MALAYSIA

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1. Introduction

Histamine is a chemical compound formed in fish muscle after the fish dies, especially for pelagic fish. The formation of histamine in fish muscles is caused by the activity of the histidine *dikarboxilase* enzyme produced by *Proteus morganiii*, a bacteria in the gill and stomach of fish. Fish species with high histidine level in its muscles, specially those from the scrombroid group like tuna and mackerel, will tend to have higher histamine content than in pelagic fish. Non scrombroid group like anchovies, sardines and herring may also have high histamine content.

Histamine formation caused by bacteria can be controlled through good handling practices and fish must be kept at a low temperature as soon as possible after capture. If fish handling practices are poor, histamine content in fish muscles will increase and exceed the safety limit for consumption, leading to histamine poisoning.

Histamine is stable at high temperatures and the accumulation of histamine beyond the safety limit could pose a threat to the consumer. The symptoms of histamine poisoning include itchness of the skin. Unsafe levels of histamine may also be harmful to the digestion and nervous system. Cases of histamine poisoning caused by fish were reported in Japan, United States of America and United Kingdom in the late 1970s. In Malaysia, cases of histamine poisoning had been reported and most of the cases were due to consumption of low quality fish that might be subjected to poor handling.

The Department of Fisheries of Malaysia carried out histamine monitoring in Malaysia for fish landing at main fish landing jetties and complexes. It started from 2001 in a program known as Sanitary and Phytosanitary (SPS) Marine Program. Histamine monitoring under this program is done in Peninsular Malaysia and will be extended to Sabah and Sarawak from 2009 onwards.

Due to the importance of histamine monitoring, the first sample of anchovies was taken from Pahang in 2007. States with many anchovies landing will be included in the next histamine monitoring plan. There is also a plan to monitor fish and fish products, especially those sold at the hypermarkets and shopping complexes, which are likely to have a content of histamine.

2. Objectives And Goals

- To carry out monitoring program to check on the content of histamine in fish for official control and generation of useful database for management control;
- Department of Fisheries Malaysia (DoFM) has implemented the SPS monitoring program in accordance with the WTO requirements to ensure that fish produced in the country is safe to eat and is of good quality; and
- To enhance the on-going SPS program through training and increasing monitoring activities with the JTF program being proposed for 2007/ 08,

3. Survey Methodologies

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

For the year of 2007, samples for histamine analysis were carried out at an accredited laboratory due to a technical problem faced by the Department of Fishery's histamine laboratory.

Fish samples were collected from the major fish landing jetties/complexes throughout Peninsular Malaysia. The fish species for the sampling of histamine analysis is based on the dominant pelagic fish species landed at the main landing jetties/complexes in the state. There were 18

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landing jetties/complexes in 11 states chosen as the sampling locations for histamine monitoring program in 2007.

For histamine sampling, 9 samples were collected at the point of sampling location. One sample may consist of 1 to 3 pieces of fish depending on fish size. The amount of fish muscles needed for the histamine analysis is about 200 g for each sample.

Total number of samples for histamine analysis in 2007 is 468, including samples of anchovies. Temperature of samples were maintained at 0-4^{o}C.

b. Method of Analysis

The method of analysis of histamine in fish is based on Journal of Chromatography A, 809 (1998), 241-245 using HPLC FD.

Sample Preparation

Reagents:

Histamine dihydrochloride, 99% purity
Trichloroacetic acid
Hydrochloric acid
Sodium hydroxide
Borate buffer (pH 9.5) [Dissolve 2.47 g boric acid (Mw = 61.83 g/mol) with 90 mL water in a beaker. Adjust the pH with 10 M NaOH to 9.5. Transfer the solution into a 100 mL volumetric flask and dilute to volume with water].
Ethyl acetate
Methanol
Derivatizing solution [Weigh 50 mg of OPA into a 5-mL volumetric flask. Add 0.2 mL of MeOH into the vial, followed by 50 µL of 2-mercaptoethanol. (Make up to 5ml with buffer solution). [Dilute the content to volume with borate buffer. Keep this solution in the refrigerator and use within a day].
Potassium dihydrogen phosphate
Sodium chloride

Apparatus and Glassware:

Volumetric flasks, pipettes, centrifuge tubes
Sartorius 0.45 micron RC-membrane filter
Whatman filter paper No. 1
Blender
Centrifuge

Standards Preparation:

Histamine Stock Solution: 1000 ppm
Weigh 166 mg of histamine dihydrochloric into a 100 mL volumetric flask. Dissolve the contents and make up to mark with 0.1 M solution of HCl.

Standard Solutions for Calibration:

Histamine Standard: 10 ppm
Pipette 1.0 mL of the stock solution into a 100 mL volumetric flask and make up to mark with 0.1 M solution of HCl.

