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Estimation of Biogenic Amines in some Fresh and Processed fish commonly consumed in Egypt

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Abstract : Biogenic amines are nitrogenous compounds that occur naturally in wide variety of food. Although the biogenic amines play some important physiological functions but high level of amines can cause toxicological effects. Biogenic amines can be produced by bacteria during amino acids decarboxylation and have been identified as one of the important agent causing seafood intoxication. Therefore, this study was carried out to estimation of biogenic amines levels in some fresh fish and processed fish products commonly consumed from Egyptian consumers by using HPLC. Freshwater fish; catfish (*Clarias gariepinus*), common carp (*Cyprinus carpio*), marine fish; mullet (*Mugil cephalus*), greasy grouper (*Epinephelus tauvina*) and processed fish products; feisiekh (*Mugil cephalus),* salted sardine (*Sardina pilchardus*), smoked herring (*Clupea harangues pallasi)* and canned mackerel (*Scomber scombrus*) were collected from fish markets in El-Kanater El-Khairia city, Egypt. According to the results of this study, the lowest values of biogenic amines were found in fresh fish especially freshwater fish samples compared with processed fish samples. Cadaverine, putrescine and spermine were detected in all collected fish samples. Histamine was detected in marine fish and processed fish samples, while not detected in freshwater fish samples. B-phenyl ethylamine, tryptamine and tyramine were detected in some investigated fresh fish and processed fish samples and not detected in another samples. Therefore, fresh catfish contains high values of putrescine (5.43 mg/kg), fresh common carp a high value of spermine (3.41 mg/kg), fresh mullet fish a high value of spermine (1.69 mg/kg), fresh greasy grouper a high value of cadaverine (11.12 mg/kg), feisiekh (fermented mullet fish) a high value of histamine(21.29 mg/kg), smoked herring a high value of putrescine (16.12 mg/kg), salted sardine was recorded a high value of putrescine (4.97 mg/kg) and canned mackerel high value of spermine (1.71 mg/kg). However, histamine and another detected amines values in investigated collected fresh fish and processed fish samples were very below the allowable limit, where the maximum permissible limit of histamine set by FDA (50 mg/kg), EOS 1996 (200 mg/kg), Commission Regulation (EU, 200 mg/kg), SABS (South African Bureau of Standard, 100 mg/kg) and AFSC (Australian Food Standard Code, 200 mg/kg). Thus, the results of this study are consistent with international references and the maximum levels of histamine in fish and processed fish products (0.49-21.29 mg/kg) is an indicator of high freshness and shelf life, which proves that fish were fresh, as raw materials, and manufacturing process was conducted properly and the recommendation, therefore all the examined samples were considered as safe for human consumption. So, in order to prevent or reduce the formation of histamine on fish and fishery products, the rapid cooling of fish after catching and the maintenance of adequate refrigeration during handling and rapidity processed or frozen storage is recommended.

Keywords: Biogenic amines; freshwater fish; marine fish; feisiekh; salted; smoked; canned fish.

Introduction

Fish is a source of easily digestible proteins and many of them are a rich source of polyunsaturated fatty acids (PUFAs) which have positive influences on the human cardiovascular and immune system moreover, it is a good source of minerals and vitamins [1]. However, it is one of the most highly perishable foods, which spoils soon after death, if not preserved properly [2]. Fish is highly susceptible to biogenic amine formation especially histamine, putrescine, cadaverine and tyramine and high levels of biogenic amines found in seafood are caused by inadequate preservation with consequent microbial decarboxylation of amino acids [3].

Biogenic amines are low molecular weight organic bases with biological activity that are formed in foods by microbial decarboxylation of the corresponding amino acids or by transamination of aldehydes and ketones by amino acid transaminases [4]. High amounts of some amines may be found in food as a consequence of the use of poor quality raw materials, contamination and inappropriate conditions during food processing and storage, in this respect, the quantity of biogenic amines is supposed to be a marker for microbiological contamination level in the food [5]. The consumption of foods containing high amounts of biogenic amines may cause problems such as headaches, nausea, hypotension, hypertension, cardiac palpitation, etc.[6].

Therefore, the presence of biogenic amines in fish are important from health and toxicological perspective since the consumption of fish or other products containing amines has been associated with some case of food poisoning. Thus, biogenic amines may be hazardous to human health if their levels in foods or beverages reach a critical threshold [7]. At any time, exposure of fish to elevated temperatures after the catch and before consumption can cause formation of histamine from histidine by bacterial histidine decarboxylases, where the low levels of biogenic amines in food are not considered a serious risk; however, when consumed in excessive amounts, they may cause distinctive pharmacological, physiological and toxic effects [8].

