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Assessment of Histamine and Putrescine Residues in Fish and Shell Fish

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A B S T R A C T

Ninety random samples of fresh fish and shellfish (45 0f each) were collected from different fish markets at different localities in Kalyobia governorate, Egypt. The fish samples were represented by *Oreochromis Niloticus, Mugil Cephalus and Sardine* (15 of each). However, the shellfish samples were represented by Sepia, Crab and Shrimp (15 of each). All the collected samples were examined for determination of their concentrations of biogenic amines (histamine and putrescine)that revealed to the mean histamine concentration (mg in fish samples (*O. Niloticus ;M. Cephalus* and Sardine) were 13.46±0.27 ; 16.80 ± 0.31 and 28.74 ± 0.52, respectively and in shellfish (Sepia, crab and shrimp) were 17.29 ± 0.40 ; 31.52 ± 0.48 and 44.96 ± 0.63, respectively In addition, the mean putrescine concentration values in fish samples (*O. Niloticus ;M. Cephalus* and Sardine) were 6.61 ± 0.21; 9.18 ± 0.24 and 14.45 ± 0.39 and in shellfish (Sepia, crab and shrimp) were 8.72 ± 0.29 ; 17.06 ± 0.42 and 25.19 ± 0.50, respectively The public health importance of biogenic amines as well as suggesting the recommendation for improving the quality of fishery products were outlined.

Key words: Histamine, Putrescine, Biogenic, Amines, Fish, Shell fish.

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1. INTRODUCTION:

Fish, and shell fish provide a healthy source of high quality proteins, essential vitamins, minerals and polyunsaturated fatty acids. A well-balanced diet that includes a variety of fish and shell fish can contribute to heart health; children proper growth and protection against certain cancers (Jaclyn *et al.*, 2010).

The biogenic amines (Bas) are low molecular weight organic bases with biological activity that are formed in foods, as fish and fish products, meat, dairy products, wine and other fermented foods; by microbial decarboxylation of the corresponding amino acids or by transamination of aldehydes and ketones by amino acid transaminases and have been identified as one of the important agent causing seafood intoxication (Kim *et al*., 2009 and Zhai *et al*., 2012). The most important BAs, histamine and putrescine, are formed from free amino acids namely histidine and

ornithine. respectively (Zarei *et al.,* 2011). Storage temperature and fish species are the most important factors contributing to BAs formation (Chong *et al*., 2011). Other parameters (i.e., pH, water activity, NaCl concentration, additives) may influence the variation of microbiota composition and lead to the differences in BAs content (Suzzi and Gardini, 2003).

Histamine is formed from histidine by bacterial action. Fish spp. implicated are invariably found to be rich in histidine. When held too long at too high temp. bacteria proliferate and *histamine* is formed. Most the bacteria capable of *histamine* production are not part of the normal flora, but represent post-harvest contamination during handling, processing and marketing. Thus, cooling, short storage and good handling practices can avoid *histamine* formation. Ababouch (1990).

Putrescine content may be a good indicator of late fish spoilage. Its formation needs an acidic pH and high concentration of ornithine (Tabor et al., 1984 and Poli et al., 2001).

Low levels of biogenic amines in products do not present in a serious risk to human health because the amine oxidases in human intestine can rapidly detoxify the amines, however, the ingestion of foods containing high levels of biogenic amines may result in severe toxicological symptoms (Mohamed *et al.,* 2010). When high amounts of biogenic amines are consumed or even the normal pathways of amines catabolism are inhibited, various pathological effects can be seen. Those effects include hypotension or hypertension, nausea, headache, rash, dizziness, cardiac palpitation, intracerebral hemorrhages and death in very severe cases (Rawles *et al.,* 1996 and Davies *et al*., 2001)

Therefore, the current study was conducted to assessment of Histamine and Putrescine residues in fish and shell fish

2. MATERIAL AND METHODS

2.1. Collection of samples:

Total of 90 samples of *fresh Oreochromis Niloticus, Mugil cephalus & Sardine* (15 of each) as fish and Sepia, Crab &Shrimp (15 of each) as shellfish (45 0f each) were collected from different fish markets at different localities in Kalyobia governorate, Egypt The.collected samples were kept separately in sterile bags and transferred to laboratory under complete aseptic condition without undue delay.

