Thailand

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1. Introduction

Scombrotoxin formation, as a result of time and temperature abuse of certain species of fish, can cause scombroid poisoning. Certain bacteria produce the enzyme histidine decarboxylase during growth. This enzyme reacts with free histidine, a naturally occurring chemical that is present in larger quantities in some fish than in others. Histamine –forming bacteria are capable of growing and producing histamine over a wide temperature range. Growth is more rapid at a high-abuse temperature of 21.1°C than at a moderate abuse temperature of 7.2°C. Growth is particularly rapid at temperatures near 32.2°C. Histamine formation is more commonly the result of spoilage due to storage at high temperatures rather than spoilage due to long term, relatively low temperature storage.

Once the enzyme histidine decarboxylase has been formed, it can continue to produce histamine in the fish even if the bacteria are not active. The enzyme can be active at or near refrigeration temperatures. The enzyme is likely to remain stable while in the frozen state and may be reactivated very rapidly after thawing. Freezing may inactivate the enzyme-forming bacteria. Cooking can inactivate both the enzyme and the bacteria. However, once histamine is formed, it cannot be eliminated by heat, including retorting, and freezing. After cooking, recontamination of the fish with the enzyme-forming bacteria will cause additional histamine to form.

The canned tuna is the first priority of export for Thailand and dried anchovies are a favourite among consumers of traditional products. The survey of histamine in the raw material of tuna, canned products and dried anchovies is important for assessing the level of safety for consumption

2. Objectives And Goals

- To survey the histamine level in the raw material of skipjack and in canned products from processing plants in 2005.
- To survey the histamine level in raw material of skipjack and dried anchovies from processing plants in 2006.
- To survey the histamine level in canned products (skipjack, sardine and mackerel) and tuna fish sauce from processing plants in 2007.
- To survey the histamine level in canned products (skipjack and mackerel) and tuna fish sauce from processing plants in 2008.

3. Survey Methodologies

a. Sampling Method, Location, Species, Number of Samples and Sampling Size

Year 2005
The central dorsal portion of imported frozen Skipjack (*Katsuwonus pelamis*) were collected during February to December. These samples originated from the Western Pacific Ocean and Indian Ocean. Canned products from the processing plant at Songkhla Province were also collected during the same period. A total number of 297 samples were collected.

The total number of samples for frozen Skipjack was 198. Eighteen replicates were used for each sample (n=18). 2-3 samples of 500g each from each body weight category (0-1.4, 1.5-1.8, 1.9-2.4, 2.5-3.4, 3.5-4.5, 4.6-6.0 and 6.1-9.0 kg) were taken. The samples were kept in a freezer at -18 °C overnight and analysed for histamine the next day.

A total of 99 samples of canned products were collected. Nine replicates were used for each sample (n=9). The samples were kept at room temperature until analysis for histamine.

Year 2006
The central dorsal portions of imported frozen Skipjack (*Katsuwonus pelamis*) were collected

during February to December. These samples originated from the Western Pacific Ocean and Indian Ocean. Dried anchovies (*Stolephorus* spp.) from processing plants were also collected during the same period. A total of 83 samples were collected.

A total of 33 frozen Skipjack samples were collected, using 3 replicates for each lot (n=3/ lot). The samples were kept in a freezer at -18 ^{o}C overnight and analysed for histamine the next day.

A total of 50 dried anchovies samples were collected, using 5 replicates for each lot of 1 kg (n=5, sampling 1 kg/lot). There were 520-2,580 pieces of dried anchovies in each kg collected and the tandard length of the dried anchovies range between 3.75-7.00 cm. The samples were kept at room temperature until analysis for histamine.

Year 2007
Canned products of Skipjack (*Katsuwonus pelamis*), Sardine (*Sardinella gibbosa*) and Mackerel (*Decapterus maruadi*) and Tuna fish sauce from processing plants in Songkhla Province were collected from April to October. The total number of samples collected was 144 samples. Nine replicates were used (n=9) for each sample for analysis. The samples were kept at room temperature until analysis for histamine.

Year 2008
Canned products of Skipjack (*Katsuwonus pelamis*), Mackerel (*Decapterus maruadi*) and Tuna fish sauce from processing plants in Songkhla Province were collected from April to May. A total of 81 samples were collected. Nine replicates (n=9) were used for analysis. The samples were kept at room temperature until analysis for histamine.

b. Method of Analysis

Purpose
Determination of histamine level using fluorometric method.

Scope and application
Quantitative test of histamine in fish and fish products.

Reference
AOAC, 2005.

