CHANGES OF HISTAMINE LEVELS AND BACTERIAL GROWTH IN LONGTAIL TUNA, *Thunnus tonggol* STORED AT DIFFERENT TEMPERATURE

NURUL AINA SYUHADA MAHUSAIN[1], FERNANDES BAYOI[1], NURUL ULFAH KARIM[1], MOHAMAD KHAIRI ZAINOL[3] AND MUHD DANISH-DANIEL[*1,2]

[1]School of Fisheries and Aquaculture Sciences, [2]Institute of Marine Biotechnology and [3]School of Food Science and Technology, Universiti Malaysia Terengganu, 21030 Kuala Nerus, Terengganu, Malaysia.

[*]Corresponding author: mdda@umt.edu.my

Abstract: Histidine is a precursor for histamine (4-(2-aminoethyl) imidazole) formation through a process called decarboxylation with the aid of histidine decarboxylase enzyme possessed by particular bacterial species. Since storage temperature highly influences the formation of histamine, a study regarding histamine and bacterial count was conducted. Longtail tuna was stored at -20, 4, 15, 25 and 37℃ and analysis of bacteria and histamine were carried out for every 48 hours till reaching 196 hours for freezing and chilling storage temperature. Histamine determination and bacterial count were performed every 0, 6, 12, 18 and 24 hours for 15, 25 and 37℃. Storage of gutted and non-gutted fish samples at day 0, -20°C showed histamine level of 3.496 mg/100 g. Reduction of histamine levels took place after 48 hours of storage but increased later throughout the storage period up to 8 days reaching a value of 4.84 and 3.78 mg/100 g for non-gutted and gutted tuna, respectively. Similar trend was seen at 4℃ where a gradual increase of histamine concentration was observed throughout the storage period. However, the histamine level in non-gutted tuna exceeded the safety level at day 8. At 15 and 25℃, the non-gutted fish meat is not safe for consumption after 24 hours. Meanwhile, the safety limit can be seen within 12 hours and 18 hours of storage in non-gutted and gutted fish stored at 37℃, respectively. For bacterial cell enumeration at -20℃, the initial count of bacteria is 5.4 Log CFU/g for both fish condition. The count then slightly increased to 6.28 and 5.91 for non-gutted and gutted fish, respectively at 48 hours till reaching last storage time. Similar trend was recorded at 4, 15, 25 and 37℃ from time to time. However, bacterial count was largely seen at 37℃. This may be due to the presence of spoilage bacteria that can deteriorate the quality of fish. This study has provided insights into the effects of storage temperature on the formation of histamine and bacterial growth in *Thunnus tonggol*.

Keywords: Histamine, Setiu, storage temperature, Terengganu, *Thunnus tonggol*

Introduction

Histamine (4-(2-aminoethyl)imidazole) is a heterocyclic amine (Ten-Brink *et al*., 1990) that is produced from the decarboxylation of free histidine catalyzed by histidine decarboxylase possessed by particular bacterial species. Histamine bacteria found in fish are dominated by the gram negative bacteria which contain pyridoxal-P-dependent histidine decarboxylase (Kimura *et al.,* 2009) that particularly belong to the family of *Enterobacteriaceae* (Joosten & Northolt, 1989). The primary contributor of histamine are *Morganella morganii* (Kim *et al.,* 2001), *Hafnia alvei* and *Klebsiella pneumonia* (Taylor *et al.,* 1979) which have been isolated from the fish incriminated in scombroid poisoning. Histamine fish poisoning multiply rapidly during improper storage, handling and processing.

Histamine can be found in various types of foods such as fish products (Chen *et al*., 2008; Tsai *et al*., 2005), sausages (Suzzi & Gardini, 2003), cheese (Joosten & Nunez, 1996), raw fish (Kim *et al*., 2009; Tsai *et al*., 2005) and fish sauce (Jiang *et al*., 2007; Kuda & Miyawaki, 2010; Park *et al*., 2001). Highly contaminated foods with histamine are prone to cause histamine intolerance in sensitive human while in fish and fish products; histamine has the tendency to cause food poisoning.

