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Biogenic Amines and Histamine of Marlin Fillet and Spotted Mackerel Fillet Sampled from Cafeteria and Anchovy from Fish Market in Keelung

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ABSTRACT

Twenty-six marlin fillet and 19 spotted mackerel fillet samples obtained from cafeteria and 22 anchovy samples collected from fish market in Keelung were analyzed for biogenic amines and histamine. The biogenic amines of samples collected in summer were greater than those in winter with levels ranging from 744 ppm to 1,234 ppm and 297 ppm to 558 ppm, respectively. Out of 67 samples analyzed, 9 samples of marlin fillet, 4 samples of spotted mackerel fillet and 1 sample of anchovy had biogenic amines above 1,000 ppm. No sample had histamine exceeded 500 ppm in summer or winter. Twenty out of 67 samples showed detectable amounts of histamine, 94% of which were less than 50 ppm. Two samples of marlin fillet and 2 samples of anchovy had histamine content greater than 50 ppm, ranging from 83 ppm to 136 ppm. It was found that 2 (6.7%) marlin fillet samples and 1 (4.5%) anchovy sample in summer exceeded the criterion of 1,000 ppm biogenic amines and 50 ppm histamine in Keelung.

Key words: biogenic amines, histamine, marlin fillet, spotted mackerel fillet, anchovy

INTRODUCTION

Biogenic amines, particularly histamine, have been considered the cause of scombroid fish poisoning[(1)]. Fish associated with scombroid fish poisoning or scombrotoxicosis are tuna, mackerel, bonito, saury, bluefish, mahi-mahi, sardine, anchovy, herring, and marlin[(2)]. Histamine seems to be formed by post-catching contamination on board, at the processing plant, in the distribution system, in restaurants, or homes[(3)]. The symptoms include rash, urticaria, edema, localized inflammation, nausea, vomiting, diarrhea, abdominal cramps, headache, palpitation, flushing and severe respiratory distress[(4,5)]. The U.S. Food and Drug Administration (FDA) revised the compliance guide for decomposition and histamine poisoning in 1995. It was announced that fishes may be considered as decomposed when histamine level reaches 50 ppm. With the exception of tuna, fishes pose health hazards when histamine level reaches 500 ppm[(6)]. The European Community (EC) has established an acceptable level of 100 ppm for histamine in tuna and other fishes in the Scombridae and Scomberesocidae families[(7)].

In addition to histamine, the FDA suggested other biogenic amines which could be used to evaluate fish freshness[(2)]. Individual susceptibility of other biogenic amines should be considered to estimate the toxic aspect of histamine. Although histamine below 50 ppm is considered safe, over half of the scombrotoxicosis cases in 1976-1986 were associated with histamine concentration below 50 ppm[(8)]. Apparently, histamine was unlikely the only causative agent of scombrotoxicosis. Bjeldanes *et al*. pointed out that not only histamine but also cadaverine were of importance in scombrotoxicosis[(9)]. Moreover, several studies indicated that putrescine and cadaverine were found relatively large quantities in toxic fish and facilitated the transportation of histamine, as well as increased the fish toxicity[(2,10)]. Therefore, low histamine might not be an assurance of a safe product. Other biogenic amines should also be concerned for human health hazards. For regulation purpose, Spanjer *et al*. suggested that the sum of histamine, putrescine and cadaverine in fish and fish products should be limited to 300 ppm[(11)]. A chemical index [(the sum of the contents of histamine, putrescine and cadaverine)/(1+ the sum of the contents of spermidine + spermine)] was proposed to evaluate the quality of canned tuna[(12)].

