

Hazard of some toxic biogenic amines and improvement the quality of some fish and fish products in alexandria city

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Twenty-five samples of canned fish (tuna and mackerel), frozen fish (mackerel and mazelli) as well as smoked fish (herring); five samples of each were randomly collected from different localities of Alexandria city. Collected samples were subjected to biogenic amine examination. Histamine and Tyramine were determined by HPLC. The highest average value (mg/100g) for histamine was 6.94 (canned tuna) and the lowest was 0.76 (Frozen Mazelli), the respective values for Tyramine were 1.63 (canned tuna) and 0.06 (frozen mazelli) mg/100g. For improvement the quality of raw fish (fresh sardine, 10 kg) during preparation the fresh sardine prior chilling or freezing was dipped into crude potato extract (as protease inhibitor) to reduce biogenic amines production. In order to test the effect of heat treatment on the concentration of the biogenic amines in fish (Frozen mackerel and sardine) were subjected to oven baking at 150°C for 20 min. This showed high reduction in the percentage of biogenic amine production due to heat treatment. The public health significance of the biogenic amines as well as the suggested measures for improving the quality of produced products has been discussed.

Amines, which are particularly important, are histamine and tyramine. Their presence in foods at high levels could be of great public health significance because of their possible involvement in various disorders such as migraine headache, gastric and intestinal ulcer and allergic response (Veciana-Nogues *et al.*, 1989 and Lang *et al.*, 2002).

Biogenic amines in food are formed during storage, spoilage and / or ripening process through degradation of protein by proteolysis together with bacterial action resulting in formation of amino acids as precursors of biogenic amines produced through the decarboxylation process (Ijamah *et al.*, 1991). The major factor leading to formation of biogenic amines are. Availability of free amino acids, presences of microorganisms that can decarboxylase the amino acids and favorable conditions for the growth of such microorganisms and production of decarboxylase enzymes (Pfannhouser and Pechneck, 1984 and El-Mossalami, 2004).

The most important histamine-producing bacteria in fish and fish products were Clostridium species and Enterobacteriaceae specially *Proteus morgani*, *Klebseilla pneum-oniae* and *Hafina alvei* (Sakaba, 1973; Taylor *et al.*, 1979 and Paarup *et al.*, 2002).

Fish have been implicated in most of the outbreaks of histamine poisoning, which the so-called scrombroid fish. The various species of tuna, mackerel, Spanish mackerel and blue fin tuna are included in the scrombroid fish category. The presence of free histidine in the tissues of scrombroid fish leads to production of high histamine levels. Other incidents of histamine poisoning have also been occurred implicating several non-scrombroid fish e.g. sardine, anchovies, herring and salmon (Taylor, 1986 and Moursy, 2001).

In correct handling and storage of processed fish can also introduce histamine-producing bacteria from personal, equipment and the environment. One outbreak was caused the contamination of canned tuna with *Proteus* species during food preparation in a German restaurant (Taylor, 1988 and Erkan *et al.*, 2001).

Histamine poisoning. The onset of symptoms is typically rapid within a few minutes to about 2 hours after eating the affected food. The common symptoms were rash, diarrhea, flushing and sweating, headache and vomiting (Bartholomew *et al.*, 1986 and Mah *et al.*, 2002). Accompanying symptoms may include nausea, burning in the mouth, abdominal pain, dizziness, palpitation and swelling of the mouth and a metallic taste. Severe poisoning has been

observed in patient taking drugs known to inhibit histamine detoxification such as isoniazid and antimalarials.

Histamine produces a range of toxicological effects, which result from its interaction with specific receptors. Histamine causes dilatation of peripheral blood vessels and can directly stimulate the heart resulting in the neurological and haemodynamic symptoms of scombrototoxic syndrome. Histamine produces contraction of the smooth muscles of the intestine and is known to influence gastric acid secretion; both these biological activities may affect the commonly seen gastrointestinal symptoms. Cutaneous symptoms are related to the ability of histamine to directly stimulate sensory and motor neurons (Scoging, 1998 and Paleologos and Kontominas, 2004).

