DETERMINATION OF HISTAMINE LEVELS IN LONGTAIL TUNA (*Thunnus tonggol*) STORED AT DIFFERENT TEMPERATURE

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ABSTRACT

Histidine is 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 is highly influenced 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 was done for every 48 hours till reaching 196 hours for freezing and chilling storage temperature. Histamine determination and bacterial count was taken for every 0, 6, 12,18 and 24 hours for 15, 25 and 37℃. At 0 day of storage for -20° C, gut and non gut fish samples showed histamine level of 3.496 mg/100. 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 gut and non gut tuna, respectively. Similar trend of result was seen at 4℃ where a gradual increase of histamine concentration was observed throughout the storage period. However, the histamine level in gut tuna exceeded the safety level at day 8. At temperature 15 and 25℃, the gut fish meat is not safe for consumption at 24 hours. Meanwhile, the safety limit can be seen within 12 hours and 18 hours of storage in gut and non gut 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 gutted and non gutted 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 is largely seen at 37℃. This may be due to the presence of spoilage bacteria that can deteriorate the quality of fish.

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 can be found in various 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., 2002; 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 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).

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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).

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 is 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. Histamine is commonly formed after exposing fish to ambient temperature after catch and increase of temperature and time during storage can rise up the levels of histamine. Low storage temperature of fish has been guaranteed as a safe level to control histamine producing bacteria.

MATERIALS AND METHODS

Samples collection and preparation

Longtail tuna was purchased from 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, half fish samples was remained non-gutted and another half was gutted and placed in individual polyethylene bags according to different storage temperature and different day of storage. The samples were used for every 48 hours for -20℃ and 4℃ for 8 days and every 6 hours for 15℃, 25℃ and 37℃ for a day respectively.

Histamine analysis

Histamine analysis was followed the protocol described by 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 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. 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 stoppered and shaken thoroughly. 2 ml of n-butanol was then added and the tubes 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 with p-phenyldiazonium sulfanate reagent. The absorbance of the color produced was measured immediately after 5 min at 496 nm using distilled water as a reference and the concentration of histamine was obtained from the standard curve.

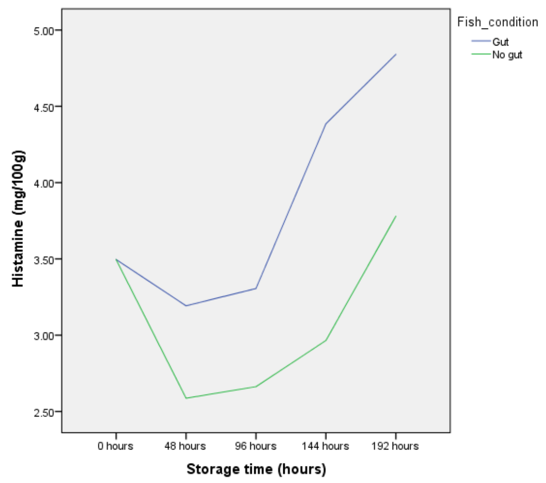
Microbiological analysis

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 expresses as $\log_{10}$ colony forming units (CFU)/g.

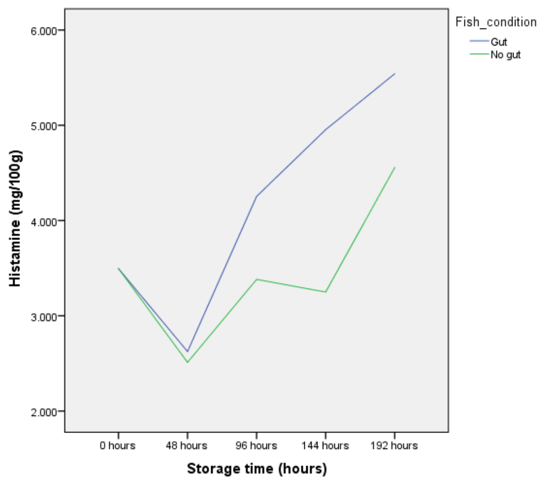
RESULTS AND DISCUSSION

The histamine concentrations of longtail tuna at -20, 4, 15, 25 and 37℃ are shown in Fig. 1. Histamine in gutted and non-gutted longtail tuna stored at -20̊ C at day 0 with a level of 3.496 mg/100. The histamine levels showed decreasing value at 48 hours and turned to increase 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. The formation of histamine at cold storage temperature (4℃) showed similar trend with the previous temperature 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 temperature 15 and 25℃, the non-gutted fish meat is not safe for consumption at 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.

