Effects of temperature and time of incubation on the formation of histamine in bonito tuna flesh

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Abstract

Nowadays, tuna related food products are usually faced to the problem of histamine contamination. Histamine causes foodborn illness and allergy due to the activities of *Morganella morganii* and *Enterobacter aerogenes*. This study tried to simulate the worse case of damaged tuna on effects of temperature and time of incubation on the formation of histamine in bonito tuna flesh. Tuna flesh samples were collected by cutting from bonito tuna and used in experiment. The result showed that microbiological contents of frozen and thawed tuna were below 1.9 x 10^3 CFU/g. Incubation of tuna flesh at 4°C for 6 h could control histamine content to 27 ppm, in which lower than USFDA regulation (50 ppm). In addition, histamine content in tuna flesh incubated at 25°C for 24 h was found to be 415.5 ppm, which was lower than the hazard level of USFDA regulation at 500 ppm. Inoculation of *E. aerogenes* in tuna flesh accelerated the formation of histamine to over hazard level within 18 h. Therefore, the information gained from this study can be applied to the process planning of tuna processing and the production of tuna related products.

Keywords : histamine; formation; bonito tuna; incubation; *Enterobacter aerogenes*

1. Introduction

Thailand is one of the biggest canned tuna producer and exporter in the world. During processing of canned tuna, a serious problem regarding hazard related to the formation of histamine produced by microorganisms. The US Food and Drug Administration (USFDA) have received several reports of scombrotoxin fish poisoning or histamine poisoning associated with tuna products. Tuna product manufacturers are trying to control the formation of histamine by strictly control the temperature and time of processing regarding USFDA practice. However, frozen tuna, the main raw material for tuna processing in Thailand, are needed to be pretreatment by thawing prior to further processing. Temperature abuse and time delay during tuna processing before heat treatment can lead to the histamine formation. Additionally, physical damage of tuna caused by handling abuse may facilitate histamine producing bacteria to grow and produce histamine in faster rate.

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Histamine is the causative agent of scombroid poisoning and a foodborne chemical hazard. Although scombroid poisoning is usually a mild illness with symptoms including rash, urticarial, nausea, vomiting, diarrhea, flushing, and tingling and itching of the skin (Taylor, *et al*., 1986). The *Scomberesocidae* and *Scombridae* families has been associated with histamine poisoning, a foodborne illness. These fish species have in common a high level of histidine in their muscle (Ovissipour *et al*., 2011; Tsai *et al*., 2004). Histidine can be transformed into histamine by decarboxylating microorganisms if microbiological risks are not avoided during processing (Ben-Gigirey *et al*., 1999; Björnsdóttir-Butler *et al*., 2010; Fernández-No *et al*., 2010; Tsai *et al*., 2004). Although refrigeration and frozen storage can extend the shelf life of seafood products, there are various psychotropic bacteria growing in their gut, which can contaminate the flesh during the gutting process and can increase in numbers if temperature abuse occurs during processing. When decarboxylating microorganisms are present in the flesh, they can induce the formation of histamine in any relating products and the level of histamine formed is generally affected by the combination of both time and temperature. The most frequently occurring microorganisms associated with fish histamine poisoning are *Enterobacteriaceae, Morganella morganii, Klebsiella pneumonia* and *Hafnia* (Kim *et al*., 2000). However, a variety of bacteria capable of producing histamine has been identified in fish (Björnsdóttir-Butler *et al*., 2010; Chen *et al*., 2008; Economou *et al*., 2007; Fernández-No *et al*., 2010; Kim *et al*., 2000). Some countries, such as USA, England, Japan and Thailand, have established legal limits, or at least tolerable maximum contents for histamine in fish and fish products. The USFDA has established a defect action level of 50 ppm for histamine in tuna, mahi-mahi and other fish species as an indication of potential health risk (FDA, 1996). The European Community has fixed 100 ppm for fish and fish products as a maximum average value in a group of 9 samples (Veciana-Nogues MT *et al*., 1995). Mexico has a maximum limit of 200 ppm of histamine for tuna and tuna products (Garcia-Tapia *et al*., 2013). Generally, the formation of histamine in tuna not only depends on the level of contamination and type of histamine producing bacteria but also the temperature and time during processing. Abuse handling of tuna during processing, particularly during thawing, may facilitate some microorganisms to produce histamine through its wounds or cuts. Therefore, the objective of this study was to investigate the effects of temperature and time of incubation as well as *Enterobacter aerogenes* inoculation on the formation of histamine in tuna flesh.