Principle of the method
The analyte wass extracted with methanol. The extract was passed through an ion-exchange column. O-Phthaldialdehyde solution was then added to the elute to form fluorescent histamine derivatives. The fluorescence intensity of derivatives was measured using a fluorometer and the level of histamine was quantified with an external standard.

Sample preparation
Whole fish should be analyzed immediately after grinding, however, if this is possible, the samples should be frozen quickly to ensure there is no further decomposition.

For products that are packed in water, brine or sauce, the products were drained for 1 minute. The samples were then blended until they were homogenous.

Apparatus
- Electronic balance
- Water bath
- Fluorometer : Turner Quantech, Excitation wavelength 360 nm and Emission wavelength 450 nm
- Homogenizer
- Beaker
- Paper filter No. 1
- Dispenser
- Volumetric flask grade A (10, 25, 100, 500 and 1,000 ml)
- Volumetric Pipette grade A (1, 2, 3, 4, 5, 10 and 20 ml)
- Erlenmeyer flask
- Chromatographic tube

Reagents
- Ion – exchange resin – Bio Rad AG 1-x8, 50-100 mesh
  The resin was convert to – OH form by adding 15 ml of NaOH for every gram of resin to a beaker. The mixture was swirled and left to stand about 30 minutes. The liquid was decanted liquid. The process was repeated with additional base. The resin was then washed throughly with H_2O.

  The resin was packed into a chromatography tube to a height of 8 cm while maintaining water above the resin bed at all times. The column was washed with 10 ml of water each time before adding an extract.

- 85% Phosphoric acid (85% H_3PO_4)

- 3.57N Phophoric acid
  12.18 ml of 85% H_3PO_4 was diluted in a 100 ml volumetric flask.

- 0.1% O-Phthaldialdehyde (OPT) solution
  0.1 g of OPT was dissolved in 100 ml methanol. The solution was stored in a refrigerator. This solution had to be prepared fresh weekly.

- Hydrochloric acid (HCl)

- 1.0N Hydrochloric acid (1.0N HCl)
  8.33 ml of concentrated HCl was diluted into a 100 ml volumetric flask with deionized water.

- 0.1N Hydrochloric acid (0.1N HCl)
  8.33 ml of concentraed HCl was diluted into a 1,000 ml volumetric flask with deionized water

- Sodium Hydroxide (NaOH)

- 1N Sodium Hydroxide (1N NaOH)

- 2N Sodium Hydroxide (2N NaOH)
  8 g of NaOH was dissolved in deionized water and diluted to 100 ml in a volumetric flask.

- Histamine dihydrochloride

- Histamine standard stock solutions (1000 ppm)
  0.0423 g of standard histamine dihydrochloride was dissolved with 0.1N HCl and diluted to 25 ml in a volumetric flask. The solution was stored in a refrigerator. The solution had to be prepared fresh weekly.

  For standard working solutions, pipette 5 ml of 0.01, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 ug/g for standard histamine. Histamine contents are 0.05, 0.25, 0.50, 0.75, 1.0, 1.25 and 1.50 ug respectively. Prepare fresh daily.)

Procedure of testing

- Sample extraction

(i) 5.000 $\pm$ 0.020 g of sample was weighted into a 50 g beaker. 20 ml of methanol was added and the mixture was blended for 2-3 minutes. The blended mixture was then transferred to a 50 ml volumetric flask.

(ii) The flask was placed for 15 minutes in a hot water bath at 60 $\pm$ 5 ^{o}C.

(iii) The contents were then cooled to room temperature and diluted to volume with methanol.

(iv) The contents were filtered and the filtrate was stored in a refrigerator until it is to be analyzed.

- Purification

(i) Impurities from the extract were removed by passing 4 or 5 ml of water through the ion exchange column. This solution was then discarded.

(ii) 1 ml of the extract was pipetted into the column and 5 ml of water was added. The column flow was initiated immediately and the eluate was collected into a 50 ml volumetric flask which contains 5 ml of 1N HCl.

(iii) Flow rate was controlled at > 3 ml/min by adjusting the height of column relative to the tubing outlet. When the liquid level is approximately 2 mm above the top of the resin, 5 ml of water was added, followed by increasingly large volumes of water until a total of 35 ml was eluted . The flow was stopped and the collected eluate was top up to 50 ml with water and mixed throughly.

- Derivatization and fluorometric measure

(i) 5 ml of sample solution and 10 ml of 0.1N HCl were pipetted into a Erlenmeyer flask. 3 ml of 1.0N NaOH was added and mixed. Within 5 minutes, 1 ml of 0.1% OPT solution was pipetted and the solution was mixed immediately. After exactly 4 minutes, 3 ml of 3.57N H_3PO_4 was added and the solution was mixed immediately. It is important to mix thoroughly after each addition and at least once during the OPT reaction.