2.2. Apparatuses and Instruments:

HPLC (Agilent 1100 HPLC system, Agilen Technologies, Waldbronn, Germany

2.3 Preperation of samples:

Accurately, 25 g of each sample were 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 3 times (2 min each) 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.

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.

2.4. Determination of biogenic amines by using HPLC

Three biogenic amines including histamine, cadaverine and putrescine were determined in all examined samples according to the protocol recommended by Krause *et al.* (1995) and Pinho *et al.* (2001).3

2.4.1. Formation of dansylamines:

One hundred µl of each stock standard solution (or sample extract) were transferred to 50ml vial and dried under vacuum. About 0.5 ml of saturated NaHCO3 solution was 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.

Actually,10 ml of distilled water was 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 pipett. 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.

2.4.2. Apparatus HPLC conditions:

High performance liquid chromatography (HPLC) used for dansylamines determination was an Agilent 1100 HPLC system, Agilen Technologies, Waldbronn, Germany, equipped with quaternary pump model G 1311A, UV detector (Model G 1314A) set at 254nm wavelength, auto sampler (model G1329A VP-ODS) and Shim pack (150× 4.6 mm) column (Shimadzu, Kyoto, Japan) was used for biogenic amines separation. Data were integrated and recorded using Chemstation Software program no. l and 10µl were injected in HPLC.

3.1. Statistical Analysis:

The obtained results were statistically evaluated by application of Analysis of Variance (ANOVA) test according to Feldman et al. (2003).

3.RESULTS:

It is evident from table (1) the results indicated that histamine level mg% in the examined samples of fish and shellfish (n=15) were ranged from 1.82 to 23.37 with a mean value of 13.46±0.27., from 2.56 to 29.14 with a mean value of 16.80 ± 0.31 and from 4.08 to 51.91 with a mean value of 28.74 ± 0.52. in *O. Niloticus*, *M. Cephalus* and sardine respectively but in sepia it was ranged from 3.14to 32.60 with a mean value of 17.29 ± 0.40 mg% but were ranged from 3.87to 54.95 with a mean value of 31.52 ± 0.48 mg% in crab and were ranged from 6.26to 71.63 with a mean value of 44.96 ± 0.63mg%in shrimp samples.

Results in Table (2) showed the maximum permissible limit of histamine in fish and shellfish according to EOS (2005) was should not exceed 20 mg %. In contrast, 30 samples, *O. Niloticus* (13.33%,2)*; M. Cephalus* (20%,3)*;* Sardine (33.33%,5)*;* sepia (20%,3)*;* crab (40%,6) and shrimp (73.33%,11), were unaccepted, as they were exceeded such safe standard limit.

The recorded results in Table (,3) revealed that the putrescine concentrations "mg %" in the examined samples of fish and shell fish samples were recorded the minimum and the maximum putrescine concentrations were

ranged from 0.79to 20.54 with a mean value of 6.61 ± 0.21*in O, Niloticus.* were ranged from 1.10 to 23.68 with a mean value of 9.18 ± 0.24 mg% in M Cephalus .and were ranged from

ranged from 1.92 to 25.43 with a mean value of 8.72 ± 0.29mg% in sepia, 2.69 to 34.77 with a mean value of 17.06 ± 0.42 mg% in crab and 3.15 to 39.82 with a mean value of 25.19 ± 0.50 mg%.in shrimp samples.

Table (4) showed that the maximum permissible limit of putrescine in fish and 2.37 to 28.15 with a mean value of 14.45 ± 0.39 mg% in sardine samples. Meanwhile, in Shell fish samples the minimum and the maximum putrescine concentrations were shellfish according to EOS (2005) should not exceed 20 mg %., *O. Niloticus* (6.67% ,1)*; M. Cephalus* (6.67% 1)*;* Sardine (20 % ,3)*;* sepia (13.33%, 2)*;* crab (26.67%,4) and shrimp (46.67%,7), were unaccepted.

Table (1): Statistical analytical results of histamine levels "mg %" in the examined samples of fish and shellfish (n=15).