2. Materials and methods

2.1 Chemicals

Histamine dihydrochloride ($C_5H_9N_3.2HCl$), O-Phthaldialdehyde (OPA) ($C_8H_6O_2$) and Dowex 1-x8 were purchased from Sigma Chemical Co. (St. Louis, MO, USA.). Plate Count Agar was procured from Merck (Darmstadt, Germany). Other chemicals used were analytical grade.

2.2 Sample preparation

Frozen bonito tuna (*Euthynnus affinis*) (~1.0 kg/fish) were obtained from Thai Union Manufacturing Co. Ltd., Samut Sakhon, Thailand. Frozen tuna was thawed by submerging in circulating tap water (18–20°C) for 4.5 h or the core temperature reached to -1°C. Both frozen and thawed tuna were subjected to microbiological analysis. Thawing water was also collected and used in the experiment. To simulate the worse condition for damaged tuna during thawing, tuna flesh was collected from thawed tuna by cutting from 8 positions of thawed tuna (Figure 1). The dimension of each tuna flesh sample was 1x1x0.5 inch^3. Tuna flesh samples from 20 fish were combined and randomly taken to experiment and analysis.

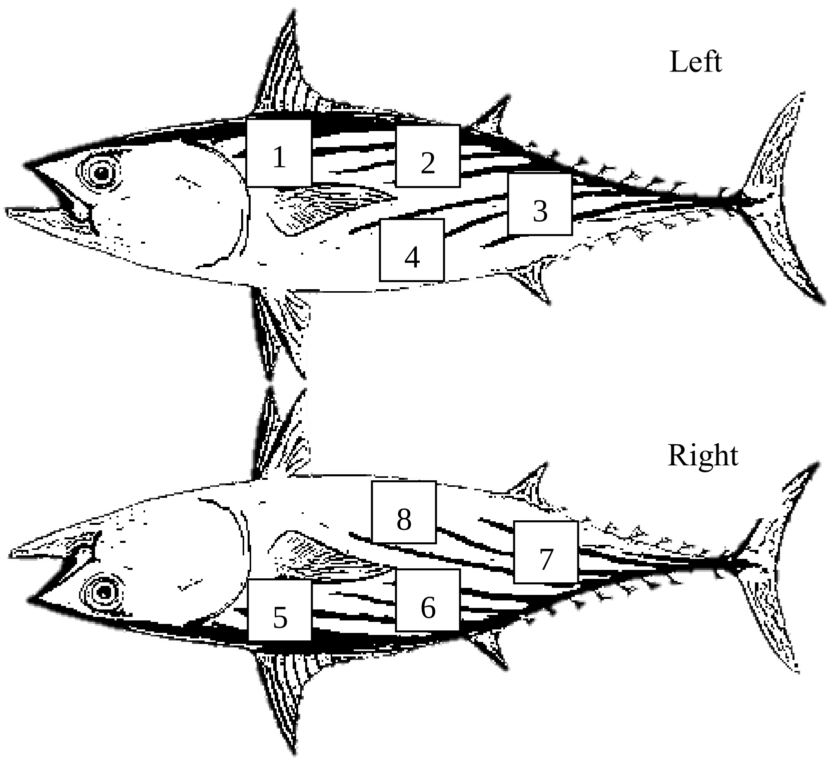

Figure 1 Sampling positions of bonito tuna flesh: 1, 2, 5 and 6 are dorsal muscle; 4 and 8 are ventral muscle; 3 and 7 are lateral line muscle

2.3 Microbiological analysis

Microbiological analysis was determined according to the method of AOAC (2000). Microorganisms were collected from the surface area of skin and gill of frozen and thawed tuna with sterile cotton and then transferred to 0.1 % (w/v) peptone water. A series of dilutions ($10–10^6$) were made properly. The prepared samples were pour-plated on Plate Count Agar and incubated at 37°C for 24 h for total plate counts.

