Benha Journal of Applied Sciences (BJAS) **print : ISSN 2356–9751**
Vol.(2) Issue(2) Nov.(2017), 25-28 **online : ISSN 2356–976x**
http:// bjas.bu.edu.eg

Assessment of Biogenic Amines in some Fishes at Kalyobia Governorate

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Abstract

A grand total of 90 random samples of fresh fish samples represented by Nile fish(*Oreochromis niloticus, Clarias lazera & Bagrus bayad*)(15 of each) and marine fish(*Sardine, Saurus, Pagrus*)(15 of each) were collected randomly from different fish markets in Benha, Kalyobia governorate for determination of histamine and putrescine using High Performance Liquid Chromatography (HPLC). The results showed that 80%, 86.67% and 100% of Nile fish samples were positive for the presence of histamine residues for *Bagrus bayad, Tilapianiloticus* and *Clarias lazera*, respectively. Concerning to marine fish, the positive samples for histamine in samples from *Pagrus, Saurus* and *Sardine* were 93.33%, 100% and 100%, respectively. While, concerning to putrescine residues in Nile fish, the positive samples were 53.33%, 60% and 80% in *Bagrus bayad*, *Tilapia niloticus* and *Clarias lazera*, respectively; and in marine fish 66.67%, 80% and 86.67%.in *Pagrus, Saurus* and *Sardine*, respectively. The public health significance of histamine and putrescine residues in fish and some recommendations to control their presence were discussed.

Keywords: Fish meat, Fresh fish, Histamine, Putrescine, HPLC .

1.Introduction

Fish meat is considered one of the most important nutrients, as they are easily digestible characterized by quality and taste and excellence in food conversion rate on many of the other types of meat in addition to the short production cycle and the low cost of breeding and feeding **[1]**.Fish meat share in solving the shortage in animal protein requirement; it is the most important single source of high-quality protein, providing nearly 16% of the animal protein consumed by the world's population **[2]**.Biogenic amines present in a wide range of food products including fish products **[3]**. The content of biogenic amines in foods can be considered as quality marker which determines the poor quality of raw materials. Accordingly, the concentrations of biogenic amines in any food of animal origin can be used as chemical indicators of thefreshness of animal origin products **[4]**. Excessive consumption of these amines can be of health concern through their actions on nervous, gastric and intestinal systems and blood pressure causing degrees of diseases **[5]**. Biogenic amines production in fish is affected by availability of free amino acids, presence of microorganisms that can decarboxylase amino acids and favorable conditions for their growth **[6]**. Histamine occurs in all types of food particularly fish. Histamine can be an indicator of defective hygiene during preparation or storage of the food**[7]** . Putrescine is one of the most common biogenic amines in food. The excessive levels ofputrescine in food may lead to alimentary poisoning **[8]**. Putrescine and cadaverine can react with nitrite forming heterocyclic carcinogenic nitrosamine, nitrosopyrrolidine and nitrosopiperidine **[9]**.

Therefore, the aim of the current study is to determine the level of histamine and putrescine residues in Nile and marine fish by using high performance liquid chromatography (HPLC).

2.Material and methods

2.1 Collection of samples

A grand total of nintey random samples of fresh fish samples represented by Nile fish (*Oreochromis niloticus, Clarias lazera* & *Bagrus bayad*, 15 of each) and marine fish (*Sardine, Saurus*& *Pagrus*, 15 of each) were collected from different fish markets in Benha, Kalyobia governorate. The collected samples were packed separately in sterile polyethylene bag and transferred directly to the laboratory without delay for determination of their biogenic amine residues.

2.2 Determination of biogenic amines by using HPLC

Histamine and putrescine were determined in all examined samples according to the protocol recommended by Krause et al. (1995) and Pinho et al. (2001).