Fish and shellfish	Min	Max	Mean ± S.E*
Fish:			
Oreochromis Niloticus	1.82	23.37	13.46 ± 0.27
Mugil Cephalus	2.56	29.14	16.80 ± 0.31
Sardine	4.08	51.91	28.74 ± 0.52
Shellfish:			
Sepia	3.14	32.60	17.29 ± 0.40
Crab	3.87	54.95	31.52 ± 0.48
Shrimp	6.26	71.63	44.96 ± 0.63

S.E* = standard error of mean

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

Fish species	Maximum Permissible Limit (mg %) *	Accepted Samples		Unaccepted Samples	
		No.	%	No.	%
Fish*:*					
Oreochromis Niloticus		13	86.67	2	13.33
Mugil Cephalus		12	80	3	20
Sardine		10	66.67	5	33.33
Shellfish:	20				
Sepia		12	80	3	20
Crab		9	60	6	40
Shrimp		4	26.67	11	73.33

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

Table (3): Statistical analytical results of putrescine levels "mg %" in the examined samples of fish and shellfish (n=15)

Fish and shellfish	Min	Max	Mean ± S.E*
Fish:			
Oreochromis Niloticus	0.79	20.54	6.61 ± 0.21
Mugil Cephalus	1.10	23.68	9.18 ± 0.24
Sardine	2.37	28.15	14.45 ± 0.39
Shellfish:			
Sepia	1.92	25.43	8.72 ± 0.29
Crab	2.69	34.77	17.06 ± 0.42
Shrimp	3.15	39.82	25.19 ± 0.50

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

Fish species	Maximum Permissible Limit (mg %) *	Accepted Samples		Unaccepted Samples	
		No.	%	No.	%
Fish:					
Oreochromis Niloticus	20	14	93.33	1	6.67
Mugil Cephalus		14	93.33	1	6.67
Sardine		12	80	3	20
Shellfish:		13	86.67	2	13.33
Sepia					
Crab		11	73.33	4	26.67
Shrimp		8	53.33	7	46.67

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

Table (5): Analysis of variance (ANOVA) of histamine levels in the examined samples of fish and shellfish

Source of variance	D. F	S. S	M. S	F. value
Total	89	663.5479		
Between Species (S)	5	317.2415	63.4481	15.39^{++}
Error	84	346.3064	4.1227	

D.F = Degrees of freedom **S.S** = Sum squares

M.S = Mean squares ++ = High significant differences (P<0.01)

Table (6): Analysis of variance (ANOVA) of putrescine levels in the examined samples of fish and shellfish

Source of variance	D. F	S. S	M. S	F. value
Total	89	387.2168		
Between Species (S)	5	144.1207	28.8239	9.96^{++}
Error	84	243.0961	2.8940	

D. F = Degrees of freedom **S. S** = Sum squares **M. S** = Mean squares
++ = High significant differences (P<0.01)

4.DISCUSSION

According to Table (1), the obtained results were disagreed with those of Yasser (2016) who detected histamine with higher concentrations ranged from 5 to 46.7, mean value 20.22±1.171 mg% and EL-Khabaz-Salwa (2017) who detected histamine with higher mean concentrations 22,68±1,96 and with those of Kulawik *et al*. (2015) who detected lower levels, from 0.104 to 2.188 mg%. For *M. Cephalus* fish samples were ranged from 2.56 to 29.14 with a mean value of 16.80 ± 0.31 mg%.

These results were disagreed with those of Rabie *et al*. (2011) and Mansour- Hayam *et al*. (2012) who detected histamine with higher concentrations 521 mg/kg and 117.6 mg/kg, respectively. Also, for Sardine fish samples were ranged from 4.08 to 51.91 with a mean value of 28.74 ± 0.52 mg%.

Nearly similar results were recorded by Abd El-Azeem- Walaa (2016) who recorded that mean value of histamine was 29.65±1.41 mg/kg but disagreed with those of Galarini *et al.* (1996), Proster (2011) and EL Khabaz-Salwa (2017) who detected histamine with higher concentrations 32.08 mg/kg, 50 mg/kg,and 59,84±3,42 , respectively. Meanwhile, for Shell fish samples; the minimum and the maximum histamine concentrations in sepia samples were ranged from 3.14to 32.60 with a mean value of 17.29 ± 0.40 mg%. For crab samples were ranged from 3.87to 54.95 with a mean value of 31.52 ± 0.48 mg%.