2.4 Effect of temperature on the formation of histamine

Tuna flesh sample (150 g) was added to 150 ml of frozen tuna thawing water. The mixture was incubated at 4, 20 and 25°C for 6 h in a temperature-controlled water bath (Memmert, Schwabach, Germany). Then, tuna flesh was collected by filtration with cheesecloth and blended for 2 min at high speed prior to determination of histamine content according to the AOAC (2005) method with the analytical no. of 977.13.

2.5 Effect of time on the formation histamine

Tuna flesh sample (150 g) was added to 150 ml of frozen tuna thawing water. The mixture was incubated at 25°C for 0, 1, 2, 4, 8, 12, 18 and 24 h in a temperature-controlled water bath. Then, tuna flesh was collected by filtration with cheesecloth and blended for 2 min at high speed prior to determination of histamine content.

2.6 Effect of Enterobacter aerogenes inoculation on the formation of histamine

E. aerogenes was obtained from the Department of Medical Sciences, Ministry of Public Health, Thailand. It was cultured and prepared as per the method of Kim *et al*. (2001). *E. aerogenes* (7 log CFU/g tuna sample) was inoculated into the mixture of tuna flesh sample (150 g) and frozen tuna thawing water (150 ml). The mixture was incubated at 25°C for 0, 1, 2, 4, 8, 12, 18 and 24 h in a temperature-controlled water bath (Memmert, Schwabach, Germany). Then, tuna flesh was collected by filtration with cheesecloth and blended for 2 min at high speed prior to determination of histamine content.

2.7 Determination of histamine content

Histamine content in tuna sample was determined according to the AOAC fluorometric method (2005) with the analytical no. of 977.13, with a slight modification. Tuna flesh sample (5 g) was homogenized in 25 ml of methanol for 2 min and the mixture was incubated in a water bath at 60°C for 15 min. After cooling to 25°C, the volume was adjusted to 50 ml with methanol and filtered through Whatman No. 1 filter paper (Whatman International, Ltd., Maidstone, England). The methanol filtrate was collected and loaded onto an ion exchange column (200 × 7 mm) packed with Dowex 1–x8 (Sigma Chemical Co., St. Louis, MO, USA), which was converted to hydroxide form by 2 N NaOH. The sample eluents and the standard solutions were derivatised with *O*-phthaldialdehyde (OPA). The fluorescence intensity of the derivatised products was then measured using a spectrofluorometer (F-2700, HITACHI, Japan) at an excitation wavelength of 360 nm and emission wavelength of 450 nm. Histamine standard solutions ranging from 0.1 to 0.3 ppm were used to prepare a standard curve. Histamine contents in the samples were calculated from the standard curve and expressed as ppm.

2.8 Statistical analysis

All data were subjected to analysis of variance (ANOVA) and differences between means were evaluated by Duncan's multiple range test (Steel & Torrie, 1980). SPSS Statistic Program (Version 10.0) (SPSS Inc., Chicago, IL, USA) was used for data analysis.

3. Results and Discussion

3.1 Microbiological analysis

Microbiological contents found in skin and gill of frozen and thawed tuna are shown in Table 1. The results showed that frozen tuna, both skin and gill, had low content of microorganism ($\leq$ 7 CFU/g). This might be due to a good practice of quality control of tuna processing plant. However, when frozen tuna was thawed by using circulating water for 4.5 h (core temperature = -1°C), microbiological counts of $1.8\ x10^2$ and $1.9x10^3$ CFU/g were found in skin and gill , respectively. The result indicated that some microorganisms grew during thawing process. Kilinc and Cakli (2004) reported that microbiological count found in thawed sardine in chill storage (4°C) over night was 4.5×10^4 CFU/g. The growth of microorganism, particularly histamine producing bacteria, may correspond to the increment of histamine in tuna flesh during thawing.