Scombroid poisoning is a seafood related illness closely linked to the formation of histamine on spoiled or bacterially contaminated Scombridae and Scomberesocidae fish family (Murray *et al*., 1982). Tuna, mackerel, bonito and saury are scombroid fish that usually associated with scombroid poisoning due to accumulation of high levels of free histidine in their muscle (Lehane & Olley, 2000). However, non scombroid fish such as mahi-mahi, bluefish, herring and sardine have no exception to implicate in histamine fish poisoning (Hwang *et al*., 2012). Histamine poisoning occurs worldwide. For instance, Gilbert *et al.* (1980) reported 150 patients were infected with scombrotoxic fish poisoning in Britain with 30 separate outbreaks, each outbreak showed similar symptoms.

Histamine poisoning commonly takes place when fish containing up to 1000 mg/kg but the cases have been reported when the levels as low as 200 mg/kg (Bortholomew *et al.,* 1987). The onset of poisoning takes place within 10 minutes to two hours after ingesting toxic fish (Murray *et al*., 1982). The symptoms of scombroid poisoning are mild illness with variety of symptoms including rash, urticaria, edema, inflammation, nausea, vomiting, diarrhea, abdominal cramps, hypotension, headache, palpitation, flushing, tingling, burning, itching (Taylor, 1986). However, suffering individual will experience only a few of these symptoms. Histamine poisoning commonly go unnoticed due to fast recovery at which infected person usually takes three hours, but in few cases they can last for several days (Kim *et al*., 2001). Severity of the symptoms differed in terms of individual's sensitivity and amount of histamine ingested.

Temperature is a fundamental in controlling quality of fish and fish products. Improper control of temperature during transport, storage, or manufacturing of food products can lead to histamine contamination which allowed multiplication of histamine forming bacteria and liberation of histamine (Takahashi *et al*., 2015). Histamine is commonly formed after exposing fish to ambient temperature after catch and increase of temperature and time during storage can raise the levels of histamine. Low storage temperature is considered as a safe level to control histamine producing bacteria.

In this study, we investigated the effects of different storage temperature on the formation of histamine and bacterial growth in longtail tuna, *Thunnus tonggol* as one of the initiatives to promote awareness on the importance of seafood safety and hygience among Setiu communities.

Materials and Methods

Sample collection and preparation

Fifty longtail tuna was purchased from fisherman at Setiu areas, Terengganu, Malaysia. After purchasing, the fish samples were stored in cool box with ice and transported to laboratory for analysis. Upon arrival at the laboratory, 25 fish samples was remained non-gutted and another 25 was gutted and placed in individual polyethylene bags according to different storage temperature and different day of storage. Samples were stored at -20 and 4 ℃ and analysed every 48 hours. In another study, samples were stored at15, 25 and 37 ℃ for one day and analysed every 6 hours.

Histamine analysis

Analysis of histamine formation was carried out according to the protocols as described in Patange *et al*. (2005). Firstly, fish muscle (5g) was taken from the dorsal part of fillet without skin and transferred to 75 ml centrifuge tube. The sample was then homogenized with 20 ml of 0.85% NaCl solution (saline) for 2 min using a high speed blender and centrifuged at 12000 x g for 10 min at 4 ℃. The supernatant was made up to 25 ml with saline. After that, the muscle extract was used immediately for further analysis. In a glass-stoppered test tube, 1 ml of the extract was diluted to 2 ml with saline and 0.5 g of salt mixture containing 6.25 g of anhydrous sodium sulfate to 1 g trisodium phosphate monohydrate was added.

The tubes were stopper and shaken thoroughly. 2 ml of n-butanol was then added and the tubes were shaken vigorously for 1 min and allowed to stand for 2 min and then shaken briefly to break the protein gel. The tubes were further shaken vigorously for few seconds and then centrifuged at 3100 x g for 10 min. The upper butanol layer (only 1 ml) was transferred into a clean and dry test tube and evaporated to dryness in a stream of nitrogen. The residue was dissolved in 1 ml of distilled water and then reacted for 5 min with p-phenyl diazonium sulfanate reagent. The absorbance was measured immediately at 496 nm. Distilled water was used as a blank. The histamine concentration was obtained from the standard curve.