(ii) Blank and standard solutions were prepared by pipetting into the Erlenmeyer flask containing 5 ml of 0.1 N HCl, 5 ml of 0.01, 0.05, 0.10, 0.15, 0.20, 0.25 and 0.30 ug/ml histamine working standards. The above procedure [(i) and (ii)] were followed.

(iii) Within 1.5 hour, fluorescence intensity(I) using excitation wavelength of 360 nm and emission wavelength of 450 nm were recorded.

(iv) If fluorescence intensity of sample is greater than the fluorescence intensity of histamine standard (0.30 ppm). The eluate must be diluted with 0.1N HCl after that preparation of the fluorophore.

- Calculation

(i) The plot of I (measured by meter deflection or recorder response and corrected for blank) against histamine/5 ml of test solution un ug should be a straight line passing through the intercept.

(ii) Results were calculated as follows:
Histamine (ppm) = (50/5) x (50/W) x (Cs-Crb) x (E/D)
Cs = Histamine solution of sample from calibration curve
Crb = Histamine solution of reagent blank from calibration curve
W = Sample weight (g)
D = Solution volum for dilution (ml)
E = Total solution volume after dilution (ml)

c. Limit of Detection and Limit of Quantification

Fish and fish product
Limit of Detection= 3.00 ppm,
Limit of Quantification = 5.00 ppm

Tuna fish sauce
Limit of Detection= 35.00 ppm,
Limit of Quantification = 50.00 ppm

d. National Regulatory Limits

Thailand	100 ppm
Australia and New Zealand	200 ppm
China	100 ppm
Canada	100 ppm
EU	m = 100 ppm, M = 200 ppm (n=9,c=2)
Russia	100 ppm
USA	50 ppm

Remarks:
n = number of units comprising the sample
c = number of sample units giving values > m or between m and M.

Results are satisfactory if:
1) mean value does not exceed m.
2) 2 samples have values more than m but less than M.
3) No samples have a value exceeding M.

4. Results And Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Year of participation	Program Name	Analyte Tested	Reported results	True value	z-score	Remarks
2005	CFIA	Histamine	7.81 (tissue) 2.02 (tissue) 14.52 (sauce) 21.06 (sauce)	7.44 2.34 14.54 20.54	0.31 -0.49 0.07 0.13	Passed
2007	FAPAS	Histamine	32.24 (canned fish)	38.1	-1.7	Passed

b. Survey Results and Discussion

Year of analysis & Sampling location	Fish sample analysed		No. of samples analysed	Min. value of results (ppm)	Max. value of results (ppm)	Average value of results (ppm)	Average Recovery (%)	Remarks
	Common name	Scientific name						
2005 & Factory of Songkhla	Skipjack	*Katsuwonus pelamis*	198	0.53 (ND)	2.65 (ND)	1.2491 (ND)	95.80%	Frozen
	Skipjack	*Katsuwonus pelamis*	99	0.83 (ND)	35.41	7.8455	88.50%	Canned
2006 & Factory of Songkhla	Skipjack	*Katsuwonus pelamis*	33	0 (ND)	6.16	1.1760 (ND)	85.00%	Frozen
	Anchovies	*Stolephorus* spp.	50	1.77 (ND)	154.87	36.7660	88.00%	Dried
2007 & Factory of Songkhla	Skipjack	*Katsuwonus pelamis*	27	0 (ND)	4.65 (<LOQ)	1.7430 (ND)	98.96%	Canned
	Long tail Tuna	*Thunnus tonggol*	9	3.79 (<LOQ)	5.88	4.6744 (<LOQ)	90.10%	
	Sardine	*Sardinella gibbosa*	36	0 (ND)	4.33 (<LOQ)	1.9450 (ND)	92.52%	Canned
	Mackerel	*Decapterus sp.*	36	0 (ND)	2.27 (<LOQ)	0.8781 (ND)	95.00%	Canned
	Tuna sauce	–	36	71.31	136.55	103.3070	110.00%	Fermented
	Skipjack	*Katsuwonus pelamis*	27	0.42 (ND)	6.76	3.1048 (<LOQ)	87.56%	Canned
2008 & Factory of Songkhla	Mackerel	*Decapterus sp.*	27	1.1 (ND)	8.13	3.5041 (<LOQ)	85.16%	Canned
	Tuna sauce	–	27	83.03	144.04	106.2130	90.16%	Fermented

c. Corrective Actions

The histamine results were in the acceptable range, therefore no corrective actions were taken.

5. Problems and Challenges Encountered

Nil.

6. Recommendations and Suggestions for Future Follow up Action

MFRD should arrange for proficiency testing in histamine for ASEAN member.