Table 1 Microbiological count found in skin and gill of frozen and thawed bonito tuna.

Raw material	Sampling area	Total count (CFU/g)
Frozen tuna	Skin	ND
	Gill	7
Thawed tuna	Skin	1.8×10^2
	Gill	1.9×10^3

Note: ND is <1 CFU/g.

3.2 Effect of temperature on the formation of histamine

Histamine contents in bonito tuna flesh after incubation for 6 h at 4, 20, and 25°C are shown in Table 2. The resulted showed that histamine content increased with increasing incubation temperature (p<0.05). This indicated that mesophilic histamine-producing bacteria contaminated in tuna and responsible to produce histamine during incubation. At 4°C, bonito tuna flesh had the lowest accumulation of histamine content (27 ppm) in which lower than USFDA regulation (50 ppm). Incubation of tuna flesh represented damaged tuna at 20 and 25°C could not control histamine level to lower than 50 ppm. Rodtong *et al*. (2005) reported that several histamine-producing bacteria including *M. morganii, E. aerogenes, and P.vulgaris* isolated from decomposed Indian anchovy observed the maximum growth at 35°C, in which *M. morganii* was the lowest histamine former at 15 and 25°C. Lin *et al*. (2012) found that production of histamine in canned tuna meat inoculated with *Raoultella ornithinolytica* was strongly inhibited when stored at 4°C even though histamine content below 1.5 mg/100g (15 ppm) was detected. Therefore, the formation of histamine in tuna could be controlled at low temperature, particularly at 4°C.

Table 2 Histamine content in bonito tuna flesh after 6 h of incubation at different temperatures

Incubation temperature (°C)	Histamine content (ppm)*
4	27.0 ± 2.83^{a†}
20	62.0 ± 9.89^{b}
25	86.5 ± 3.54^{c}

Note: * Mean ± standard deviation (n=3).

† Different superscript letters in the same column indicate significant differences (p<0.05)

3.3 Effect of time on the formation of histamine

Histamine contents in tuna flesh during incubation at 25°C for 0, 1, 2, 4, 8, 12, 18 and 24 h are presented in Table 3**.** The resulted showed that histamine content increased when incubation time increased. Histamine content significantly increased 2 fold after 12 h of incubation at 25°C (p<0.05). The formation of histamine in tuna flesh was found up to 415.5 ppm within 24 h of incubation. However, it was lower slightly than the hazard level of USFDA regulation at 500 ppm. Ben-Gigirey *et al*. (1999) reported that *Stenotrophomonas maltophilia*, histamine producing bacteria exhibited optimum temperature at 20–30°C to produce histamine in fresh albacore tuna during storage for 24–48 h. However, the rate of histamine formation could be reduced for longer time of storage, which was related to histidine decarboxylase activity (Ben-Gigirey *et al*., 1999; Eitenmiller *et al*., 1981; Wei *et al*., 1990). In general, production of canned tuna is fully processed from thawing to retorting within 18 h. The result revealed that treatment of tuna during processing within 18 h at 25°C could control the formation of histamine to lower critical hazard level.

Table 3 Histamine content in bonito tuna flesh during incubation at 25°C for different times

Incubation time (h)	Histamine content (ppm)*
0	53.5 ± 6.36[a†]
1	63.0 ± 1.41[ab]
2	65.0 ± 1.41[ab]
4	72.0 ± 1.41[ab]
8	90.0 ± 7.77[bc]
12	113.0 ± 1.41[bc]
18	147.5 ± 14.84[c]
24	415.5 ± 62.93[d]

Note: * Mean ± standard deviation (n=3).