Histamine is potentially hazardous and the causative agent of histamine intoxication associated with the consumption of seafood [9]. Moreover, histamine levels in freshly caught fish are generally very low, usually below 0.1mg/100g [10]. Besides histamine, such secondary amines as putrescine and cadaverine are good indices of spoilage of marine fish [11]. Therefore, Food and Drug Administration (FDA) has set the maximum action level of histamine as 50 mg /Kg for fish [12] while, a level of 200 mg /Kg of food was ruled by the European Community[13] that established in Germany. Also, 100 mg /Kg of food was accepted in Canada, Finland and Switzerland. In Egypt, Egyptian Standard Specifications [14, 15, 16] evident the maximum permissible limit of histamine content must not exceed 200 mg/kg for salted, smoked and frozen fish products. In the same trend, a maximum limit of total biogenic amines (BAs) levels of 750–900 mg/kg in foods has been proposed by [7]. The European Food Safety Agency [17] has established a daily maximum intake of 50 mg of histamine and 600 mg of tyramine in foods for healthy adults.

High potential risk for human health can be caused by miss handling of fresh fish or by traditionally processed fish products as results of halophilic pathogenic bacteria and biogenic amines bacteria [18].Various biogenic amines when subjected to heat, can give rise to the formation of secondary amines and in the presence of nitrites, these can generate nitrosamines, chemical agents considered to possess major carcinogenic properties [19]. Also, some technological processes such as salting, ripening, fermentation or marinating can increase the possibility of formation of biogenic amines, as well as during storage of the final product if improper holding temperatures are employed [20]. In addition, feisiekh is the traditional name for the salted-fermented bouri fish (*Mugil cephlus*) produced in Egypt. It is popular not only as an appetizer, but also as the main dish at some feasts in Egypt [21]. There are two types of feisiekh on the Egyptian markets, the first type having a low salt content and being suitable for consumption after 15-20 days of maturing, whilst the second has a high salt content and can be eaten after 2-3 months of storage. From the nutritional point of view, Feisiekh is a rich source of high quality protein, essential amino acids, vitamins, and minerals[22].

Therefore, the objective of the current study was to estimation the level of contamination by biogenic amines causing histamine poisoning in fresh fish and processed fish products available commercially in Egyptian fish markets and to estimation the level of safety for the Egyptian consumers.

Materials and methods

Collection of fish samples

A total of 80 fish samples of freshwater fish; catfish (*Clarias gariepinus*), common carp (*Cyprinus carpio*) and marine fish; mullet (*Mugil cephalus*), greasy grouper (*Epinephelus tauvina*) were used as fresh fish. Fish products; feisiekh (*Mugil cephalus)*, salted sardine (*Sardina pilchardus*), smoked herring (*Clupea harangues pallasi)* and canned mackerel (Scomber scombrus) (10 of each) were collected from fish markets at various localities in El-Kanater El-Khairia city, Egypt during May, 2016 by using two ice box container and transported to Fish Processing Technology Laboratory, National Institute of Oceanography and Fisheries, El-Kanater El-Khiria, Egypt. Upon arrival fresh, fermented, salted and smoked fish samples were beheaded, gutted, skins and bones were removed, washed with tap water, filleted and mixed to get the homogenized mixture of edible tissue sample from each fish or product. Imported 10 cans of canned mackerel samples were collected also from various markets from the same company namely sunshine, Thailand, total weight 350g of each can, after opening each can, oil was drained off and meat was ground in a food blender and then were mixed well in order to take also homogenized mixture from the different parts of the fish. The prepared samples were stored immediately at -18°C in deep freezer until biogenic amine analysis.

Determination of Biogenic Amines

Seven biogenic amines included histamine, tyramine, tryptamine, cadaverine, putrescine, spermine and β-phenylethylamine were estimated in both fresh and processed fish samples at The National Research Center, Dokki, Cairo, Egypt by using HPLC (High performance liquid chromatography) according to [11, 23]. The results were expressed as mg/kg wet weight of sample.

Statistical analysis

All measurements of biogenic amines were performed in triplicate for each fresh fish or processed fish product, and the mean values ± standard deviation was reported for each case.

Results and discussion

The study was carried out to survey and investigate the concentrations (mg/kg) of biogenic amines (histamine, tyramine, tryptamine, cadaverine, putrescine, spermine and β-phenylethylamine) in some Egyptian fresh fish and processed fish products as freshwater fish; catfish and common carp, marine fish; mullet and greasy grouper, processed fish products; feisiekh, salted sardine, smoked herring and canned mackerel fish and the results are shown in (Table 1) and illustrated in figures (1-8).