Spermidine and spermine are found as minor components in fish which change slightly during storage[(13)]. On the other hand, putrescine and cadaverine increased steadily with spoilage, thus, they are considered good indicators of fish quality[(14)]. Good correlation was found between sensory evaluation and levels of putrescine and cadaverine[(15)]. Albrecht-Ruiz *et al*.[(16)] developed an enzyme-based colorimetric method which allowed accurate quantification of biogenic amines, called “Amine Index” (histamine + putrescine + cadaverine), for fish meal. It may be inferred that amine index could be an indicator of fish quality. Besides scombroid fish poisoning, the carcinogenic nitrosamine formed by nitrite and diamine should be concerned. The putrescine and cadaverine can react with nitrite to form carcinogenic nitrosamine. Nitrosamines are produced by salting or smoking while cooking or frying

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enhanced their formation[17]. The formation of heterocyclic amines in dressed fried fish fibre was also reported[18,19].

Tuna, mackerel and marlin were associated with the occasional food poisoning cases in Taiwan. Similarly, sailfish fillets (Istiophorus platypterus) were implicated in a 1994 scombroid poisoning outbreak in western Taiwan[20]. In 1996, two incidents of scombroid poisoning in cafeterias in northern Taiwan were reported on non-scombroid fish of Makaira and scombroid fish of skipjack tuna (Euthynnus pelamis) with histamine content as high as 84.13 mg/100g and 118.5 mg/100g, respectively[21]. In view of these incidents, the histamine level and biogenic amines of marlin and mackerel fillet and raw anchovy were investigated.

MATERIALS AND METHODS

I. *Materials*

Cooked marlin fillets (26 samples) and spotted mackerel fillets (19 samples) were collected from local cafeterias in seven districts of Keelung, Taiwan during winter and summer between 2001 and 2003. Meanwhile, anchovies (*Engraulis anchoita*, 22 samples) were collected from the same fish markets in different seasons. The average weight of marlin fillet, spotted mackerel fillet and anchovy was about 150g, 100g and 150g, respectively. Marlin fillet and spotted mackerel fillet were made from the middle section of the fish. All samples were collected with sampling bags (Whirl-Pak Bags, Nasco), kept in a temperature-holding bag and transferred to the laboratory immediately. The samples were stored at -20˚C until use.

II. *Methods*

(I) *Biogenic amines determination*

The biogenic amines were determined by a modified enzyme-based colorimetric method[16]. Diamine oxidase (EC1.4.3.6), horseradish peroxidase type VI-A (EC1.11.1.7), histamine dihydrochloride, 4-aminoantipyrine and trichloroacetic acid were purchased from Sigma Chemical Co. (U.S.A.). Color developing reagent was prepared by mixing 4 parts of 1.5 M Tris buffer pH 9.0, 1 part of 400 mM 4-aminoantipyrine and 1 part of 40 mM phenol. Reagent was prepared freshly before use. Diamine oxidase and horseradish peroxdase solution were prepared in concentration of 300 mU/mL and 175 U/mL, respectively.

Fifty milliliters of 20% trichloroacetic acid was added to 5 g of fish sample and homogenized (Ultra-Turrax T25, Germany) in a plastic bottle for 10 min. Ten milliliters supernatant was taken and diluted to 100 mL with distilled water. The precipitant was filtered. The supernatant was adjusted to pH 9 with 50% KOH. The extract was further clarified by centrifugation at 700 ×g for 3 min. A stock solution of histamine dihydrochloride was prepared in distilled water and diluted to 1, 5, 10, 20 ppm to obtain a standard curve. Distilled water was used as blank. One milliliter of extract, distilled water or standard solution, 0.45 mL of color developing reagent, 0.5 mL of diamine oxidase solution and 0.05 mL of horseradish peroxidase solution were mixed vigorously in a tube. The tubes were incubated at 50˚C in water bath for 1 hr. The absorbance was read with a UV-VIS spectrophotometer at 505 nm. The concentration of test solution was determined according to the standard curve. The biogenic amines were calculated as below:

$$\text{Biogenic amines (ppm)} = X\ (\text{mg/L}) \times 100\ (\text{mL}) / 10\ (\text{mL}) \times 55\ (\text{mL}) / 5\ (\text{g})$$