Other amines (Putrescine and Cadaverine) may act as potentiators of histamine toxicity by their inhibition of histamine detoxification. Heat stable of histamine and the lack of organoleptic indicators of histamine contamination added to the difficulties of disease prevention (Morrow *et al.*, 1991 and Lehane and Olley, 2000).

Tyramine and Migraine Headache. Tyramine may be involved in the initiation of migraine headache and hypertension crises in patients on monoamine oxidase therapy (Taylor *et al.*, 1994). The enzyme monoamine oxidase deaminates Tyramine, Tryptamine and phenylethylamine and plays a major role in their degradation in man (Shalaby, 1996). Drugs known as monoamine oxidase inhibitors (MAOI) have been commonly prescribed for the treatment of mental depression and there have been numerous reports of hypertension crises of patients taking MAOI drugs since the pathway for inactivation of amines is blocked. (Hanna *et al.*, 1988 and Lang and Wittmann, 2002).

A significant reduction in the level of biogenic amines was found as a result of precooking of fish. Loss of histamine during cooking (baking or steaming) had no appreciable effect on the residual histamine level of spoiled fish (Santos-Buelg *et al.*, 1986).

The autolysis of fresh fish during storage is due to the proteolytic enzymes in the stomach and intestines. These proteolytic enzymes leak from their tissue compartments and cause proteolysis of the fish proteins (Azudin and Sari, 1990), which are degraded to soluble peptides and free amino acids (Aksnes, 1988). This gives a small physical barrier for the microorganisms to penetrate and a good medium in which they may

grow. The bacterial metabolism decreases the contents of amino acids and produces metabolites such as histamine and cadaverine. Perfect preservation could be achieved by successfully inhibiting of the fish enzymes (Aksnes and Berken, 1988).

Dipping fish into a solution of protease inhibitors from Indian red wood seeds has been found to suppress the production of total volatile nitrogen and trimethylamine nitrogen (Aksnes, 1989 and Paarup, 2002).

The potato extract preserved the contents of total arginine, tyrosine and lysine in the stored herring samples and values of histamine, putrescine, tyramine and cadaverine were reduced. It also gave a somewhat low content of total volatile basic nitrogen and total bacterial count (Azudin and Sari, 1990).

This study was planned to fulfill the following points.

1- Quantitative determination for biogenic amines especially histamine and tyramine in raw fish and fish products sold in Alexandria markets using high performance liquid chromatography (HPLC).

2- Effect of Heat treatment (Oven Baking) on the level of histamine and tyramine in some frozen fish.

3- Improvement the quality of raw fish during preparation by dipping in Crude Potato Extract (as protease inhibitor), which decrease of histamine and tyramine contents during storage .

Material and Methods

Collection of samples. 25 samples of canned fish (tuna and mackerel), frozen fish (mackerel and mazelli) as well as smoked fish (herring); five samples of each were randomly collected from different localities of Alexandria City .

Fresh sardine samples (*Sardinella aureate*) were purchased (about 10 kg) from El-Maadia region. Both frozen fish and fresh sardine samples were transported to the laboratory in ice box.

I. Determination of biogenic amine content .

A- Extraction of Biogenic Amines. Biogenic amines were extracted by using the method previously described by (Mietz and Karmas, 1977 and Gardana *et al.*, 1999).

Ground sample (50 g) was extracted with 5 % trichloroacetic acid (TCA) by 3 x 75 ml using a Warring blender. Each blended mixture was centrifuged and the clear extracts were combined. The volume was adjusted to 250 ml with TCA (5 %) solution. The equivalent of 2 g

of samples as the TCA extract (10 ml was made alkaline by adding 1 ml 50% sodium hydroxide) and then extracted with n-butanol/chloroform mixture (1:1 v/v) 3 x 5 ml. The combined organic phase after addition of an equal amount of n-heptane (15 ml) was extracted with several portions of 0.02N HCl (1ml each), and the aqueous extract was dried by using current of air and water bath at 30°C.