The results in Table 1 showed that there were variations between all samples regarding to biogenic amines contents depended on the fish species or kind of processed fish product. Generally, fresh fish samples recorded the lowest values of biogenic amines compared with processed fish products, also fresh freshwater fish recorded lowest values of biogenic amines compared with marine fish except spermine was higher in fresh freshwater fish than marine fish and processed fish products. Cadaverine, putrescine and spermine are predominant amines in all collected fish samples and the highest values were recorded (12.08, 16.12 and 3.49 mg/kg) in fresh greasy grouper, smoked herring and fresh catfish, respectively.

Also, it can be seen that feisiekh product contains a relatively higher concentrations of histamine (21.29 mg/kg), followed by salted sardine (1.69 mg/kg), canned mackerel (0.62 mg/kg), fresh greasy grouper (0.52 mg/kg), smoked herring (0.49 mg/kg) and fresh mullet fish (0.13 mg/kg), while it could be not detected in fresh catfish and common carp. A higher content of tryptamine was found in fresh greasy grouper (2.28 mg/kg) and feisiekh product (2.09 mg / kg), while not detected in another investigated fish samples. Tyramine was detected by lowest concentrations in fresh common carp (0.17 mg/kg), feisiekh (0.25 mg/kg) and smoked herring (0.12 mg/kg). Also, B-phenylethylamine was recorded a low value that 1.3, 0.99 and 0.18 mg/kg in fresh common carp, smoked herring and salted sardine, respectively.

Therefore, the present data revealed that fresh catfish contains high values of putrescine followed by spermine and cadaverine (Fig 1), fresh comman carp contains high values of spermine followed by putrescine, β-phenylethylamine, tyramine and cadaverine (Fig 2), fresh mullet fish recorded a high value of spermine

followed by cadaverine, putrescine and histamine (Fig 3), fresh greasy grouper contains a high value of cadaverine followed by putrescine, tryptamine, histamine and spermine (Fig 4), feisiekh (fermented mullet fish) recorded a high value of histamine followed by cadaverine, putrescine, spermine, tryptamine and tyramine (Fig 5), smoked herring was recorded a high value of putrescine followed by cadaverine, β-phenylethylamine, spermine and tyramine(Fig 6), salted sardine was recorded a high value of putrescine followed by histamine, spermine, cadaverine and β-phenylethylamine (Fig 7) and canned mackerel recorded high value of spermine, followed by putrescine, histamine and cadaverine(Fig 8). Therefore, it should be noted that the production of amines in fish muscles or processed fish products can be vary according to the muscle type (white or dark), different slices (in area of tail or near it) and environment temperature, fishing season and fish size, rate of pollution, bacterial infestation of fish and fish processing method [24, 25] .

Table 1Biogenic amines levels (Mean ± SD) of some fresh fish and processed fish products marketed in Egypt

Fish samples	**Biogenic amines (mg/kg)**							**Total BAs**
	TRY	**B-PHE**	**PUT**	**CAD**	**HIS**	**TYR**	**SPM**	
Fresh fish								
Fresh water fish:								
Catfish	ND	ND	5.43 ±1.05	2.40 ± 0.31	ND	ND	3.49 ± 0.88	11.32
Common carp	ND	1.30 ± 0.35	1.81 ± 0.22	0.13 ± 0.06	ND	0.17 ± 0.01	3.41 ± 0.51	6.82
Marine fish:								
Mullet fish	ND	ND	0.70 ± 0.23	0.88 ± 0.08	0.13 ± 0.01	ND	1.69 ± 0.28	3.40
Greasy grouper	2.28 ± 0.65	ND	11.12 ± 1.66	12.08 ± 1.08	0.52 ± 0.10	ND	0.23 ± 0.06	26.23
Processed fish products								
Feseekh*;* Mullet fish	2.09 ± 0.12	ND	9.39 ± 0.92	10.19 ± 1.21	21.29 ± 1.80	0.25 ± 0.03	2.98 ± 0.20	46.19
Smoked herring	ND	0.99 ± 0.13	16.12 ± 2.03	2.87 ± 1.05	0.49 ± 0.04	0.12 ± 0.00	0.65 ± 0.22	21.24
Salted sardine	ND	0.18 ± 0.05	4.97 ± 1.10	0.23 ± 0.02	1.69 ± 0.30	ND	0.49 ± 0.12	7.56
Canned mackerel	ND	ND	0.78 ± 0.11	0.46 ± 0.08	0.62 ± 0.16	ND	1.71 ± 0.60	3.57

ND: Not detected; TRY: Tryptamine; B-PHE: B- Phenylethylamine; PUT: Putrescine;
CAD: Cadaverine; HIS: Histamine, TYR: Tyramine; SPM: Spermine.