3. Extraction of samples

Twenty five grams of each sample was blended with 125 ml of 5% Trichloro acetic acid (TCA) for 3 min using a warning blender then filtration was achieved using filter paper Whatman No1. Thus, 10 ml of the filtrate were transferred into a suitable glass tube with 4g NaCl and 1 ml of 50 % NaOH. The filtrate was extracted three times (2min each) by using 5 ml n-butanol: chloroform (1:1 v/v) and the upper clear layer was transferred to 100 ml separating funnel by using disposable Pasteur pipette. To combine the organic extracts (upper layer), 15 ml of n-heptane was added in separating funnel and extracted three times with 1.0 ml portions of 0.2 NHCl, the HCl layer was collected

in a glass Stoppard tube. Solution was evaporated just to dryness using water bath at 95°C with aid of a gentle current of air. About 0.5 ml of saturated NaHCO3 solution was added to the residue of the sample extract (or the standard). Vial was stoppered and carefully mixed to prevent loss- due to spattering. Carefully, 1.0 ml dansyl chloride solution was added and mixed thoroughly using vortex mixer. The reaction mixture was incubated at 55°C for 45 min. About 10 ml of distilled water were added to the reaction mixture, then vial was stoppered and shaked vigorously using vortex mixer, the extraction of dansylated biogenic amines was carried out using 5ml of diethyl ether for 3times again vial was stoppered, shaked for 11.0 min and the ether layers were collected in a culture tube using disposable Pasteur pipette. The combined ether extracts were carefully evaporated at 35°C in dry bath with aid of current air. The obtained dry material was dissolved in 1ml methanol and 10µl were injected in HPLC.

4. Statistical analysis

The obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) test **[10] .**

4.1 Results and discussion

It is evident from the results recorded in table (1) that the histamine levels were varied in Nile fish from 1.3 to 29.7 with an average of 13.75 + 1.14 for Bagrus bayad, 2.8 to 41.5 with an average of 22.68 + 1.96 for Tilapia niloticus, 4.0 to 62.1 with an average of 37.14 + 2.79 for Clarias lazera. However, in marine fish, the histamine levels were varied from 3.4 to 47.9 with an average of 28.51 + 1.83, from 6.2 to 65.1 with an average of 46.09 + 3.25 and from 7.8 to 91.6 with an average of 59.84 + 3.42 for Pagrus, Saurus and Sardine, respectively**.**

The results achieved in table (2) declared the acceptability of the examined samples based on their levels of histamine according to **[11]** which stated their maximum permissible limit of histamine was 20 mg%. Therefore, the number of the unaccepted samples of Nile fish were 3 (20%) in Bagrus bayad, 4 (26.67%) in Tilapia niloticus and 7 (46.67%) in Clarias lazera, while the number of the unaccepted samples of marine fish were 6 (40%), 8 (53.33%) and 9 (60%) in Pagrus, Saurus and Sardine respectively.

Table (3) revealed that the concentrations of putrescine (mg %) in the examined samples were ranged from 1.0 to 22.1 with a mean value of 8.83 ± 0.72 for Bagrus bayad,1.0 to 28.6 with a mean value of 11.29 ± 1.05 for Tilapia niloticus and 2.2 to 36.9 with a mean value of 20.53 ± 1.66 for Clarias lazera. While in marine fish, putrescine concentration (mg %) in the examined samples were ranged from 1.9 to 30.5 with a mean value of 17.15 ± 1.45, 3.1 to 39.7 with a mean value of 23.70 ± 1.89 and 4.5 to 46.4 with a mean value of 27.98 ± 2.12 for Pagrus, Saurus and Sardine respectively.

The results achieved in table (4) declared the acceptability of the examined fish samples based on their levels of putrescine according to**[11]** which stipulated their maximum permissible limit in fish samples was 20 mg%. Therefore, the number of the unaccepted samples of Nile fish was 2 (13.33%) in *Bagrus bayad*, 2 (13.33%) in *Tilapia niloticus* and 4 (26.67%) in *Clarias lazera*. While, in marine fish the number of the unaccepted samples were 3 (20%), 5 (33.33%) and 6 (40%) in *Pagrus, Saurus and Sardine* respectively.