Preparation of Samples:

(i) Extraction:

TCA procedure: Homogenize 5 g of sample with 10 ml of TCA extracting solution and centrifuge for 15 minutes at 13 rpm (r=0.3m). The aqueous layer was recovered and the organic layer was discarded; the procedure was repeated twice on the sample. The collected aqueous fractions were filtered into a 50 ml flask and then made to volume with 10% TCA.

(ii) Derivatization:

2.0 ml of solution, prepared according to the above method, or standard histamine solutions are transferred into a 20 ml test tube and 1 ml of 1M NaOH. After 5 minutes, 1 ml of derivatizing solution is added. Allow the solution to stand for 10 minutes, and then add 0.5 g NaCl. The solution is extracted with 3 ml of ethyl acetate twice. The aqueous and organic layers are separated by centrifugation. The organic layer is evaporated under N_2 to 100 µl and reconstituted to 2 ml with mobile phase.

Analysis by HPLC:

HPLC system:
Column: Spherisorb C18, 5 micron, 4.6 x 250 mm
Mobile phase: Eluent A (20mM KH_2PO_4 at pH 3): MeOH=50:50
Flow rate: 1.0 mL/min
Detector: Fluor LC305, Ex:216 nm, Em:416 nm
HPLC settings: Range:5, rise time:10, lamp flash rate:100, PMT voltage:500
Injection volume: 20 µL

Calibration:

Calibration is done prior to actual running of the analysis. Standard concentration solutions use for calibration are 0.1 ppm, 0.5 ppm, 1.0 ppm, 5.0 ppm and 10.0 ppm. Calibration curve is then plotted based on the data from these standards.

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c. Limit of Detection and Limit of Quantification

Limit of detection and limit of quantification in the analysis are 0.1 ppm and 10 ppm respectively.

d. National Regulatory Limits

There is no regulatory limit for histamine in Malaysia. For the histamine monitoring purposes under the SPS Marine program, the safety limit used is 50 ppm and it is based on the USFDA standard. Fish with histamine content exceeding this limit may cause histamine poisoning to the consumers. Compared to the European Union (EU) standard, histamine limit for the fish products from fish species associated with a high amount of histidine is between 100 – 200 mg/kg (100-200 ppm).

4. Results and Discussion

a. Participation in Inter-laboratory Proficiency Testing and Results

Malaysia did not participate in any inter-laboratory proficiency testing for Histamine.

b. Survey Results and Discussion

Based on the results of histamine analysis in 2007, all of the samples with histamine detected were within the standard used by the Department of Fisheries Malaysia, the competent authority for fish monitoring in Malaysia. The limit of histamine detected in fish samples are between not detected to 347 ppm. Only 3 landing jetties/complexes at Tanjung Sedeli, Johor; Sg. Besar and Sg. Buloh, Selangor had samples of slightly higher content of histamine in fish. The highest limit detected was at 347 ppm.

Fish samples from the northern states (Perlis, Kedah, Penang and Perak) comprised of Japanese threadfin beam fish (kerisi), mackerel (kembung) and a mixture of a few pelagic species. The histamine content detected for fish samples from the northern states is between 10 - 35 ppm.

Fish samples from Selangor, Melaka, Negeri Sembilan are mainly Dorab wolf-herring (Parang), Narrow-barred Spanish Mackerel (Tenggiri), mackerel (kembung) which are dominant species in this area and also a mixture of few other species. The highest histamine content was detected in the samples from Selangor (Sungai Buloh jetty and Sungai Besar landing jetty) in March 2007 . The average content of histamine for the 9 samples from each of the landing jetty range from not detected to 347 ppm. The other sampling locations reported histamine content of under 77 ppm.

For the southern state (Johor), fish species were taken from a mixture of few pelagic species. 32 samples were taken from two landing jetties, Pontian Besar and Tanjung Sedeli in March, April, July and August with histamine content between 9 ppm - 260 ppm.

The east coast states (Pahang and Terengganu) contributed nearly 50% (225 samples out of 468 samples) of the total histamine samples in 2007. Dominant species from these two states are Shortfin scad (Selayang), Hardtail Scad (Cencaru), Big Eye Scad (Lolong), Round Herring (Tamban), Yellow-striped trevally (Selar kuning), anchovies and also a mixture of a few pelagic fish species. There are also 18 samples of anchovies collected for histamine analysis from Kuantan landing complex in Pahang. The highest histamine content for east coast states was reported at 101 ppm in Kemanaman and Terengganu which was collected in June 2007. Samples from the other sampling locations had low histamine levels.

Table 1. Names of species analysed.