† Different superscript letters in the same column indicate significant differences (p<0.05)

3.4 Formation of histamine in tuna flesh as affected by *E. aerogenes* innoculation

The Formation of histamine content in bonito tuna flesh inoculated with *E. aerogenes* (7 log CFU/g sample) during incubation at 25°C is depicted in Figure 2. It was found that histamine content, within 4 h of tuna flesh incubation, gradually increased below the defect action level of 50 ppm for histamine in tuna (USFDA, 1996). A sharply increase in histamine content was found after 8 h of incubation. Histamine content in tuna flesh increased from 240 ppm to over critical hazard level (>500 ppm) at 12 and 24 h of incubation, respectively. Lee *et al*. (2012) reported that once the frozen tuna dumpling stuffing, inoculated with *E. aerogenes* (5 log CFU/g), stored at -20°C for 8 weeks were thawed and then held at 25°C, they started to show rapid increase of *E. aerogenes*, reaching the levels in 12 h. Therefore, with contamination of *E. aerogenes* at 7 log CFU/g and 25°C, bonito tuna should be fully processed within 4 h and must be rejected after 18 h of delay prior to heat treatment, such as steaming and retorting.

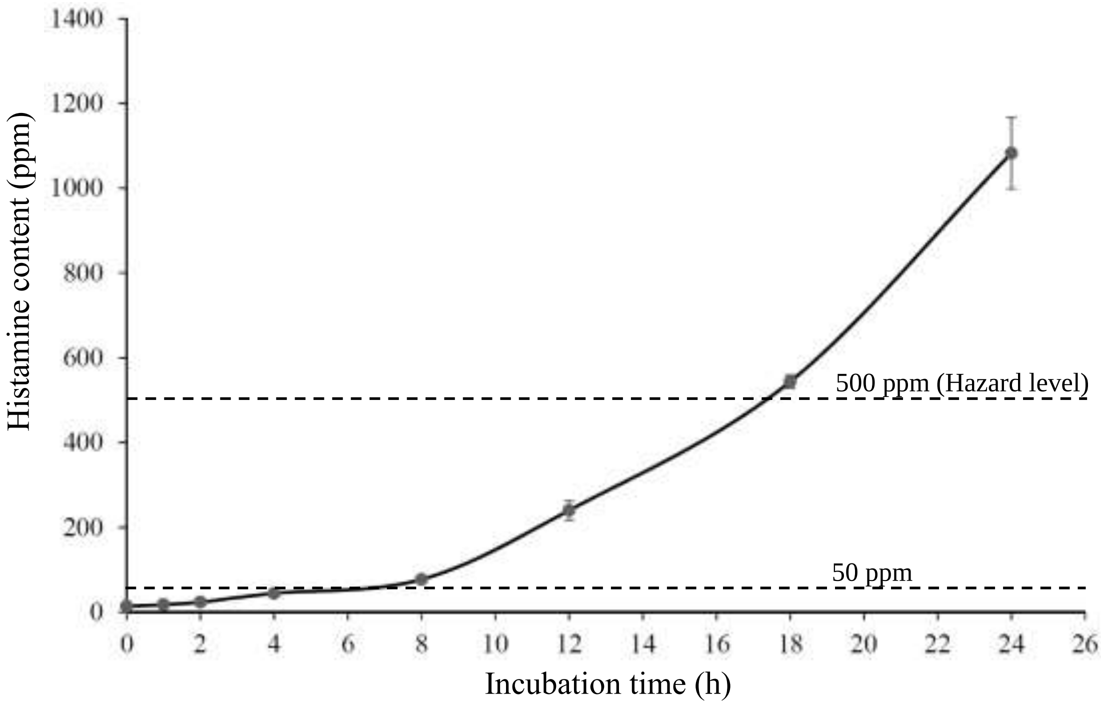

Figure 2 Formation of histamine content in bonito tuna flesh inoculated with *Enterobacter aerogenes* (7 log CFU/g sample) during incubation at 25°C

4. Conclusion

A simulation of damaged tuna incubation with histamine-producing bacteria contaminated thawing water was investigated. Histamine content could be controlled at 4°C of incubation. Bonito tuna flesh sample without and with *E. aerogenes* inoculation (7 log CFU/g) could be treated at 25°C for 24 h and 12 h, respectively, in which histamine content lower than hazard level limited by USFDA. The information gained form this study is useful to control histamine level for tuna processing.

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