Histamine Determination in Five Commercial Fish Species of the Peninsular Malaysia Under the Sanitary and Phyto-Sanitary (SPS) Program

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Abstract: Histamine, a biogenic amine produced in the tissue of some fish as a result of microbial decarboxylation of amino acid, histidine, is often associated with food poisoning. Five commercially species of fish i.e. Indian mackerel (*Rastrelliger kanagurta*), Japanese threadfin bream (*Nemipterus japonicus*), Spotted sardinella (*Amblygaster sirm*), Round scad (*Decapterus maruadsi*) and Tigertooth croaker (*Otolithes rubber*) which were obtained fresh from various landing jetties throughout Peninsular Malaysia were analysed using the Kawabata method to determine the histamine levels in fish flesh. In general, all samples analysed had low levels of histamine. The highest mean concentration of 2.66 mg/100 g was found in Round scad whereas the lowest mean concentration of 1.63 mg/100 g of flesh was found in Japanese threadfin bream. The results showed that pelagic fish had high levels of histamine compared to demersal fish ($p<0.05$). There is a significant difference in the histamine levels in samples taken from different locations ($p<0.05$). This preliminary study indicates that landed fishes from the study sites were generally well handled at sea after being harvested.

Keywords: histamine, commercial fishes, pelagic, demersal

Abstrak: Histamin merupakan biogen amina yang terhasil dalam tisu kebanyakan ikan semasa pendekarboksilan asid amino, histidina, oleh mikrob yang boleh menyebabkan keracunan makanan. Lima spesis ikan komersial iaitu Kembong (*Rastrelliger kanagurta*), Kerisi (*Nemipterus japonicus*), Tamban (*Amblygaster sirm*), Selayang (*Decapterus maruadsi*) dan Gelama (*Otolithes rubber*) yang diperolehi dari jeti pendaratan ikan di seluruh Semenanjung Malaysia telah dianalisa menggunakan kaedah Kawabata untuk menentukan paras histamin di dalam isi ikan. Secara keseluruhannya, semua sampel yang dianalisa mempunyai paras histamin yang rendah. Paras histamin yang paling tinggi iaitu 2.66 mg/100 g diperolehi daripada ikan selayang. Paras histamin yang paling rendah iaitu 1.63 mg/100 g diperolehi daripada ikan kerisi. Keputusan menunjukkan bahawa ikan pelagik mempunyai paras histamin yang lebih tinggi berbanding dengan ikan demersal ($p<0.05$). Terdapat perbezaan yang signifikan antara paras histamin ikan yang diambil dari lokasi yang berbeza ($p<0.05$). Kajian awal ini menunjukkan bahawa ikan yang didaratkan telah dikendalikan dengan baik selepas ditangkap.

Introduction

Histamine produced by microbial activities in fresh, semi-preserved or preserved fish has been reported as one of the principal compounds leading to fish poisoning (Taylor, 1986; Veciana-Nogues *et al.*, 1989). Histamine fish poisoning occurs throughout the world and is perhaps the most common form of toxicity caused by the ingestion of fish (Mines *et al.*, 1997). Since 1970, the countries with the most reported incidents of histamine fish poisoning are Japan, the United States of America and Great Britain. Less frequent outbreaks have been reported in various other countries including Canada, New Zealand, France, Germany, Norway, Sweden, Czechoslovakia, the Netherlands, Australia, Sri Lanka, Indonesia, South Africa and Egypt (Taylor, 1986). Since only a few of these countries keep official records on incidents of fish poisoning, it would be appropriate to assume that many incidents in these countries are not reported.

The number of histamine toxicity outbreaks has continued to increase in most European countries (Lopez-Sabater *et al.*, 1996) and is one of the major seafood-borne illnesses reported in United States, Japan and Canada. The presence of high level of histamine in fish and fish product is also causing product retention and thus a trade loss to the industry. Histamine is not destroyed by heat, therefore food authorities/agencies had to list this compound as one of the important safety criteria to regulate the safety of seafood products.

Histamine is formed mainly by the decarboxylation of the amino acid, histidine, through exogenous decarboxylation released from microorganisms associated with fish or environment (Rawles *et al.*, 1996). Fish of the family Scombridae such as tuna, mackerel and bonito are commonly implicated, hence the term scombroid fish poisoning. However, other types of fish from the family Clupeidae, Scombersocidae, Pomatonidae and Engraulidae have also been reported to cause histamine poisoning such as albacore (Kim *et al.*, 1999), bluefish (Karolus *et al.*, 1985), mackerel (Ritchie and Mackie, 1980; Wendakoon *et al.*, 1990), sardine (El Marrakchi *et al.*, 1990), horse mackerel (Okuzumi *et al.*, 1990), anchovy (Veciana-Nogues *et al.*, 1990), mahi-mahi (Baranowski *et al.*, 1990) and flying fish (Vijayan *et al.*, 1994).

