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Determination of Histamine and Tyramine Levels in Canned Salted Fish by Using HPLC

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Abstract: To determine the levels of histamine and tyramine in canned salted mackerel, sardine and tuna fish and to compare these levels with the maximum allowable levels, a total of 90 fish samples, 30 samples from every studied species were analyzed and the histamine and tyramine levels were estimated. Revealed that 46.7%, 80% and 73.3% of mackerel, sardine and tuna samples were positive for histamine residues and contain levels higher than 50 ppm, respectively. About 60%, 86.7% and 80% of mackerel, sardine and tuna samples were positive for tyramine residues and contain levels higher than 100 ppm respectively. Overall percentage of histamine and tyramine in all samples are 66.7% and 71.1% respectively. High levels of histamine and tyramine in canned salted samples can pose a health risk to consumers, where histamine can cause scombroid fish poisoning and tyramine is a potent mutagen precursor. All histamine and tyramine levels exceeded the maximum allowable levels determined by Food and Drug Administration (FDA). Therefore, quality control programs in the fish canning industries must recommend removing contaminated fish and improving the quality of canned fish.

Key words: Histamine · Tyramine · Biogenic Amines · Fish · Intoxication

INTRODUCTION

Histamine and tyramine are biogenic amines produced in fish by microbial decarboxylation of amino acids histidine and tyrosine that are of importance in fish intoxication and an index of fish spoilage [1]. Biogenic polyamines such as putrescine, cadaverine, spermidine, spermine and agmatine are potential carcinogens which converted to nitrosamines when reacted with nitrites [2]. Biogenic amines can produce mild to severe sings of food intoxication such as urticaria, headache, flushing, abdominal pains, cramps, heart failure, brain hemorrhage and hyper- or hypotension [3]. Families Scombridae and Scomberesocideae such as mackerel, tunas, saury, bonito, seerfish, butterfly kingfish and non-scombroid fish such as sardine, pilchards, anchovies, herring and marline are commonly implicated in biogenic amine intoxication especially histamine poisoning [4]. Some food technological treatments such as salting, ripening, fermentation and marination can increase the levels of biogenic amines in fish [5]. In Iranian study, high levels of histamine and tyramine are found in canned tuna fish [6]. Some authors recorded that a significant increase of histamine level was noticed in selected fish after commercial frying process [7], histamine represents the major cause of scombroid poisoning and other biogenic amines such as tyramine, cadaverine and putrescine acts as potentiators of histamine toxicity [8]. In June2007, in Southern Taiwan, food poisoning in 347 victims was occurred due to ingestion of fried fish cubes containing high levels of histamine and given the allergic signs in victims [9]. The toxicity level of biogenic amines is very difficult to establish because it depends on the individual characteristics and the presence of other amines, however, a maximum total biogenic amines level of 750 – 900 mg/kg has been proposed [10].

The types of bacteria that are associated with histamine production are commonly present in the salt-water environment which naturally present on the gills, external surfaces and in the gut of live salt water fish with no harm to the fish. Up on death, the defense mechanisms of the fish no longer inhibit bacterial growth in the muscle tissue and histamine-forming bacteria may start to grow resulting in the formation of biogenic amines [11]. Storage temperature is the most important factor contributing to biogenic amines formation [12].

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In this study we deterimine the amount of two highly toxic biogenic amines; histamine and tyramine, in different types of canned salted fish by using highly sensitive and reliable method; HPLC. Also, we compare these levels with maximum allowable levels set by Food and Drug Administration (FDA) to assess the risk of these toxic amines.

MATERIALS AND METHODS

Samples: A total of 90 canned salted fish; 30 canned mackerel, 30 canned sardine and 30 canned tuna of different factories were purchased from supermarkets. After opening each can, oil was drained off and the meat was ground in a food blender with stainless steel cutters for 3 minutes. Samples were then taken for determination of biogenic amines concentration.

Reagents: All reagents were of analytical grade. Standard histamine and tyramine were obtained from Sigma (St. Louis, MO, USA). Trichloroacetic acid, sodium-1-heptanesulfonate, monobasic potassium phosphate, orthophosphoric acid and acetonitrile were purchased from Merck (Darmstadt, Germany).

Extraction: Ten grams from each sample were homogenized with 25 ml of 5% trichloroacetic acid for three minutes. The homogenates were centrifuged for 15 minutes at 3000 rpm then filtered through0.45 µm whatman filter paper [13].