B- Derivative Formation (Lapa-Guimaraes pickova, 2004). The dansyle derivatives of the biogenic amines were formed by adding saturated sodium bicarbonate solution (0.5 ml) to the residue (dry film), stoppered and carefully mixed using vortex mixer, then carefully adding 1 ml dansyle chloride solution (500 mg in 100 ml acetone) and thoroughly mixed. After standing for more than 10 hours at room temperature, the dansyle-amines were extracted by adding 15 ml HPLC grade water and then the mixture was extracted with several portion (5ml each) of diethyl ether (HPLC grade). The combined ether extracts were evaporated to dryness by the aid of current of air and water bath at 35°C. The residue was dissolved in 1ml acetonitrile.

C- Preparation of standard Solution.

Histamine. Aliquot 41.40 mg of histamine dihydrochloride (sigma chemical Co., N. 7505) was dissolved in 50 ml water HPLC grade. (Stock solution 0.5mg/ml).

Tyramine. Aliquot 31.39 mg of tyramine (4-hydroxyphenylethylamine) hydrochloride (N, T-2879) was dissolved in 50 ml water HPLC grade. (stock solution 0.5mg/ml). 200 µl of each stock standard solution was transferred to glass tube (using micropipette), then evaporated using current of air. The residue was subjected to dansylation as described above. The residue was dissolved in 5 ml acetonitrile. (1ml = 20 µg or 10 µl = 0.2 µg each amine as derivative).

D- Detection (Takagi and Shikata, 2004). Shimadzu HPLC was used for the quantitative estimation of biogenic amines. The conditions used as follows:

Mobile solvent: Solvent A: acetonitrile: 0.02 N acetic acid (1: 9 v/v).

Solvent B: 0.02 N acetic acid: acetonitrile: methanol (2: 9: 9 v/v/v).

Program: gradient program 60% solvent B in solvent A to 100% solvent B using linear program over 30 min period and 1 ml constant flow rate.

Detector: UV-Vis at 254 nm.

Column: Reversed phase, C 18 Shim Pack. CLC. ODS, 0.15 m x 6.0.

Injection. 10 µl of standard solution (as derivative) or sample was injected into HPLC apparatus.

II. Effect of Heat treatment on the level of Histamine and Tyramine.

Fish products (frozen mackerel and sardine) were prepared as described before, then subjected to extraction and analysis of histamine and tyramine. Each sample was subjected to baking at 150°C for 20 min and after baking treatment was subjected to analysis of histamine and tyramine as described before.

III- Preparation of Proteolytic Inhibitors from Crude Potato Extract.

Crude potato extract was prepared according to the method of Aksnes (1989) as follows:

Extract of proteolytic inhibitor was obtained by grinding whole potato tubers with skin using a rotating knife. The liquid fraction was centrifuged for 20 min at 10000-x g. The supernatant was used as a source of proteolytic inhibitors.

Fish treatment. Fresh sardine were divided into two batches. The first batch (5 kg) was treated by dipping the fish in the potato extract for 10 min, then drained (Gowda and Karunasagas, 1985). The second batch was left without treatment. Both treated and untreated sardine samples were kept in polyethylene bags. The two batches (treated & untreated) were then divided into two groups. The first group was stored in a deep freezer at -18°C for 56 days and the other one was stored in a refrigerator at 4°C for six days. Chilled fish was subjected to periodically analysis each two days, while frozen one was subjected to analysis each 14 days.

Results and discussion

From the data in Table (1) it could be observed that histamine concentration was the highest in canned tuna and smoked herring followed by canned mackerel and finally frozen fish (mackerel and mazelli).

With respect to tyramine, a relatively high level was detected in tuna samples. However, in the other investigated samples, Tyramine was very low.

The high level of histamine in canned tuna and canned mackerel could be related to belonging of these species of fish to scrombroid fish category, which contain free histidine in their tissue (Lemke, 1982 and Erkan, 2001).

Table (1): Histamine and tyramine content in imported fish and fish products.