(II) *Histamine content determination*

Histamine content was determined by a commercial enzyme immunoassay kit[22] (Veratox Quantitative Histamine Test Kit, Neogen, U.S.A.). Rogers and Staruszkiewicz[23] reported that the Veratox kit can accurately quantify histamine ranging from 2.5 to 50 ppm. Procedures were conducted according to the kit protocol. Ten grams of sample along with 90 mL of deionized water were homogenized and settled. The supernatant was filtered through Neogen filter syringe into a clean plastic tube. One-hundred microliters of the filtrate were added to a 10 mL of sample extract diluent buffer in a clean plastic container. Then, the sample extract was ready for histamine immunoassay.

One-hundred microliters of histamine-horseradish peroxidase conjugate were added to each red-marked mixing well. One-hundred of controls and sample extracts were then transferred to each mixing well and mixed thoroughly by pipetting the mixture liquid up and down for 3 times. Transferring 100 µL of the mixed sample liquid to the antibody coated well and incubated for 10 min at room temperature. Shake out the contents of the antibody wells. Each well was washed with diluted wash buffer and tapping out the remaining liquid. One-hundred of substrate were added into each well and incubated for 10 min. One-hundred microliters of red stop solution were added into each well and mixed by sliding back and forth. The histamine concentration was determined using a microwell reader with 650 nm filter.

(III) *Statistical analysis*

Data were analyzed using T-test to determine the significance of difference at $p < 0.05$ on biogenic amines in different products and seasons.

RESULTS AND DISCUSSION

The standard curve of histamine is shown in Figure 1. The regression equation is $Y = 0.0165X + 0.0483$ ($r^2 = 0.9996$).

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Table 1. Distribution of histamine level of marlin fillet, spotted mackerel fillet and anchovy sampled in winter and summer

Sample	Season	Sample no.					
		Total	< 50	50-99	100-500	> 500	Detected range (ppm)
Marlin	Winter	16	16	0	0	0	4 - 24
	Summer	10	8	1	1	0	4 -125
Mackerel	Winter	11	11	0	0	0	11 - 25
	Summer	8	11	0	0	0	10 - 44
Anchovy	Winter	14	13	1	0	0	41 - 83
	Summer	8	7	0	1	0	14 -136
Total		67	63	2	2	0	4 -136

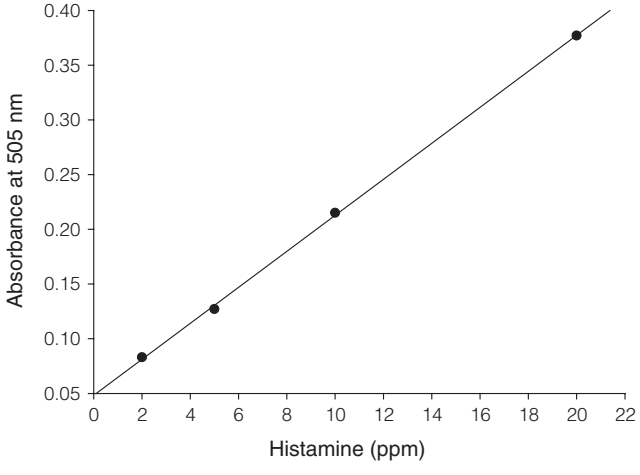

Figure 1. Standard curve of histamine.