No.	Common Name	Scientific Name
1.	Hardtail scad	*Megalaspis cordyla*
2.	Rainbow sardine/Round herring	*Sardinella fimbriata*
3.	Yellow-striped trevally	*Selaroides leptolepis*
4.	Big eye scad	*Selar crumenophthalmus*
5.	Shortfin scad	*Decapterus macrosoma*
6.	Narrow-barred spanish mackerel	*Scomberomorus commerson*
7.	Dorab wolf-herring	*Chirocentrus dorab*
8.	Swordfish	*Platybelone orgalus platyura*
9.	Mackerel	*Rastrelliger* spp.
10.	Japanese threadfin bream	*Nemipterus* spp.
11.	Indian Anchovy	*Stolephorus indicus*

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Table 2. Results of Analysis in 2007.

State	Sampling Location	Month	Common Name	No. of Samples	Average Results (ppm)
Perlis	Kuala Perlis	March	Mix species	9	25 – 35
		August	Mix species	9	10 – 18
Kedah	Kuala Kedah	February	Mix species	9	12 – 24
		June	Japanese threadfin bream (Kerisi)	9	18 – 28
			Mackerel (Kembung)	9	22 – 31
Penang	Teluk Bahang	April	Mix species	9	19 – 31
Perak	Hutan Melintang	March	Japanese threadfin bream (Kerisi)	9	15 – 22
	Pangkor		Mix species	9	25 – 35
Selangor	Sg. Besar	March	Mix species	9	ND – 347
	Sg. Buloh		Mix species	9	ND – 269
	Sg. Buloh	June	Mix species	9	16 – 26
	Sg. Besar		Mackerel (Kembung)	9	19 – 27
	Sg. Buloh	September	Mix species	9	8 – 14
	Sg. Besar		Mackerel (Kembung)	9	8 – 15
N. Sembilan	Port Dickson	May	Dorab wolf-herring (Parang)	8	ND – 11
			Narrow-barred spanish mackerel (Tenggiri)	9	9 – 56
			Swordfish (Todak)	9	23 – 77
Melaka	Kuala Sungai Baru	March	Dorab wolf-herring (Parang)	9	7 – 23
			Narrow-barred spanish mackerel (Tenggiri)	9	ND – 10
		June	Dorab wolf-herring (Parang)	9	17 – 24
			Narrow-barred spanish mackerel (Tenggiri)	9	17 – 25
		September	Dorab wolf-herring (Parang)	9	8 – 14
			Narrow-barred spanish mackerel (Tenggiri)	9	8 – 14
Johor	Pontian Besar	March	Mix species	18	10 – 110
	Tanjung Sedeli	April	Mix species	9	9 -112
		July	Mix species	5	99 – 260
	Pontian Besar	August	Mix species	5	6 – 31
Pahang	Rompin	May	Shortfin scad (Selayang)	9	8 – 16
			Hardtail scad (Cencaru)	9	7 – 13
			Big eye scad (Lolong)	9	7 – 12
	Kuantan		Hardtail scad (Cencaru)	9	7 – 14
	Kuantan	June	Rainbow sardine, Round herring (Tamban)	9	29 – 36
	Rompin		Rainbow sardine, Round herring (Tamban)	9	27 – 37
	Kuantan	September	Rainbow sardine, Round herring (Tamban)	9	17 – 28
	Rompin		Yellow-striped trevally (Selar kuning)	9	17 – 23
	Kuantan		Anchovies (Ikan bilis)	9	35 – 44

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State	Sampling Location	Month	Common Name	No. of Sample	Average Result (ppm)
Pahang	Kuantan	September	Mix species	9	9 – 20
	Rompin		Mix species	9	10 – 18
	Kuantan	October	Rainbow sardine/Round herring (Tamban)	9	17 – 28
	Kuantan	November	Shortfin scad (Selayang)	9	9 – 20
			Anchovies (Ikan bilis)	9	36 – 44
	Rompin		Rainbow sardine/Round herring (Tamban)	9	10 – 18
			Anchovies (Ikan bilis)	9	31 – 42
Terengganu	Dungun	June	Mix species	9	ND – 28
	Kuala Terengganu		Mix species	9	30 – 91
	Marang		Mix species	9	7 – 80
	Kemaman		Mix species	9	35 – 101
	Dungun	July	Mix species	9	17 – 28
	Kemaman		Mix species	9	18 – 24
	Kuala Terengganu		Big eye scad (Lolong)	9	14 – 19
	Chendering		Rainbow sardine/Round herring (Tamban)	9	17 – 26
	Kuala Besut		Mix species	9	17 – 24

Recovery of analysis = 80%

5. Problems and Challenges Encountered

- Lack of technical support staff.
- Insufficient information pertaining to fish preservation technique which might affect the fish quality.
- Implementation of good handling practices to assure fish safety and quality.
- Lack of regulatory enforcement.
- Implementation of good hygiene practices on fishing vessel.

6. Recommendations and Suggestions for Future Follow Up Action

- More allocations should be provided to cover for more samples during testing.
- Sampling points should be increased to cover more areas so as to have a more representative sampling distribution for the whole country.
- The frequency of the sampling should be increased depending on the amount of dominant species landed at the sampling locations.

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