Microbiological analysis

A 25-g portion of the longtail tuna sample was homogenized at high speed for 2 min in a sterile blender with 225 ml sterile potassium phosphate buffer (0.05 M, pH 7.0). The blender was sterilized by autoclaving for 15 min at 121 ℃. The homogenates were serially diluted with a sterile phosphate buffer, and 1.0 ml aliquots of the dilute were poured into aerobic plate count (APC) agar containing 0.5% NaCl. Bacterial colonies were counted after the plates were incubated at 35 ℃ for 48 h. Triplicates were taken for bacterial analysis. Bacterial numbers in the samples were expressed as $\log_{10}$ colony forming units (CFU)/g.

Results and Discussion

The contents of histamine in gutted and non-gutted longtail tuna samples

The histamine concentrations of longtail tuna at -20, 4, 15, 25 and 37 ℃ are shown in Figure 1. Histamine in gutted and non-gutted long tail tuna stored at -20° C at day 0 with a level of 3.496 mg/100. The histamine levels showed decreasing value at 48 hours and then increased throughout the storage period, reaching a value of 4.84 and 3.78 mg/100 g for non-gutted and gutted tuna respectively at 192 hours which was considered as a safe level by FDA standard (must not exceed 5mg/100 g). The formation of histamine is not only influenced by histamine forming bacteria but also histamine degrading bacteria.

Therefore, presence of histamine degrading bacteria may be a possible reason for any decline for earlier stage of every temperature. The formation of histamine at cold storage temperature (4 ℃) showed similar trend with -20 ˚C at which gradual increase was observed throughout the storage period. However, the histamine level in non-gutted tuna exceeded the safety level at 192 hours. At 15 and 25 ℃, 24 hours storage, the non-gutted fish meat is not safe for consumption. Meanwhile, the safety limit can be seen within 12 and 18 hours of storage in non-gutted and gutted fish stored at 37 ℃, respectively.