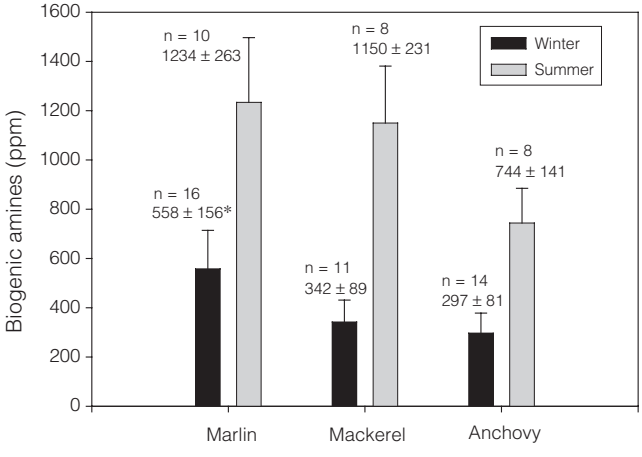

Figure 2. Biogenic amines of various fishes in different seasons. *: Mean ± S.E.

Results of samples analyzed for histamine were summarized in Table 1. Eight out of 26 marlin fillet samples, 5 out of 19 mackerel fillet samples and 7 out of 22 anchovy samples had detectable amounts of histamine, ranging from 4 to 125 ppm, 10 to 44 ppm, and 14 to 136 ppm, respectively. Among the total of 67 samples analyzed, histamine was detected in 20 samples with levels from 4 to 136 ppm. Histamine was not detected in 70% of the samples.

Nout (1994) indicated that 50 to 100 ppm of histamine was proposed for Good Manufacturing Practice (GMP) for fishery product[24]. As shown in Table 1, 1 sample of anchovy collected in summer, 1 sample of anchovy collected in winter and 2 samples of marlin fillet collected in summer reached the decomposition level of 50 ppm. One sample of marlin fillet and 1 sample of anchovy did not meet the requirement of the European Community. One sample of marlin fillet and 1 sample of anchovy did not meet the suggested requirement for GMP for fishery product. Apart from the one anchovy sample, no sample contained histamine greater than 50 ppm in winter. Rawles *et al*. (1996) showed that histamine in spoiled fish is extremely variable. The factors include holding time, temperature, body section of fish, fish species, type and level of microorganisms present[6]. In this study, no sample of spotted mackerel fillet collected in winter and summer had histamine above 50 ppm. It might be inferred that the mackerel fillet had low post-catching contamination or contained low histamine originally[3,6]. Table 1 also indicated that some anchovy in Keelung may contain histamine higher than 50 ppm. Histamine contents of 94% (63/67) of the samples collected in Keelung were below the 50 ppm defect action level, while 6% (4/67) was above the action level, according to the U.S. FDA Compliance Policy Guide. Fletcher, *et al*.[25] reported that 8 out of 107 (7.5%) of the smoked fish from New Zealand markets had histamine levels above 50 ppm.

The biogenic amines of various fishes in different seasons are shown in Figure 2. All anchovy, spotted mackerel fillet and marlin fillet samples collected had significantly less biogenic amines in winter than in summer ($p < 0.05$). It could be ascribed to the low temperature that reduced the rate of biogenic amines formation during sample handling since microorganisms are responsible for biogenic amines formation. Both in winter and summer, the amounts of biogenic amines of marlin were higher than mackerel followed by anchovy. Silla Santos[26] reported that 1,000 ppm of the amines in food could be considered as a hazard criterion for health. In this study, all samples except marlin and mackerel fillets collected in summer had biogenic amines level below 1,000 ppm. It was obvious that most of the samples collected were safe for consumption. The average level of biogenic amines of marlin and mackerel fillets collected in summer was 1234 ppm, and 1150 ppm, respectively. Mendes[27] reported that cadaverine was produced in mackerel at the concentration of 900 ppm after two days of storage at room temperature (20-23˚C). The concentration of putrescine above 200 ppm was also observed. It seemed that some marlin and mackerel

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Table 2. Distribution of biogenic amines of marlin fillet, mackerel fillet and anchovy

Sample	Season	Sample no.	Biogenic amines (ppm)		
			< 300	300 - 1000	> 1000
Marlin	Winter	16	6	6	4
	Summer	10	1	4	5
Mackerel	Winter	11	7	3	1
	Summer	8	0	5	3
Anchovy	Winter	14	8	6	0
	Summer	8	1	6	1
Total		67	23	30	14