These results were disagreed with those of Yanshan-Xu *et al.* (2009) who detected histamine with higher concentrations 95.22ppm and with Abimannan *et al.* (2017) who detected lower levels, 7.55±0.46 µg/kg and 17.68 ± 1.30 µg/100g at 4 and 20°C in crab samples.

Also, for shrimp samples were ranged from 6.26to 71.63 with a mean value of 44.96 ± 0.63mg%. Nearly similar results were recorded by Carolina *et al*. (2012) who recorded that mean value of histamine was 48.29 µg/kg but disagreed with those of Tsal (2006) who detected histamine with higher concentrations 382 µg/kg and with those of Rigg (1997) who failed to detect it in examined samples of shellfish.

From the obtained results, it showed that histamine level was higher in sardine samples than other species of fish as sardine muscles have high level of histamine as reported by) (lee *et al*. 2012**);** also, sardine contain high amount of oils which play an important role in growth of Lipolytic M.O. which convert

histidine to histamine or may be attributed to poor quality of raw material, improper handling or other changes during storage **(**Rodriguez *et al*.,**2014).** But in shell fish, the level of histamine was higher in shrimp than sepia and crab samples; it may refer to that shrimp may be exposed to contamination quicker than sepia and crab.

Concerning to table (3), These results were disagreed with those of Yasser (2016) who detected putrescine with higher concentrations ranged from 2.8 to 21.7 with a mean value of 12.18±0.74 mg/kg and ELKhabaz-Salwa (2017) who detected the mean concentration of putrescine was 11,29±1,05 and with those of Kulawik *et al*. (2015) who detected lower levels, from 0.538 to 1.876 µg/100g. For *M. Cephalus* fish samples were ranged from 1.10 to 23.68 with a mean value of 9.18 ± 0.24 mg%. These results were disagreed with those of Mansour- Hayam *et al*. (2012) who detected putrescine with higher concentrations 130.5 µg/100g. Also, for Sardine fish samples were ranged from 2.37 to 28.15 with a mean value of 14.45 ± 0.39 mg%. These results were disagreed with those of Abd El-Azeem- Walaa (2016) who detected putrescine with higher concentrations 18.83±0.97 mg% and with Fatih and Yasin (2006) who detected lower levels, from 10 to 12.2 µg/100g.Meanwhile, for Shell fish samples; the minimum and the maximum putrescine concentrations in sepia samples were ranged from 1.92 to 25.43 with a mean value of 8.72 ± 0.29mg%. For crab samples were ranged from 2.69 to 34.77 with a mean value of 17.06 ± 0.42 mg%. Also, for shrimp samples were ranged from 3.15 to 39.82 with a mean value of 25.19 ± 0.50 mg%. These results were disagreed with those of Saaid *et al*. (2009) who detected putrescine with higher concentrations 331ppm.

5.CONCLUSION AND RECOMMENDATIONS

From the obtained result it was concluded that histamine recorded the highest level in sardine samples in samples of fish and shrimp in samples of shell fish, so in order to achieve good quality fish and fish products with a low bacterial count and minimize BAs contents, the following regulations should be followed:

- Hygienic handling of fish from the moment of capture to the point of consumption is also crucial to reduce the bacterial counts and the formation of BAs.
- The easiest method of prevention BAs accumulation is rapid chilling and freezing of harvested fish and maintenance of low temperature until the point of consumption.
- The use of good quality fish raw material is required to produce a safe and a good quality canned and salted fish product.
- Not only Enterobacteriaceae and pH were responsible for the formation of BAs, but also other many factors (storage temperature, availability of amino acid precursors and activity of decarboxylase enzymes.
- Hazard analysis critical control points (HACCP) should be applied under the control and supervision of the controlling authorities throughout:
- Good manufacturing practices (GMP) should be applied under the control and supervision of the controlling authorities.

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