HPLC Determination: Analysis was performed by using Wasters HPLC-Millipore System. The mobile phase was a buffer and organic solvent, acetonitrile. The buffer composed of 0.01M sodium-1-heptanesulfonate in 0.05M monobasic potassium phosphate, pH to 3.2 with phosphoric acid. The proportion of buffer to acetonitrile was 75:25. The mobile phase was prepared with deionized water and filtered by 0.45 µm filter paper. The column used was aplha Bond C18, 10 µm, 300 X 3.9 mm, the flow rate was 1.0 ml/minute at ambient temperature, the injection volume was 20 µl and the analyte was detected by UV-detector at 220 nm wavelength [14].

Calculations: Measure peak area for histamine and tyramine standard solutions and for test samples; prepare standard curve of histamine and tyramine standard solutions concentrations versus peak areas by using data from the same standard solutions. From measured peak areas of test samples, calculate histamine and tyramine concentrations from regression equation as follows:

$$y = m x + b$$

where:

y = peak area x = histamine (or tyramine) concentration (µg/g)
m = slope of curve b = intercept of y

RESULTS

Results revealed that salted fish contain variable amounts of biogenic amines, histamine and tyramine. Histamine levels was 54-82 ppm (Mean 68 ppm), 57-67 ppm (Mean 62 ppm) and 55-237 ppm (Mean 146 ppm) in mackerel, sardine and tuna respectively. Tyramine levels was 71-128 ppm (Mean 99.5), 111-326 ppm (Mean 318.5 ppm) and 98-275 ppm (Mean 201.5 ppm) in mackerel, sardine and tuna respectively (Tables 1, 2). About 46.7%, 80% and 73.3% of mackerel, sardine and tuna samples were positive for histamine residues and contain levels higher than 50 ppm, respectively, while 53.3%, 20% and 26.7% of mackerel, sardine and tuna samples were free from histamine residues, respectively. About 60%, 86.7% and 80% of mackerel, sardine and tuna samples were positive for tyramine residues and contain levels higher than 100 ppm, respectively, while 0%, 13.3% and 26.6% of mackerel, sardine and tuna samples were free from tyramine residues, respectively (Tables 2, 4). The overall frequency distribution rate of histamine and tyramine levels in salted fish was 66.7% and 86.7%, respectively, while 33.3% and 13.3% of salted fish samples were free from histamine and tyramine residues, respectively (Table 3). Overall rate of histamine levels in salted fish higher than 50 ppm was 66.7% while overall rate of tyramine levels in salted fish higher than 100 ppm was 71.1 ppm (Table 5).

Table 1: Histamine and Tyramine levels in Salted Fish

	Histamine Levels (ppm)			Tyramine Levels (ppm)		
Sample	Min.	Max.	Mean	Min.	Max.	Mean
Mackerel	54	82	68	71	128	99.5
Sardine	57	67	62	111	326	318.5
Tuna	55	237	146	98	275	186.5
Total	54	237	92	71	326	201.5

Table 2: Frequency Distribution of Histamine and Tyramine levels in Mackerel, Sardine and Tuna

	Histamine Levels (ppm)			Tyramine Levels (ppm)		
Sample	Range	Frequency	%	Range	Frequency	%
Mackerel (n=30)	0 (Free)	16	53.3	0 (Free)	0	0
	50-60	4	13.3	70-90	4	13.3
	60-70	4	13.3	90-110	12	40
	70-80	2	6.6	110-120	4	13.3
	80-90	4	13.3	120-140	8	26.6
Sardine (n=30)	0 (Free)	6	20	0 (Free)	4	13.3
	50-60	16	53.3	100-150	6	20
	60-70	8	26.6	150-250	6	20
	70-80	0	0	250-300	8	26.6
	80-90	0	0	300-350	6	20
Tuna (n=30)	0 (Free)	8	26.6	0 (Free)	8	26.6
	50-100	12	40	90-150	12	40
	100-150	0	0	150-200	4	13.3
	150-200	4	13.3	200-250	4	13.3
	200-250	6	20	250-300	2	6.6

Table 3: Overall Frequency Distribution of Histamine and Tyramine levels in Salted Fish

	Histamine Levels (ppm) (n=90)		Tyramine Levels (ppm) (n=90)	
Range	Frequency	%	Frequency	%
0 (Free)	30	33.3	12	13.3
50-60	26	28.8	0	0
60-70	12	13.3	0	0
70-80	4	4.4	2	2.2
80-100	8	8.8	12	13.3
100-150	0	0	34	37.7
150-200	4	4.4	8	8.8
200-250	6	6.6	6	6.6
250-300	0	0	10	11.1
300-350	0	0	2	2.2

Table 4: Frequency Distribution of Histamine and Tyramine levels in Mackerel, Sardine and Tuna according to Maximum Allowable Levels (MAL)