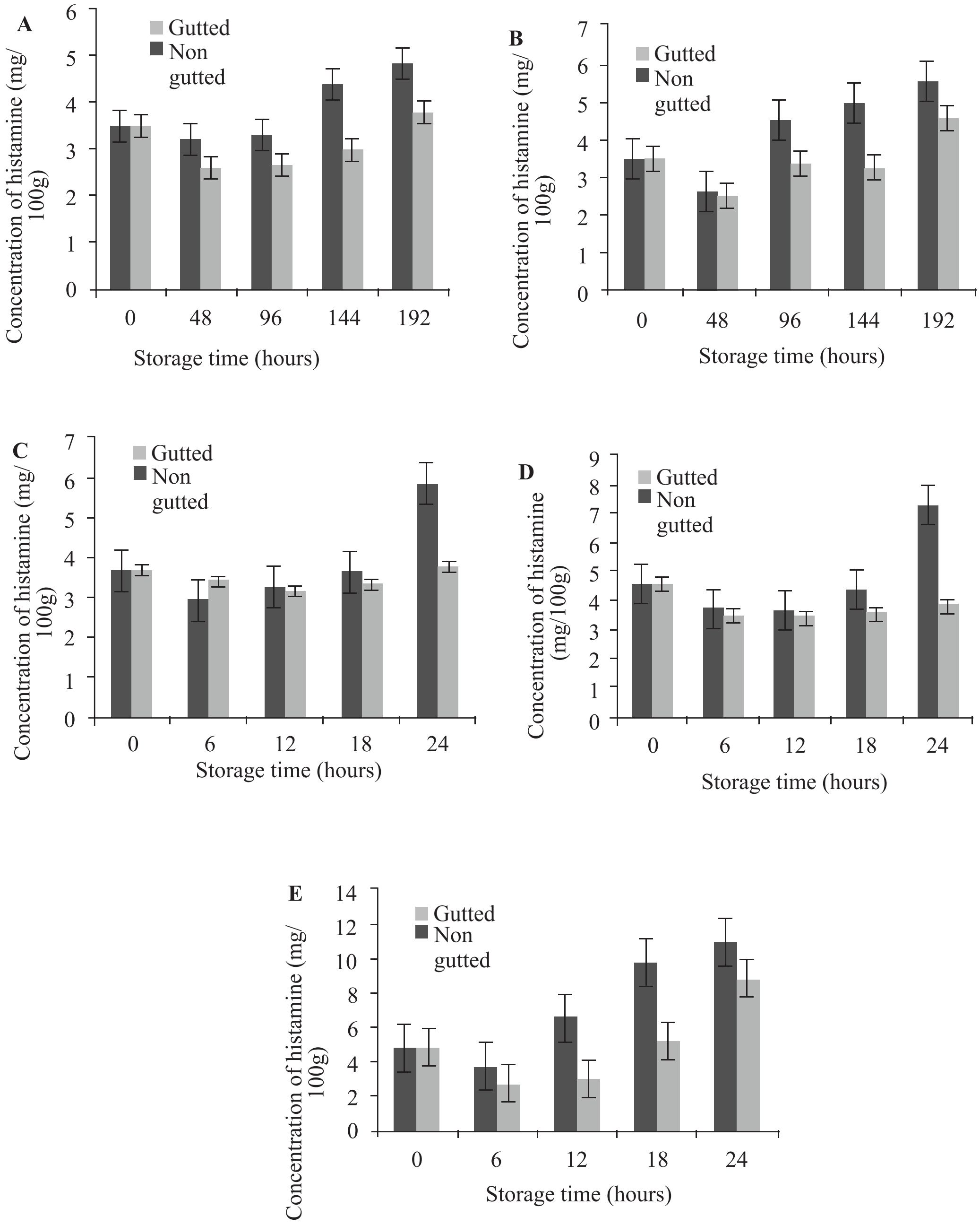

Figure 1: Histamine accumulation in gutted and non-gutted tuna meat at five different temperature; -20℃ (A), 4 ℃ (B), 15℃(C), 25℃(D) and 37℃ (E). *There was no statistically significant difference between gutted and non-gutted fish ($p<0.05$).

Temperature is the major determinant for histamine formation. Combination of time and temperature affect concentration of biogenic amine at which longer times and higher temperatures will lead to greater microbial growth and biogenic amine formation (FAO & WHO, 2012). Low storage temperature of fish has been guaranteed as a safe level to control histamine producing bacteria. A storage temperature of -20℃ may inactivate some of the decarboxylase enzyme bacteria (FDA, 2001). Kim *et al*. (2009) found no change of histamine concentrations in mackerel, saury, spanish mackerel and amberjack samples when stored at -20˚C.

The finding from the previous study agreed with that of Afsharmanesh *et al*. (2013) who found no histamine level in *Thunnus albacores* which are stored in frozen method. In the present study, histamine concentration increased both in gutted and non-gutted tuna. This may be due to initial contamination during and after catching. Prolong exposure to room temperature during defrost might increase histamine concentration in tuna. Silva *et al*. (1998) reported rise of histamine content at 10 and 4 ℃, but notable amounts were detected after 3 days at 10℃ and 6 days at 4℃. A study conducted by Rossano *et al*. (2006), are in agreement that histamine level increase with time of storage when stored at 4℃.

Storage at 15, 25 and 37℃ encouraged unsafe level of histamine mainly in non-gutted sample. Tsai *et al*. (2005) reported that in milkfish, the highest histamine content was detected at 399mg/100g after 24hours at 25 ℃ and 419mg/100g after 12hours at 37 ˚C. When stored at 15 ℃, the fish samples continued to accumulate histamine up to 96h, reaching 199mg/100g in sailfish. The primary contributor of histamine at high temperature is *Morganella morganii* (Klausen & Huss, 1987) which can provide large amount of histamine in fish. In the present study, all non-gutted tuna exhibited high concentration of histamine in all temperatures compared to gutted sample. This probably due to large amount of bacteria dominated in viscera area rather than other fish parts. In fact, not much difference ($p<0.05$) can be seen between gutted and non-gutted fish maybe because of short time interval.