Type of sample	Histamine (mg/100g)		Tyramine (mg/100g)	
	Range	M ± SD	Range	M ± SD
Canned tuna	0.02 – 18.36	6.94 ± 1.69	0.22 – 8.36	1.63 ± 0.53
Canned mackerel	0.13 – 11.58	3.88 ± 0.79	0.0 – 0.30	0.06 ± 0.02
Frozen Mackerel	0.11 – 6.06	1.57 ± 0.41	0.0 – 0.35	0.09 ± 0.03
Frozen Mazelli	0.01 – 3.22	0.76 ± 6.21	0.0 – 0.30	0.06 ± 0.02
Smoked herring	0.03 – 19.10	6.36 ± 1.99	0.0 – 1.78	0.51 ± 0.53

Table (2): Effect of Crude Potato Extract (as protease inhibitor) on histamine and tyramine contents (mg/100g) of fresh sardine stored under freezing conditions (-18°C).

Storage time (days)	Histamine (mg/100g)		R (%)	Tyramine (mg/100g)		R (%)
	Untreated	Treated		Untreated	Treated	
0	0	0	0	0	0	0
14	1.57 ± 0.41	0.91 ± 0.33	42.04	0.48 ± 0.13	0.33 ± 0.04	31.25
28	1.94 ± 0.42	1.54 ± 0.12	20.6	0.67 ± 0.12	0.51 ± 0.53	23.88
42	2.21 ± 0.11	1.63 ± 0.53	26.24	0.96 ± 0.21	0.76 ± 0.21	20.8
56	3.00 ± 0.72	2.15 ± 6.78	28.33	1.18 ± 0.09	0.95 ± 0.25	19.50

Table (3): Effect of Crude Potato Extract (as protease inhibitor) on histamine and tyramine contents (mg/100g) of fresh sardine stored under refrigeration conditions (4°C).

Storage time (days)	Histamine (mg/100g)		R (%)	Tyramine (mg/100g)		R (%)
	Untreated	Treated		Untreated	Treated	
0	0	0	0	0	0	0
2	1.34 ± 0.05	0.44 ± 0.10	67.74	0.71 ± 0.29	0.06 ± 0.02	91.55
4	2.51 ± 0.71	1.31 ± 0.02	47.81	1.06 ± 0.28	0.72 ± 0.04	32.08
6	4.34 ± 0.06	2.08 ± 0.17	52.07	1.27 ± 0.02	0.92 ± 0.06	27.55

Table (4): Effect of heating treatment (oven baking) on the level of histamine and tyramine (mg/100g) in raw fish .

Product	Histamine			Tyramine		
	Before heating	After heating	R (%)	Before heating	After heating	R (%)
Frozen Mackerel	6.36 ± 1.71	4.02 ± 0.07	36.79	0	0	0
Frozen Sardine	6.94 ± 1.69	5.26 ± 1.99	24.21	0	0	0

On the other hand, tuna would be netted in water of approximately 29°C and the fish must be held in the net matter of minutes or hours. The fish would then be transferred to well containing refrigerated sea water (-1°C) and could remain at this temperature for 2-21 days until the well is filled and then subjected to frozen. Tuna are often stored frozen for several months before thawing, evisceration and processing (Behling and Taylor, 1982). This could be also another reason of such elevated biogenic amines in canned tuna. The detected

histamine level in canned tuna was ranged from 0.02 to 18.36 mg/100g (Table.1), which is higher than the levels of (1.60 – 7.41 and 1.87 – 6.30 mg / 100 g), which reported by Taylor *et al.*, (1978) and Ali (1985), but lower than 30.39, 66.50 and 18.7 mg/100g as average concentrations of histamine in canned tuna stated by (Middlbrook *et al.*, 1988; Negendra and Indrani-karusagor, 1990 and Tasi, *et al.*, 2005). Similar results were recorded by Gajewska *et al.*, (1991), which ranged from 0.0 – 16.0 mg/100 g.