A



B



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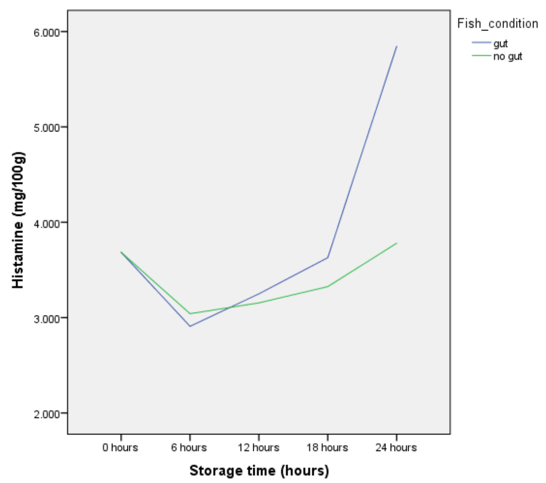

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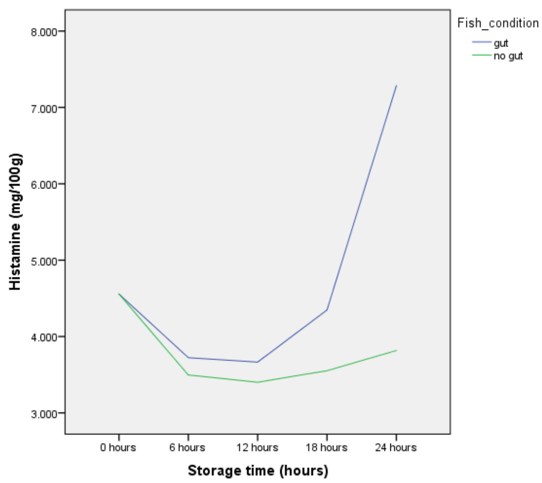

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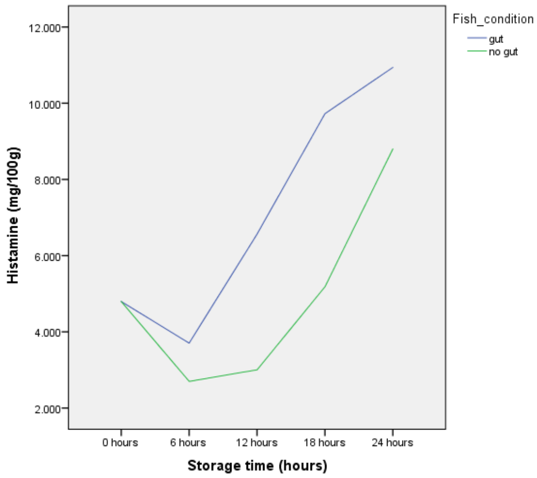

Fig 1. Histamine accumulation in gutted and non-gutted tuna meat at -20℃ (A), 4 ℃ (B), 15℃(C), 25℃(D) and 37℃ (E)

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. (2010) 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 albacares* which stored in frozen method. In the present study, histamine concentration increased both in gut and non gut 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 samples. Tsaiet al. (2005) reported that in milkfish, the highest histamine content was detected at 399 mg/100 g after 24 h at 25℃ and 419 mg/100 g after 12 h at 37 ℃. When stored at 15℃, the fish samples continued to accumulate histamine up to 96 h, reaching 199 mg/100 g 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 gut tuna exhibited high concentration of histamine in all temperatures compared to non-gut 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 gut and non gut fish maybe because of short time interval.

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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 gutted and non-gutted respectively at 48 hours till reaching last storage time. Similar trend was recorded at 4, 15, 25 and 37℃ from time to time.

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

A

Storage time (hours)	Bacterial count (Log CFU/g)	
	Gutted	Non 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)	
	Gutted	Non 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)	
	Gutted	Non 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)	
	Gutted	Non 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)	
	Gutted	Non 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

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.

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