Fresh fish contains very low levels of histamine but the content increases with the progress of fish decomposition (Frank *et al.*, 1981; Fernandez-Salguero and Mackie, 1987). Hence, it has been proposed as a chemical index of fish spoilage (Lopez-Sabater *et al.*, 1994; Yoshinaga and Frank, 1982), poor hygienic quality of raw materials used and poor manufacturing conditions (Hui and Taylor, 1983). Since histamine is colourless and odourless, the consumer is unlikely to be aware of them when buying fish. Chilling the fish is one way of preventing the production of histamine because the bacteria responsible for the producing the enzyme is not active below about 10°C.

Histamine is a chemical hazard monitored by the Food and Drug Administration of the USA for the safety of seafood products. Regulatory guidelines have not been established for all the various fishes of concern, but 50 ppm is inferred from the U.S. FDA's defect action level for tuna (USFDA, 1998). The Codex Alimentarius has specified the level of more than 10 mg/100 g as decomposition level and 20 mg/100 g as the hygienic level. In Malaysia, no guidelines on histamine levels of toxicity are established in Malaysian Food Regulations (Food Regulations, 1985) due to lack of information on histamine levels in local species.

The challenges facing by the fishery industry in Malaysia are not only to increase production but also to produce safe and high quality seafood both for domestic and international markets. The World Trade Organisation (WTO) has set rules for each member country to implement legitimate measures to protect the life and health of their people from hazards in food under the Agreement on Sanitary and Phytosanitary Measures (SPS).

The Department of Fisheries (DOF) Malaysia has already taken steps towards adhering to WTO by initiating a programme for marine SPS in 1998. This programme aims to ensure that fish/shellfish are caught from safe areas, are of high quality and safe to consume. At present it is only implemented in Peninsular Malaysia but will be extended to the states of Sabah and Sarawak in East Malaysia. Eleven sampling sites comprising of landing ports/jetties were identified for this purpose. This paper will describe the results of histamine monitoring of five selected fish species under the DOF's Marine SPS programme carried out from 2000 to 2002 and will discuss the implications of the initial findings on the fishery industry.

Materials and Methods

Sample preparation

Five commercial fish species, namely Indian mackerel (*Rastrelliger kanagurta*) (n=32), Japanese threadfin bream (*Nemipterus japonicus*) (n=37), Spotted sardinella (*Amblygaster sirm*) (n=23), Round scad (*Decapterus maruadsi*) (n=31) and Tigertooth croaker (*Otolithes rubber*) (n=32) were obtained fresh from various landing jetties throughout Peninsular Malaysia, i.e. Tg. Sedeli (Johor), Kuala Kedah/Kuala Perlis (Kedah/Perlis), Bachok (Kelantan), Port Dickson/Kuala Sg. Baru (N.Sembilan/Melaka), Batu Maung (Penang), Kuantan (Pahang), Hutan Melintang (Perak), Bagan Sg. Besar (Selangor) and Kuala Besut

(Terengganu). Samples were collected by the state's extension officer, packed in whirl pack bags and were transported in ice-cooled insulated box. Samples were immediately sent to the Food Chemistry Laboratory at the Fisheries Research Institute, Penang. The sample temperature range was 3.0 - 4.9°C during receiving. The samples were kept chilled (<10°C) until analysis commenced.

Histamine analysis

The histamine content was analysed in duplicate according to the Kawabata method (Kawabata *et al.*, 1960). The fishes were filleted and the skin removed. Extracts for analyses of fresh fishes were prepared by blending 10 g of minced fish with 20 ml of 10% Trichloro-acetic acid (TCA) solution and 20 ml of distilled water (DW) in a homogenizer. The volume was adjusted to 100 ml with DW and filtered through Whatman No. 1 filter paper. Then the sample extract was added with 0.4 N acetate buffer to stabilise the pH. The histamine was separated from the interfering substances such as histidine, tyrosine, tyramine etc. by Cation Exchange Chormatography with Amberlite CG 50 resin. It was then determined by using a UV/VIS spectrophotometer (Cary 50) at wavelength 510 nm.

Statistical Analysis

All assays were determined in duplicate and the results were reported as means. The significance of the differences in formation of histamine among families of fish was determined by descriptive statistics, T-test, one-way analysis of variance (ANOVA) and multiple comparisons by the Duncan test (SPSS for Windows, version 11.5, SPSS *Inc.*, Chicago, Ill., USA). The significance difference was established at $p < 0.05$.