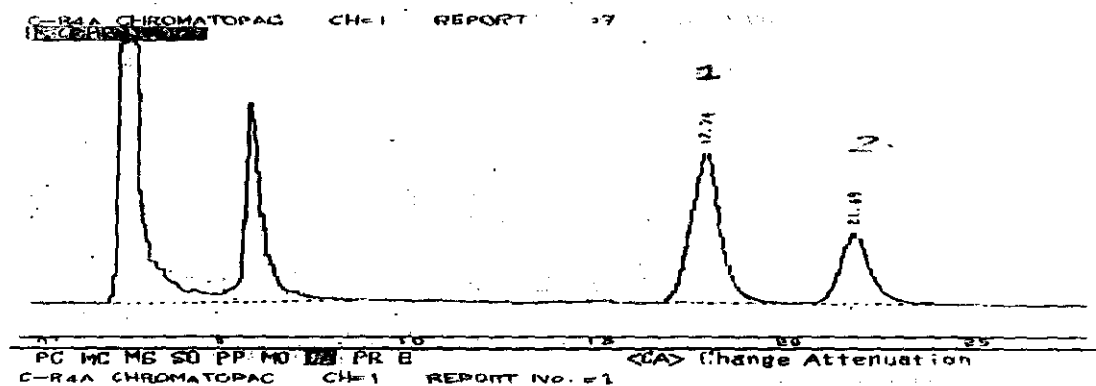


Fig. (1): HPLC-Chromatogram of standard solution consisting of 2 selected biogenic amines.

Peak Results

	Name	Ret. time (min)	Area (UV sec)	Concentration	Height (UV)	Int. Type
1	Hist	17.74	979578	15.5969	18022	TV
2	Tyr	21.69	495669	7.8921	8545	TV

Hist (histamine), Tyr. (tyramine)

As for tyramine, it was ranging between (0.22 – 8.36 mg/100g) (Table, 1) in canned tuna, which is higher than the level of 0.0 to 3.35 mg / 100g that reported by Ali (1985). Similar results were obtained by Gajewska *et al.*, (1991), which ranged from 0.0 – 10.0 mg/100g.

Histamine content in canned mackerel was in the range of 0.13 – 11.58 mg / 100g. Ali, (1985), reported a relatively high range of histamine in canned mackerel 2.0 – 16.20 mg/100 g, but low level of histamine with average 2.05 and 3.70 mg/100g respectively were reported by Taylor *et al.* (1978) and Ababouch *et al.* (1988).

The highest levels of histamine in canned mackerel (153.9 mg/100 g) were obtained by Tasi *et al.* (2005). Tyramine was very low in all the studied samples of canned mackerel while it was reported to reach 0.6 mg/100g as a mean level of 42 samples of canned mackerel Veciana-Nogues, *et al.* (1989).

The variable concentration of amines noticed in the studied sample may be related to the differences in the quality of raw materials used before canning process. Since, Biogenic amines are thermally stable and canning has no effect on the contents of such amines (Tsai *et al.*, 2005).

In frozen mackerel, it can be noticed from the result that frozen mackerel contained a relatively higher level of histamine and tyramine than that recorded in frozen mazelli. This could be attributed to the belonging of mackerel to the scrombroid fish family, while mazelli fish is

non-scrombroid fish (Davidek and Davidek, 1985, and Lapa-Guimaraes and Pickova, 2004).

Frozen mackerel recorded histamine concentration in the range of 0.11 – 6.02 mg/100g (Table 1), while mazelli recorded 0.01 – 3.22 mg/100g. Khalafala, (1993), reported nearly the same level of histamine in frozen mackerel 2.5 mg/100g, while Ali, (1985), reported histamine level in frozen tilapia (non scrombroid fish) in the range of 1.35 – 1.75 mg/100g. Higher results was recorded by Gajewska *et al.* (1991) were (0:0 – 8.0 mg/100g). On the other hand, traces of tyramine were detected in frozen fish (mackerel and mazelli) in the present investigation. The same traces level of tyramine was also obtained in frozen bouri and tilapia fish in the study conducted by Ali, (1985). High results were recorded by Gajewska *et al.* (1991) which ranged from 0.0 – 2.6 mg/100g.

Variable concentration of histamine and tyramine in frozen fish could be due to the quality of raw fish material and the delay of fish freezing. (Venugopal, 2002).

In smoked herring, the level of histamine and tyamine were ranged between 0.03 – 12.10 and 0.0 – 1.78 mg/100g; respectively. Lower level of both amines were reported in an Egyptian survey on smoked herring ranging of 0.0 – 1.6 mg histamine/100g and 0.0 – 0.8 mg tyamine/100g; respectively (Shalaby, 1995).