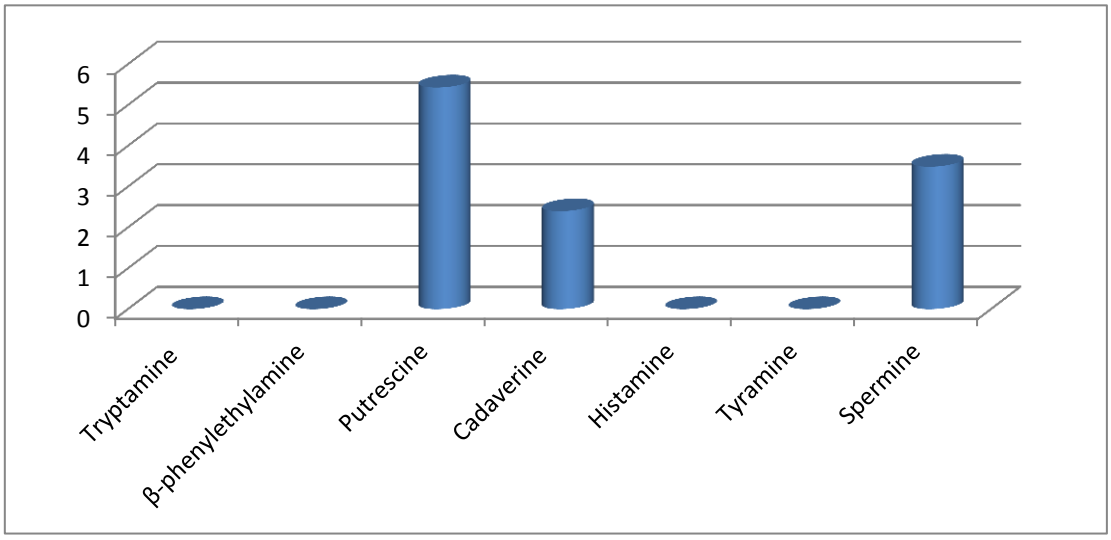

Fig. 1 Biogenic amine contents (mg/kg) in fresh catfish

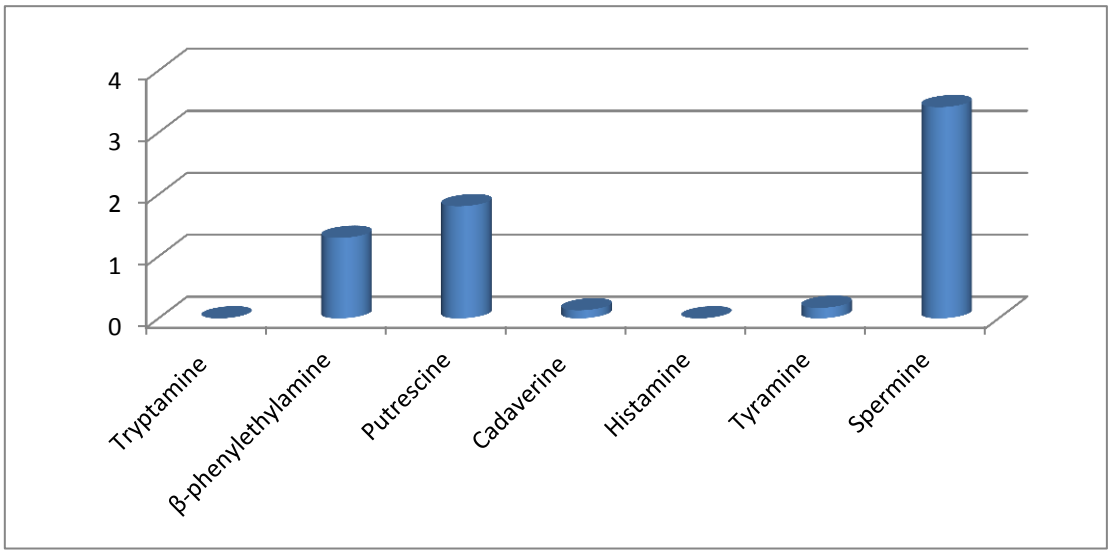

Fig. 2 Biogenic amines contents (mg/kg) in fresh common carp

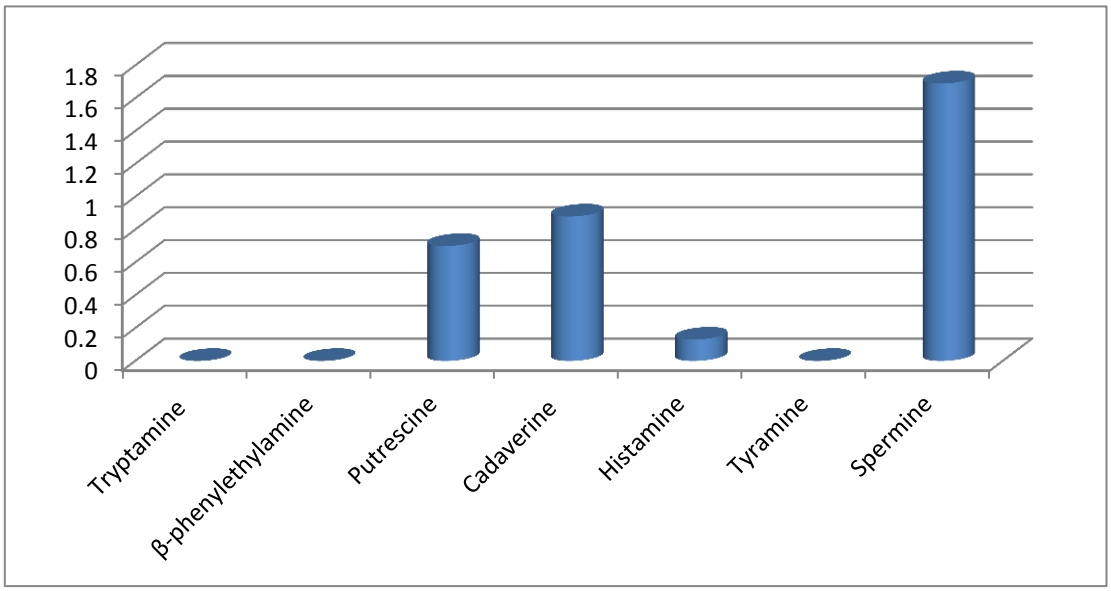

Fig. 3 Biogenic amines contents (mg/kg) in fresh mullet

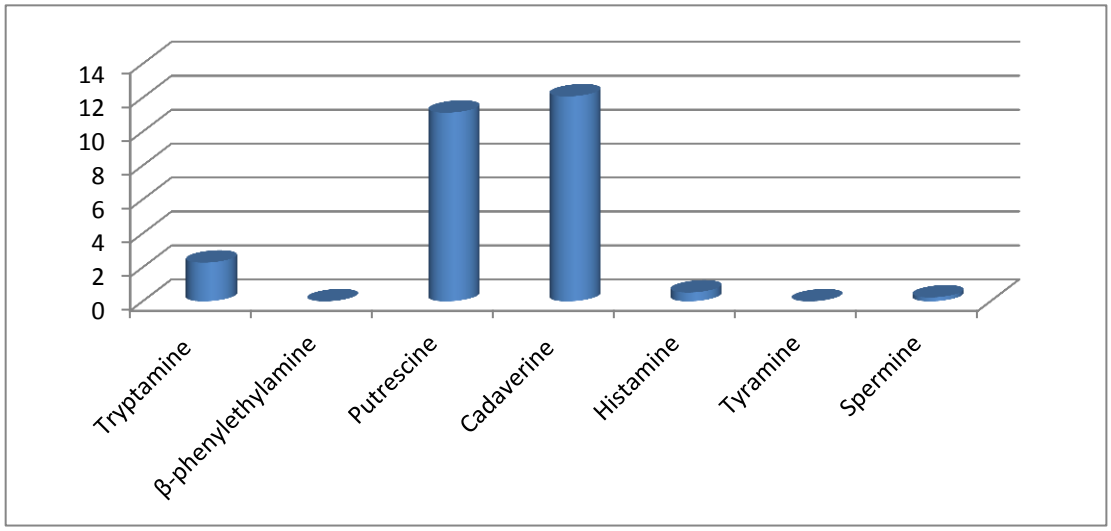

Fig. 4 Biogenic amines contents (mg/kg) in fresh greasy grouper

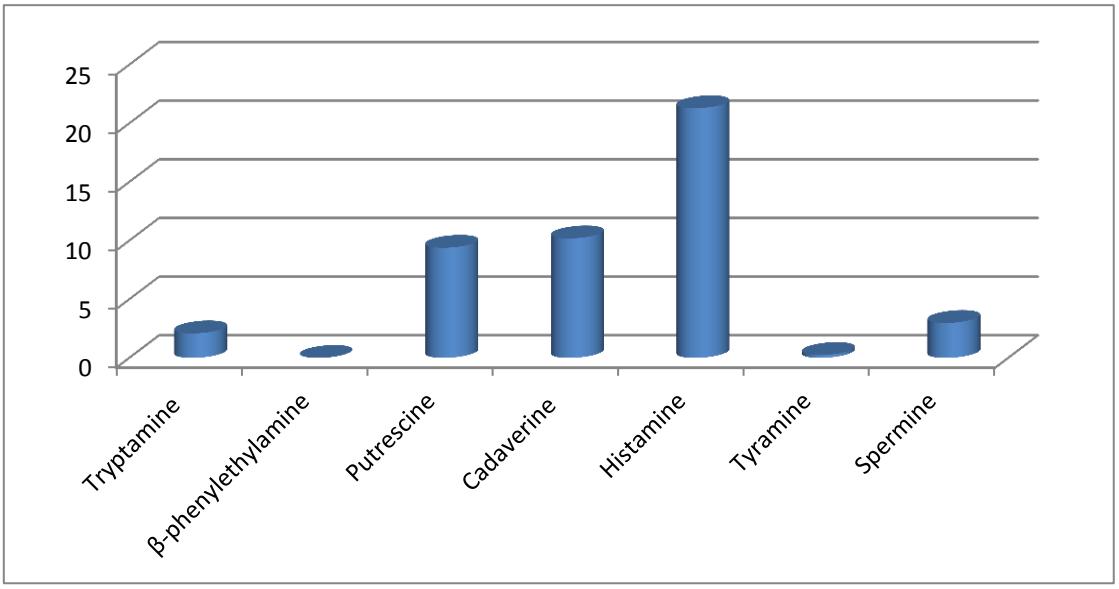

Fig. 5 Biogenic amines contents (mg/kg) in feisiekh

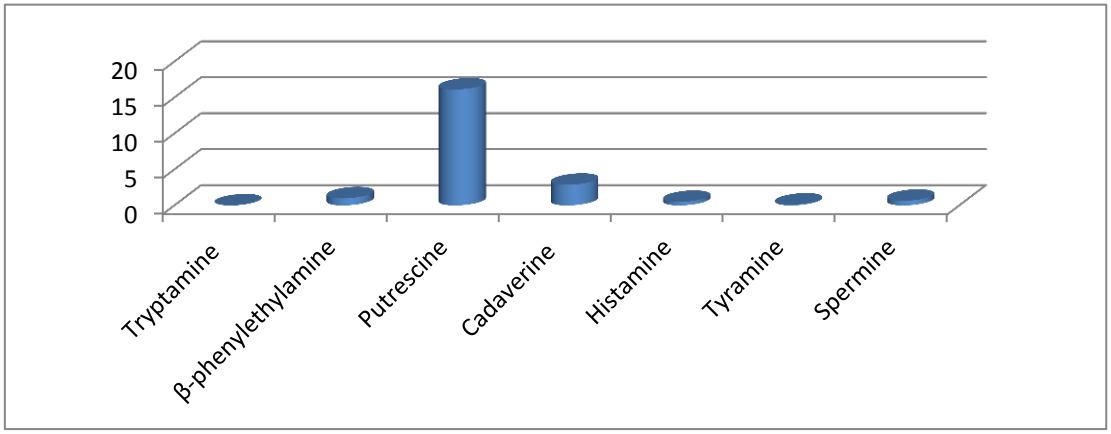

Fig. 6 Biogenic amines contents (mg/kg) in smoked herring

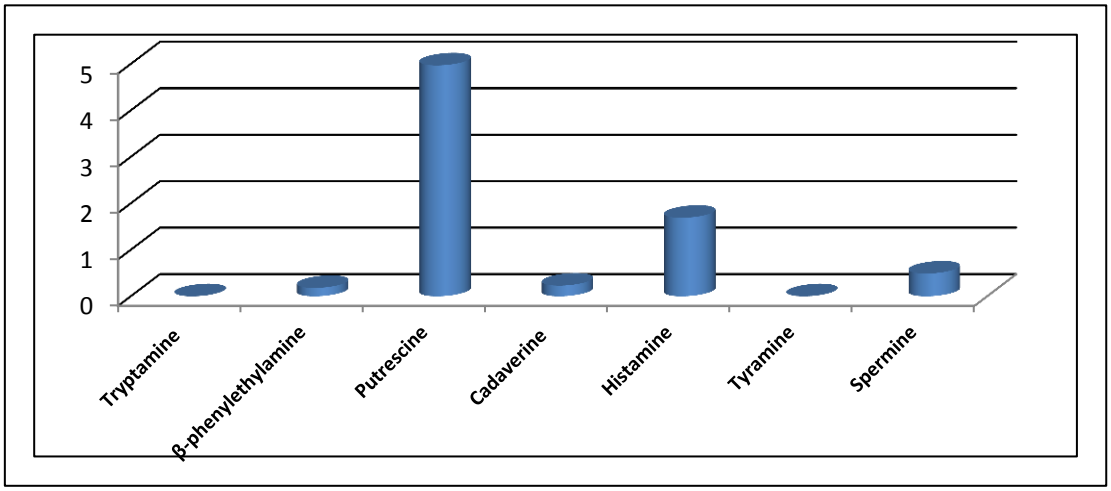

Fig. 7 Biogenic amines contents (mg/kg) in salted sardine

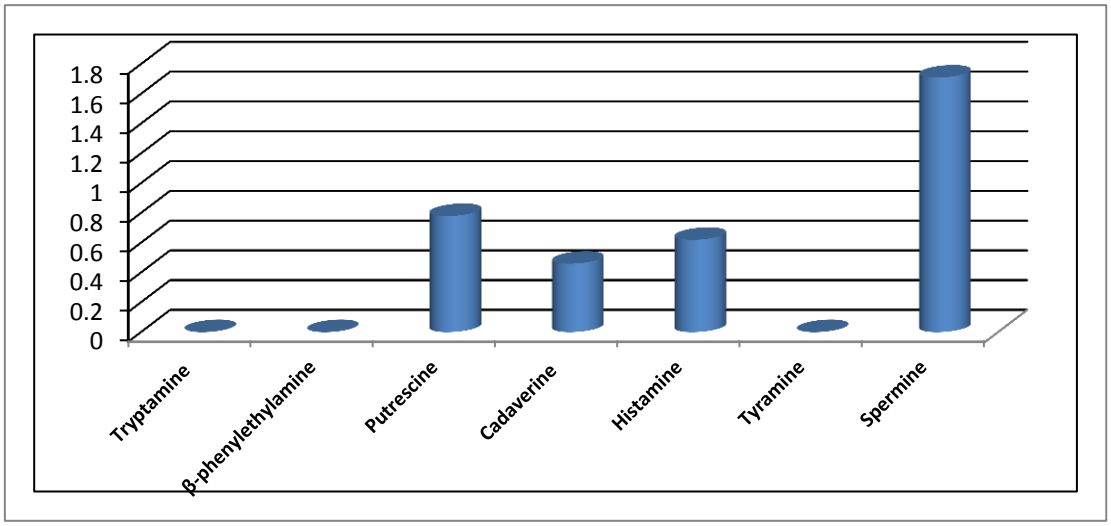

Fig. 8 Biogenic amines contents (mg/kg) in canned mackerel

Also, it can be seen that feisiekh product contains a relatively higher concentrations of histamine (21.29 mg/kg), followed by salted sardine (1.69 mg/kg), canned mackerel (0.62 mg/kg), fresh greasy grouper (0.52 