Microbiological analysis of gut and non gut longtail tuna samples

Table 1 indicated bacterial count in gutted and non-gutted longtail tuna at -20, 4, 15, 25 and 37 ℃. At -20 ℃, the initial count of bacteria is 5.4 Log CFU/g for both fish condition. The count then slightly increased to 6.28 and 5.91 for non-gutted and gutted respectively at 48 hours till reaching last storage time. Similar trend was recorded at 4, 15, 25 and 37℃ from time to time. Bacterial count is largely seen at 37℃. This may be due to the presence of spoilage bacteria that can deteriorate the quality of fish. In fact, the fish stored at 37℃ undergo earlier deterioration rather than other temperature by having foul smell at 12 days and protruding belly and eyes at 24 days.

Table 1: Bacterial count in gutted and non-gutted tuna meat at five different temperature, -20℃ (A), 4 ℃ (B), 15℃(C), 25℃(D) and 37℃ (E).

A

Storage time (hours)	**Bacterial count (Log CFU/g)**	
	Non-gutted	**Gutted**
0	5.4	5.4
48	6.28	5.91
96	6.4	6.1
144	6.59	6.43
192	6.64	6.46

B

Storage time (hours)	**Bacterial count (Log CFU/g)**	
	Non-gutted	**Gutted**
0	5.4	5.4
48	6.4	6.26
96	6.53	6.35
144	6.61	6.37
192	7.45	7.3

C

Storage time (hours)	**Bacterial count (Log CFU/g)**	
	Non-gutted	**Gutted**
0	5.26	5.26
6	5.35	5.3
12	5.71	5.68
18	6.69	6.6
24	6.76	6.71

D

Storage time (hours)	**Bacterial count (Log CFU/g)**	
	Non-gutted	**Gutted**
0	5.68	5.68
6	5.16	4.8
12	5.39	5.35
18	6.59	6.49
24	7.23	7

E

Storage time (hours)	**Bacterial count (Log CFU/g)**	
	Non-gutted	**Gutted**
0	3.43	3.43
6	6.53	6.01
12	7.62	7.5
18	7.71	7.61
24	9.75	9.7

Fish bacteria are very sensitive to temperature, higher temperatures promotes the fish bacteria to grow and multiply rapidly using the organic compounds of fish as source of energy, while lower temperatures did not totally stop the growth of bacteria but they might inhibit the bacterial growth and activities (Humaid & Jamal, 2014). Elevated temperature leads to promote the bacterial growth (Pan & James, 1985). Many studies showed that the load of bacterial growth almost similar in the case of different fish species in the same environment, especially when fish are exposed to similar treatments (Humaid & Jamal, 2014; Paleologos *et al*., 2004; Rossano *et al*., 2006). The present study indicated the histamine levels of longtail tuna for each temperature are not in relation with the total bacterial count as increased of storage time occurred. According to Edmunds and Eitenmiller (1975), the total number of bacteria present in fish is not directly related to the formation of histamine but greatly influenced by the microflora capable of synthesizing histidine decarboxylation. However, Chong *et al*. (2014) reported that correlation was observed between concentration of histamine and aerobic plate count in sample stored at ambient temperature but such correlation was not found at 0°C.

The high concentration of histamine in fish stored at ambient temperature could be explained by the proliferation of mesophilic bacteria under optimum growth condition as compared to ice storage. Emmanuelle *et al*. (2012) found that strong correlation existed between the aerobic plate count and histamine. In order to maintain the freshness of fish, proper storage is necessary. Low storage temperature can delay the formation of histamine meanwhile high storage temperature provide accumulation of high levels of histamine which result in histamine fish poisoning.

Conclusion

The increase of storage temperature can increase histamine concentration and bacterial count for both gutted and non-gutted fish. However, non-gutted fish indicated higher level of histamine and bacterial count compared to gutted sample. It is recommended that bacteria producing histamine should be identified and their role in histamine accumulation shall be investigated. Strategies to improve the shelf-life of longtail tuna by inhibiting the histamine forming bacteria can be carried out in the future.

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