Results and Discussion

In this experiment, the fish species can be classified according to the fish group namely pelagic fish (Indian mackerel, Round scad, Spotted sardinella) and demersal fish (Tigertooth croaker, Threadfin bream). Table 1 shows the mean and standard deviation of histamine levels in different fishes. Comparison of the overall fishes means indicates there is evidence to conclude at the 5% level of significance that the mean histamine levels is different between fishes ($p<0.05$). The range histamine level for overall fishes is 5.78 mg/100 g. Irrespective of the locations, the lowest histamine level was observed in Tigertooth croaker ranging from 0.37 to 3.33 mg of histamine per 100 g flesh, while the highest was observed in round scad, from 0.44 to 6.10 mg/100 g. The highest histamine level was obtained from Round scad with 2.67 mg/100 g and differed statistically ($p<0.05$) from Tigertooth croaker (1.66 mg/100 g), Threadfin bream (1.63 mg/100 g) and Indian mackerel (1.74 mg/100 g). The lowest was obtained from Japanese threadfin bream with 1.63 mg/100 g and differed statistically ($p<0.05$) from those of Round scad. The histamine content in all species was less than 5 mg/100 g. This study confirms previous observations that fresh fish contain very little histamine (Frank *et al.*, 1981).

The results (Table 2) show that most of the fish from the pelagic group (Round scad, Spotted sardinella) had high levels of histamine compared to demersal group (Tigertooth croaker, Threadfin bream) ($p<0.05$). However, Indian mackerel which is from pelagic fish was not differed statistically ($p>0.05$) from Tigertooth croaker and Threadfin bream (demersal group) (Table 1). On the average, mean histamine levels in pelagic fish is between 0.17 mg/100 g and 0.94 mg/100 g higher than demersal fish as shown by the 95% confidence interval of the mean difference (Table 2). The histamine concentration was higher in pelagic fish probably because of the presence of red meat in this fish. The spoilage in round scad was rapid, as these fishes contain a high proportion of dark meat, which is readily available for spoilage by microorganisms. Kimata and Kawai (1953) reported histamine production is higher in fish species with high proportion of red muscle. The conclusions made by Takagi *et al.* (1969) are consistent with those of other researchers in that more histamine is produced in the red muscle fish such as tuna and mackerel than in white muscle species such as rockfish. Murray *et al.* (1982) reported that the recommended limit for quality purposes for histamine in pelagic fish is less than 5 mg/100 g.

Table 1: The mean histamine contents in different species of fish

Descriptives

Species	Group	N	Histamine (mg/100 g) Mean	S. D.	S. E.	95% Confidence Interval Lower	Upper
Tigertooth croaker (*Otolithes ruber*)	Demersal	32	1.66258	0.928753	0.164182	1.32773	1.99743
Threadfin bream (*Nemipterus japonicus*)	Demersal	37	1.63275	1.197732	0.196906	1.23341	2.03209
Indian mackerel (*Rastrelliger kanagurta*)	Pelagic	32	1.73750	1.042871	0.184355	1.36150	2.11350
Round scad (*Decapterus maruadsi*)	Pelagic	31	2.67480	1.184775	0.212792	2.24022	3.10938
Spotted sardinella (*Amblygaster sirm*)	Pelagic	23	2.20036	1.704318	0.355375	1.46336	2.93736
Total		155	1.95317	1.257836	0.101032	1.75358	2.15276

Histamin (mg/100 g)

	Species	Group	N	Subset for alpha = 0.05 1	2
Duncan (a,b)	Threadfin bream (*Nemipterus japonicus*)	Demersal	37	1.63275	
	Tigertooth croaker (*Otolithes ruber*)	Demersal	32	1.66258	
	Indian mackerel (*Rastrelliger kanagurta*)	Pelagic	32	1.73750	
	Spotted sardinella (*Amblygaster sirm*)	Pelagic	23		2.20036
	Round scad (*Decapterus maruadsi*)	Pelagic	31		2.67480
	Sig.			0.097	0.128

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 29.157.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.

Table 2: Histamine levels per 100 g of flesh in pelagic and demersal fishes

T-Test

Group Statistics

	Group	N	Mean	S. D.	Mean S. E.
Histamin (mg/100 g)	Pelagic	86	2.19915	1.344105	0.144939
	Demersal	69	1.64659	1.073748	0.129264

Independent Samples Test

		Levene's Test for Equality of Variance		test for Equality of Means						
									95% Confidence Interval	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	S. E. Difference	Lower	Upper
Histamin (mg/100g)	Equal variances assumed	4.008	0.047	2.777	153	0.006	0.55257	0.199001	0.159422	0.945711
	Equal variances not assumed			2.845	152.999	0.005	0.55257	0.194207	0.168893	0.936240