From the obtained results, the collected fish samples (Nile and marine fish) contained high levels of toxic biogenic amines (histamine and putrescine). The present results are lower than those reported by **[12]** who detect high histamine level in *Sardine* samples exceeding 50 ppm. Also the present results are lower than those reported by **[13]** who detect high histamine level (2350 ppm) in *Sardine* samples after 24 hours at ambient temperature and similar levels after 8 days storage in ice. Histamine alone may not cause toxicity at a low level, but the presence of other biogenic amines such as putrescine and cadaverine, at concentrations 5 times higher than histamine, enhance the toxicity of histamine through the inhibition of histamine oxidizing enzymes **[14]**. Histamine poisoning has a short incubation period, ranging from minutes to a few hours after ingestion with syptoms of headache, facial flushing and sweating, rash and itching, nausea, vomiting, diarrhea and heart palpitations **[15]**. The present results came inaccordance with those reported by **[16]** who found that putrescine was detected in all tested samples of fish commonly consumed in southern China within a range of (0.19 - 45.16 mg/kg). The present results came in agreement with Bunka et al. (2013) who reported that putrescine was the most abundant biogenic amine observed in raw fresh water and sea fish samples, and its concentrations up to 10 mg/kg and between 10 and 100 mg/kg were recorded in 26 and 69 samples, respectively. Higher levels were detected by **[13]** who found that putrescine accumulated to level of 300 ppm in sardine after 24 hours at ambient temperature and similar levels after 8 days on ice.

The present study allows confirming the bad hazard using of biogenic amines in fish. Accordingly, the concerned authorities must take extra efforts for corrective methods of decreasing or even elimination their levels for solving the problem of such residues in fish meat.

Table (1) Statistical Statistical analytical results of histamine levels (mg/kg) in the examined samples of Nile and marine fish (n=15).

Fish species	**Min.**	**Max.**	**Mean ± S.E***
Nile Fish:			
Bagrus bayad	1.3	29.7	13.75 ± 1.14
Tilapia niloticus	2.8	41.5	22.68 ± 1.96
Clarias lazera	4.0	62.1	37.14 ± 2.79
Marine Fish:			
Pagrus	3.4	47.9	28.51 ± 1.83
Saurus	6.2	65.1	46.09 ± 3.25
Sardine	7.8	91.6	59.84 ± 3.42

***S.E =** Standard Error of the mean**.**

Table (2) Acceptability of the examined samples of fish based on their levels of histamine (n=15).

Fish species	**Maximum Permissible Limit (mg %)***	**Positive samples**		**Unaccepted Samples**	
		No.	**%**	**No.**	**%**
Nile Fish:					
Bagrusbayad	20	12	80	3	20
Tilapia niloticus	20	13	86.67	4	26.67
Clarias lazera	20	15	100	7	46.67
Marine Fish:					
Pagrus	20	14	93.33	6	40
Saurus	20	15	100	8	53.33
Sardine	20	15	100	9	60

* Maximum Residual Limit stipulated by Egyptian Organization for Standardization "EOS" (2005).

Table (3) Statistical Statistical analytical results of putrescinelevels (mg/kg) in the examined samples of Nile and marine fish (n=15).

Fish species	**Min.**	**Max.**	**Mean ± S.E***
Nile Fish:			
Bagrusbayad	1.0	22.1	8.83 ± 0.72
Tilapia niloticus	1.0	28.6	11.29 ± 1.05
Clarias lazera	2.2	36.9	20.53 ± 1.66
Marine Fish:			
Pagrus	1.9	30.5	17.15 ± 1.45
Saurus	3.1	39.7	23.70 ± 1.89
Sardine	4.5	46.4	27.98 ± 2.12

***S.E =** Standard Error of the mean**.**

Table (4) Acceptability of the examined samples of fish based on their levels of putrescine (n=15).

Fish species	Maximum Permissible Limit (mg %)*	Positive samples		Unaccepted Samples	
		No.	%	No.	%
Nile Fish:					
Bagrusbayad	20	8	53.33	2	13.33
Tilapia niloticus	20	9	60	2	13.33
Clarias lazera	20	12	80	4	26.67
Marine Fish:					
Pagrus	20	10	66.67	3	20
Saurus	20	12	80	5	33.33
Sardine	20	13	86.67	6	40

* Maximum Residual Limit stipulated by Egyptian Organization for Standardization "EOS"(2005).

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