mg/kg), smoked herring (0.49 mg/kg) and fresh mullet fish (0.13 mg/kg), while it could be not detected in fresh catfish and common carp. A higher content of tryptamine was found in fresh greasy grouper (2.28 mg/kg) and feisiekh product (2.09 mg / kg), while not detected in another investigated fish samples. Tyramine was detected by lowest concentrations in fresh common carp (0.17 mg/kg), feisiekh (0.25 mg/kg) and smoked herring (0.12 mg/kg). Also, B-phenylethylamine was recorded a low value that 1.3, 0.99 and 0.18 mg/kg in fresh common carp, smoked herring and salted sardine, respectively.

Therefore, the present data revealed that fresh catfish contains high values of putrescine followed by spermine and cadaverine (Fig 1), fresh comman carp contains high values of spermine followed by putrescine, β-phenylethylamine, tyramine and cadaverine (Fig 2), fresh mullet fish recorded a high value of spermine followed by cadaverine, putrescine and histamine (Fig 3), fresh greasy grouper contains a high value of cadaverine followed by putrescine, tryptamine, histamine and spermine (Fig 4), feisiekh (fermented mullet fish) recorded a high value of histamine followed by cadaverine, putrescine, spermine, tryptamine and tyramine (Fig 5), smoked herring was recorded a high value of putrescine followed by cadaverine, β-phenylethylamine, spermine and tyramine(Fig 6), salted sardine was recorded a high value of putrescine followed by histamine, spermine, cadaverine and β-phenylethylamine (Fig 7) and canned mackerel recorded high value of spermine, followed by putrescine, histamine and cadaverine(Fig 8). Therefore, it should be noted that the production of amines in fish muscles or processed fish products can be vary according to the muscle type (white or dark),

different slices (in area of tail or near it) and environment temperature, fishing season and fish size, rate of pollution, bacterial infestation of fish and fish processing method [24, 25] .

According to the above mentioned conclusion, generally our data showed that all detected amines in all the investigated fresh fish and processed fish products samples were much lower than the hazard levels and results of some authors [26, 27], histamine that potentially hazardous not detected of fresh catfish and common carp, the low values of histamine that found in the fresh greasy grouper (0.52 mg/kg) and fresh mullet fish (0.13 mg/kg) may be due to this fish species belonging marine fish [28] or to the mass handling by exposure the fish to elevated temperatures after the catch and before consumption can cause formation of histamine from histidine by bacterial histidine decarboxylases [25]. Smoked herring contains low values of histamine (0.49 mg/kg) may be due to that heating step during smoking could eliminate bacteria which able to convert the histidine to histamine [29]**.** In the same direction, [4] found the maximum histamine level in canned anchovies (26.95 mg/kg), 22.38 mg/kg in canned sardines; these values were higher than our result of investigated canned mackerel (0.62 mg/kg).