In another study in Spain, the mean histamine and tyamine levels of 48 samples of smoked herring were 0.84 and 0.20 mg/100g; respectively (Veciana-Nogues *et al.*, 1989).

The wide variation of histamine and tyramine in smoked herring could be attributed to the quality of raw material and the used method of smoking (cold or hot) (Silva *et al.*, 2002 and Venugopal, 2002). The Food and Drug Administration (FDA, 1996) established a defect action level for histamine in fish (an amount that signifies some mishandling of the fish) at a concentration of 5 mg/100g and the hazard action level (the amount constituting a known human health hazard) at a concentration of 20mg / 100g. Accordingly, it appears that the histamine level in all the investigated fish samples was lower than the hazard level, while its level in canned tuna, canned mackerel as well as smoked herring was above the defect action level (5mg/100g). It could be predicted that consumption of 136 g canned tuna and 167 g canned mackerel may reach the hazard level, while very large amounts that an individual can not consume from other studied samples may reach the hazard level. As for tyramine, none of the investigated samples reached the level of 6 mg, which was reported to be a dangerous dose for patients receiving MAOI (Blackwell and Mabbit, 1965 and Lang and Wittmann, 2002).

Crude Potato Extract As Protease Inhibitor.

The data tabulated in (Tables 2, 3) summarized the effect of crude potato extract on the level of histamine and tyramine in fresh sardine during storage at -18°C (freezing condition) and 4°C (Refrigeration condition). The data revealed that sardine stored at -18°C had lower level of both amines than that stored at 4°C. This may be due to the sub-lethally injures effect of freezing on histamine-producing bacteria. These results are in agreement with those reported by Barawaskii *et al.* (1990), who mentioned that frozen storage of fresh mahi-mahi at -20°C inhibited histamine formation, but did not affect the extent of quality loss. The level of histamine in fresh sardine stored at both temperatures (4°C and -18°C) was below the defect action level (5mg /100g) according to the limit established by FDA, (1996). Tyramine level in all the studied samples was below the level 6mg, which has been reported to cause health problem in patients receiving MAOI drugs (Blackwell and Mabbit, 1965 and Coisson, *et al.*, 2004). On the other hand, the highest tyramine level among all the studied samples was 1.31mg/100g, therefore, consumption of 458 g of sardine fish (large Aksnes, A. (1989): Effect of proteinase inhibitors from potato on the quality of stored herring. *J. Sci. Food Agric.*, 49: 225-234

amount for an individual to consume) may be sufficient to reach the 6 mg of tyramine capable of producing health problem.

Effect of Heat Treatment (Oven Baking) on Histamine and Tyramine Levels in Some frozen fish.

It could be noticed from the data of (Table 4) that heating had apparent effect on the reduction of both amines in some raw fish samples (mackerel and sardine). In fish samples, frozen mackerel recorded higher reduction percentage (33.51%) than that of frozen sardine (28.29%). Similar results since they concluded that the subsequent heat processing of canned products significantly lowered histamine and putrescine. By analogy, it was reported that biogenic amine production was reduced due to precooking of fish (baking or steaming) to a temperature 160-180.

As for the effect of heat treatment on the concentration of the Biogenic amines, it could be noticed from the result that fish products showed high reduction percentage due to heat treatment (oven baking). This could be explained by the high release of such amines as soluble substances in fish samples (Sekiguchi *et al.*, 2004).

The key to keeping bacterial numbers and biogenic amine levels low is the rapid cooling of fish after catching and the maintenance of adequate refrigeration during handling and storage. Despite the huge expansion in trade in recent years, great progress has been made in ensuring the quality and safety of fish products. This is largely the result of the introduction of international standards of food hygiene and the application of risk analysis and hazard analysis and critical control point (HACCP) principles. Heat treatment had apparent effect on the reduction of biogenic amines in some raw fish. Potato extract could be used for treating raw fish prior chilling or freezing to reduce biogenic amine production. Fish products subjected to curing, aging or smoking should be avoided for sensitive people (bronchial asthma) hypotension or patients used MAOI drugs.

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