The results in Fig. 1 show that there is a significant difference in the histamine levels in samples taken from different locations ($p<0.05$). Irrespective of the fishes, the highest mean concentration of 3.05 ± 0.66 mg of histamine per 100 g flesh was found in the fish analysed from Johor. The lowest of 1.19 ± 1.41 mg of histamine per 100 g flesh was formed in fish analysed from Negeri Sembilan/Melaka followed by Penang with 1.27 ± 0.69 mg/100 g. The highest histamine level in Johor was obtained from tigertooth croaker (3.17 ± 0.18 mg/100 g). However, the same species showed different histamine levels in N.Sembilan/Melaka, Kedah/Perlis, Perak and Selangor with 1.95 mg/100 g, 1.78 mg/100 g, 1.22 mg/100 g and 1.57 mg/100 g respectively. Histamine levels are also known to vary greatly, depending on the location and species of the fish (Arnold and Brown, 1978; Lerke *et al.*, 1978). The variation in concentration of histamine among samples might be due to their origin, handling conditions, inadequate refrigeration and degree of microbiological contamination. The reason for this variation is also because of some fish may have been caught at different times and different locations. This is in agreement with Fernandez-Salguero and Mackie (1987), Ritchie and Mackie (1980), Veciana-Nogues *et al.* (1990) and Mackie *et al.* (1997).

In this study, all samples analysed had low levels of histamine. These levels were probably lower due to the fact that the samples were frozen or chilled on board and immediately chilled after reaching the dock and kept below 10°C until analysis. These concentrations are well below the maximum level (5 mg/100 g) required by the FDA. Frank *et al.* (1981) also reported that histamine content of fresh fish is very low. The levels of histamine in fresh fish are normally between 10-50 ppm (Halasz *et al.*, 1994). It is known to have relatively long shelf-life and contains negligible quantities of histamine immediately after catch. There are a few reports that fresh fish do contain histamine up to a level of 5 ppm (Gopakumar *et al.*, 1985; Vijayan *et al.*, 1994). The low levels of histamine were in agreement with those in the literature. Veciana-Nogues *et al.* (1990) reported that histamine production in muscle of anchovy (*Engraulis encrasicholus*) was practically inhibited at 0°C. Toyama *et al.* (1982) reported 0 ppm of histamine in muscle of fresh sardine, while El Marrakchi *et al.* (1990), studying the correlation of storage on ice of Morocco sardine (*Sardinops pilchardus*) with the production of histamine and other biogenic amines, found levels of 1.12 mg/100 g of fish on the first day of storage and < 20 mg/100 g after 18 days. In addition to storage temperature, biogenic amines level may differ depending upon the moment of capture and stomach contents at the time of death, since microbial flora varies seasonally (Rodriguez *et al.*, 1994).

High histamine levels in fish muscle is associated with spoilage and is used as an indicator of toxicity of raw materials and it indicates poor processing conditions. Since the most important exogenous factor on histamine formation is temperature, the rapid chilling of fish after catch avoids its production. The results suggested that the optimum postcatch handling conditions used in this study assured a low microbial population on the fish surface before ice-storing at 0°C.

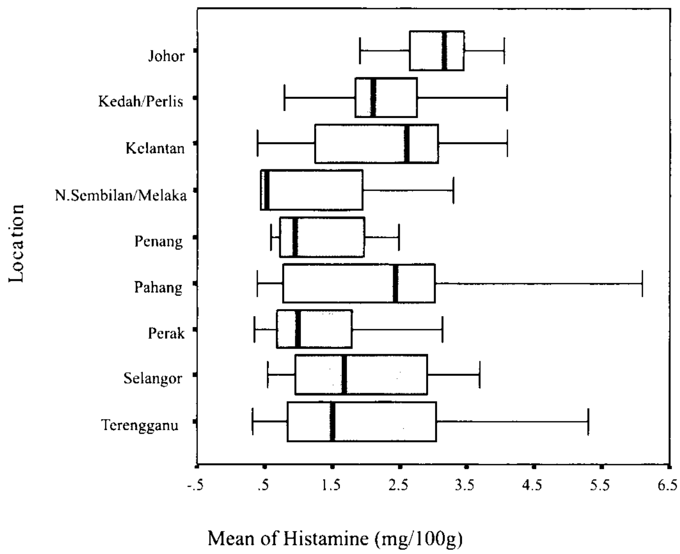

Figure 1: Mean histamine concentrations at different locations

Conclusion

In general, histamine concentrations in freshly caught fish are low. The fish analysed contained low levels of histamine, well below hazard action level (< 50 mg/100 g) which might constitute a known human health hazard. It has been found that there are variations in the formation of histamine in fishes belonging to the same species of different regions. There are also differences in the maximum level of histamine formation among the five fishes, with round scad showing the highest (2.67 mg/100 g) and threadfin bream showing the lowest histamine content (1.63 mg/100 g). This preliminary monitoring indicated that landed fish generally well preserved after catch at sea.

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