In addition to, highest histamine value which found in investigated feisiekh sample (21.29 mg/kg) marketed in Egypt considered low than 50 mg histamine /kg, the allowable limit suggested by FDA [12] and Egyptian Standards allows 200 mg/kg as a permissible limit for histamine content in salted, smoked and frozen fish products. In the same trend, [30] considered that the best quality fish has histamine values less than 10 ppm, while histamine values between 10 to 30 ppm are accepted as middle quality and 30-50 ppm histamine value is critical. Europe Union has suggested that the average concentration of histamine in fish should not be more than 10 mg of histamine per 100 g of fish muscle and it seems to be good for general health [31]. As well as the SABS (South African Boreau of Standard) and AFSC (Australian Food Standard Code), respectively considered 10 mg/100g and 20 mg/100g of histamine levels in fish muscle has been suggested as a safe limit. Thus, the results of this study are consistent with international references.

On the other hand, in same Table 1, it could be observed that a maximum total biogenic amines level (3.40 – 46.19 mg/kg) of each fresh fish or processed fish products was lower than that proposed (750–900 mg/kg) by [7] and this agreement with reported by [32], stated that the total amount of all three biogenic amines; putrescine, tyramine and cadaverin should not be increase than 10.1 mg/100g of fresh fish, this indicated the high quality and safety of investigated fresh fish and processed fish products in our study. In addition to, although histamine and another amines values in investigated samples was very low however, in order to prevent or reduce the formation of histamine on fish and fishery products, the rapid cooling of fish after catching and the maintenance of adequate refrigeration during handling and storage is recommended [31, 12].

These results are in agreement with reported by [33] who found that the mean contents of biogenic amines in fresh common carp fish fillets were putrescine (2.3 mg/kg), cadaverine (ND), spermine (2.5 mg/kg), histamine (ND), tyramine (1.2 mg/kg) and (ND) tryptamine. From the study, regarding fresh greasy grouper fish, Our results are in accordance with some those findings by[34] that mean values of the concentrations of putrescine, cadaverine, histamine and tyramine (mg/100g) in greasy grouper fish samples collected from Thuel markets in Saudi Arabia were 1.73, 1.084 and ND, respectively, while histamine was higher (0.932 mg/ 100g) than found in our study (0.52 mg/kg). However, histamine, cadaverine and putrescine contents for investigated feisiekh, salted sardine and smoked herring were lower than that found by [26] that the mean values for histamine content were (33.12, 28.14, 23.14 mg/100g) for feisiekh, salted sardine and smoked herring respectively, mean values for cadaverine content were (13.80, 11.05 and 7.78 mg/100g) for feisiekh, salted sardine and smoked herring respectively and (9.99, 8.81 and 6.12 mg/100g) for Putrescine respectively. Also, these results were much lower than that notes by [35] that histamine values were 18.06 and 23.51mg/100gm in Egyptian feisiekh and salted sardine respectively. Moreover, histamine content of investigated samples in this study was lower than the reported by [27] who showed that mean histamine values were 126.65and 4.44 mg/100gm in feisiekh and smoked herring respectively. While, these results are higher than that reported by [36] which evident that mean values for histamine content were 117.6, 110.8, 3.3, 2 (µg/100g) for feseikh, molouha, smoked herring. In addition to, this observation was supported by a finding of [37] which showed that the concentrations levels of histamine, tyramine, and putrescine in local canned mackerel were (36.72, 0.393 & 2.1) and (29.04, 0.368 and 1.894 mg/kg) for imported canned mackerel, these values were much higher than our results in this study that 0.62, ND and 0.78 mg/kg of collected imported canned mackerel**.** Therefore, these results are indicated the safety of fresh fish and processed fish products collected from Egyptian markets for consumers.

Conclusion

Based on these results, it could be concluded that all investigated fresh fish species; catfish, common carp, mullet fish, greasy grouper and processed fish products; feisiekh, salted sardine, smoked herring and canned mackerel samples obtained from fish markets at various localities in El-Kanater El-Khairia city, Egypt were high safety due to a much lower of determined biogenic amines values than the allowable limit suggested by international references and many results of many authors Thus, fresh fish and processed fish products available on El-Kanater El-Khairia markets at the time of this work was carried out do not exceed the safety limit of histamine and another biogenic amines and safe for the consumers.

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