	Histamine Levels (ppm)			Tyramine Levels (ppm)		
Sample	Range	Frequency	%	Range	Frequency	%
Mackerel (n=30)	< 50 ppm	16	53.3	< 100 ppm	12	40
	> 50 ppm	14	46.7	> 100 ppm	18	60
Sardine (n=30)	<50 ppm	6	20	< 100 ppm	4	13.3
	>50 ppm	24	80	> 100 ppm	26	86.7
Tuna (n=30)	<50 ppm	8	26.7	< 100 ppm	6	20
	>50 ppm	22	73.3	> 100 ppm	24	80

Table 5: Overall Frequency Distribution of Histamine and Tyramine levels in Salted Fish according to Maximum Allowable Levels

	Histamine Levels (ppm) (n=90)		Tyramine Levels (ppm) (n=90)	
Range	Frequency	%	Frequency	%
> 50 ppm	30	33.3	-	-
< 50 ppm	60	66.7	-	-
< 100 ppm	-	-	26	28.9
> 100 ppm	-	-	64	71.1

DISCUSSION

All samples of salted fish were positive for the presence of biogenic amine residues, histamine and tyramine. Levels of tyramine were higher than levels of histamine in all samples. Tuna fish contained the highest histamine levels, then mackerel and sardine while sardine contained the highest tyramine levels, then tuna and lowest levels in mackerel. Histamine contents ranged from 62 to 146 ppm while tyramine contents ranged from 318.5 to 99.5 ppm, these results were lower than results reported by authors; 1220 ppm [15]. Histamine content in allpositive salted fish samples exceeded the maximum allowable level of 50 ppm designed by US FDA [16, 17]. Tyramine content in positive samples also exceeded the maximum allowable level of 100 ppm but not reached the toxic level of 800 ppm [18].

Scombroid fish poisoning is related with the intake of fish from Scombroid family such as tuna, mackerel and bonito [19]. However, certain non-scombroid fish are also implicated in histamine poisoning including blue fish and sardines [20]. Tyramine has been identified as the major mutagen precursor [21]. Biogenic amines accumulation in fish results mainly from decarboxylation activity of bacteria toward free amino acids [22]. Proteolytic bacteria play an important role in releasing amino acids from the protein tissues offering the substrate for decarboxylation activity by amines producing bacteria. Pearson`s correlation analysis of the data revealed a good relationship between the aerobic and proteolytic bacteria counts and biogenic amines contents [23].

Fish are rich sources of high-quality proteins which represents a risk in the decomposition process. The disintegration of proteins yields peptides and amino acids, which are susceptible to further decay, resulting in biogenic amines [24]. Histamine is identified by the Food and Drug Administration (FDA) as the major chemical hazard of seafood. It is the causative agent of scombroid poisoning and is formed by time/temperature abuse of fish muscle [16]. Quality loss and histamine accumulation often occur after frozen fish are thawed and kept for long periods of time at room temperature before canning. Since histamine is heat resistant, it can remain intact in canned fish [25]. Most bacterial isolates isolated from salted fish and secrete decarboxylase enzymes tolerated the salt concentration of up to 15% and temperature of up to 45°C [23]. Both the temperature of storage and the type of meat processing have statistically important effects on the amine content in fish [26]. Fresh fish contained negligible amounts of biogenic amines, upon storage, significant increases in the concentrations of amines were noticed especially between 6 and 12 hours of storage at 30°C, then histamine and tyramine accumulation and cause toxicity [27]. Samples of good and acceptable quality do not contain toxicologically significant concentrations of histamine or tyramine[28].

It seems that the use of poor quality fish as raw material for canning and/or defective handling techniques of fish during processing are the main reasons of this high percentage of unacceptable canned fish samples. Histamine and tyramine production is associated with the growth of bacteria producing decarboxylase enzymes. These bacteria can produce hazardous amounts of histamine in a very short period of time when the fish are kept at elevated temperatures. Fish can contaminated by these bacteria during defrosting handling for processing, from the environment or equipment in the processing plants [29, 30]. A variety of techniques can be combined together to control the microbial growth and enzyme activity during and storage for better shelf life extension and food safety [12]. Secondary control measures to prevent biogenic amine formation in fish or to reduce their levels once formed can be used such as approaches to limit microbial growthas hydrostatic pressures, irradiation, controlled atmosphere packaging, or the use of food additives. Histamine may potentially be degraded by the use of bacterial amine oxidase or amine-negative bacteria [31].

CONCLUSION

High levels of histamine and tyramine in 66.7% and 71.1% of canned salted samples can pose a health risk to consumers, where histamine can cause scombroid fish poisoning and tyramine is a potent mutagen precursor. The presence of high levels of amines in canned salted fish showed that the products lacked necessary hygiene during production. Therefore, quality control programs in the fish canning industries must recommend removing contaminated fish and improving the quality of canned fish.

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