Table 3. Samples collected in summer contained histamine above 50 ppm and biogenic amines above 1000 ppm

Sample	Total samples collected	Sample no.	Biogenic amines (ppm)	Histamine (ppm)
Marlin	10	2	2873	125
			1312	89
Anchovy	8	1	1599	136

fillets in cafeterias in Keelung were not carefully stored, handled, and processed because of temperature abuse in summer. The climate conditions of high humidity and high temperature in Keelung contribute to those potential hazard results.

According to the suggested levels of 300 ppm and 1,000 ppm in previous literature, the distribution of biogenic amines of marlin fillet, spotted mackerel fillet and anchovy was investigated and shown in Table 2. Seven out of 26 samples of marlin fillet, 7 out of 19 samples of mackerel fillet, and 9 out of 22 samples of anchovy had biogenic amines below the control level of 300 ppm. For marlin fillet, mackerel fillet and anchovy, 4, 5 and 6 summer samples had biogenic amines between 300 ppm and 1,000 ppm, respectively. On the other hand, 6, 3, and 6 samples had this range of biogenic amines in winter, respectively. Meanwhile, 9 out of 26 samples of marlin fillet, 4 out of 19 samples of mackerel fillet, and 1 out of 22 samples of anchovy had biogenic amines over 1,000 ppm.

Rossi *et al*.[28] presented that histamine level was higher than that of cadaverine and putrescine in bigeye tuna and skipjack tuna stored in ice or under room temperature. Ozogul *et al*.[29] also reported similar result in herring. Meanwhile, they pointed out that the cadaverine and putrescine levels increased significantly during ice storage, but the level of histamine did not. On the contrary, Baixas-Nogueras *et al*.[30] and Ruiz-Capillas and Moral[31] reported levels of cadaverine and putrescine were higher than that of histamine in hake samples stored in ice or under room temperature. Hwang *et al*.[32] reported that the level of each biogenic amine in marlin fillet varied during storage at room temperature depending on the different bacteria strain contaminated. Although the histamine level is often detected to be higher in the scombroid fish, the level of other biogenic amines (such as cadaverine and putrescine) in all fish increased more easily. Which biogenic amine will significantly appear depends on the bacteria species contaminated, storage temperature, storage time and conditions. Judging from our data, the contamination of histamine-producing bacteria was less than other spoilage bacteria or the storage condition was not favorable to histamine-producing bacteria.

Hwang *et al*.[20,32] reported marlin fish involved in histamine poisoning in Taiwan, and the histamine content reached 180 mg/100g and 82.2 mg/100g in 1995 and 1999, respectively. More attention should be paid when fish products reach the defect action level of 50 ppm of histamine. Table 3 summarized the samples collected in summer containing histamine above 50 ppm and biogenic amines above 1,000 ppm. The biogenic amines and histamine of one marlin fillet collected in summer were 2,873 ppm and 125 ppm, respectively, another were 1,312 ppm and 89 ppm, respectively. One anchovy sample collected in fish market in summer contained biogenic amines of 1,599 ppm and histamine of 136 ppm. It indicated that 2 out of 10 samples of marlin fillet collected from cafeterias in summer and 1 out of 8 samples of anchovy collected in fish market in summer in Keelung exposed a potential hazard for consumption.

According to the investigation results, most of the fishery products analyzed in this study were safe from histamine poisoning. However, 6.7% (2/26) marlin fillet from cafeteria and 4.5% (1/22) anchovy in fish market in Keelung posed potential risk. In addition, the high level of biogenic amines in some marlin and mackerel fillets indicated that some fishery products in the cafeteria and fish market in Keelung were not properly processed and handled. More attention is needed during handling and processing of raw fishes before serving for consumption in Keelung.

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