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Biogenic Amines in Seafood

Nariman A. Helmy[1], Mohamed A. Hassan[2], Faten S. Hassanien[2] and Ahmed A. Maarouf[1]

Animal Health Research Institute, Benha Branch[1].

Food Hygiene Control Department, Faculty of Veterinary Medicine, Benha University[2]

A B S T R A C T

Ninety random samples of fresh fish (*Claris gariepinus*, *Oreochromis niloticus* and *Mugil cephalus*, 15 of each) and shellfish (Oyster, Shrimp and Crab, 15 of each) were collected from different fish markets in Kalyobia governorate, Egypt, for determination of histamine and cadaverine levels in their tissues. The obtained results revealed that, the mean value concentrations of histamine in fish samples were 21.59 ±1.72;18.31 ±1.45 and11.64 ±1.19 for *Claris gariepinus*, *Oreochromis niloticus* and *Mugil cephalus*, respectively. Meanwhile, in shellfish samples and they were 41.75 ±3.26;33.08 ± 2.57 and 19.92 ± 2.02 for Oyster, shrimp and crab, respectively. In addition, mean value concentrations of cadaverine in fish samples were 17.86 ±1.40;16.57 ± 1.24 and 8.94 ± 0.76 for *Claris gariepinus*, *Oreochromis niloticus* and *Mugil cephalus*, respectively. The average concentrations of cadaverine in shellfish samples were of 29.16 ± 2.05;21.83 ± 1.61 and 13.09 ± 1.14 for Oyster, shrimp and crab, respectively. It could be inferred that regarding the products contamination, the highest histamine contamination was in oyster followed by shrimp followed by *Claris gariepinus* then crab then *Oreochromis niloticus* finally *Mugil cephalus*. Whereas the highest cadaverine contamination was in oyster followed by shrimp followed by *Claris gariepinus* then *Oreochromis niloticus* then crab finally *Mugil cephalus*.

Key words: Seafood, Residues, histamine, cadaverine

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1. INTRODUCTION:

Seafood are a major part of food, consumed by a large percentage of population in the world, as they contain the most important nutritional components that supplies all essential elements, especially proteins and essential polyunsaturated fatty acids, required for life processes in a balanced manner and serve as a source of energy for human beings, as well as they can contribute to heart health and children's growth and development (Sutharshiny and Sivashanthini, 2011).The biogenic amines (Bas) 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 (Zhai *et al*., 2012). The most important BAs, histamine, tyramine, putrescine, and cadaverine, are formed from free amino acids namely histidine,

tyrosine, ornithine and lysine, respectively (Zarei *et al*., 2011). The free amino acids either occur as such in foods or may be liberated through proteolysis. In addition to the availability of precursors (amino acids), BAs accumulation in foods requires the presence of microorganisms with amino acid decarboxylases and favorable conditions for their growth and decarboxylation activity (Zarei *et al*., 2011). Storage temperature is the most important factor contributing to BAs formation (Chong *et al*., 2011). Histamine and other biogenic amines can be used as indicators for product decomposition as these compounds are detected at non-significant concentrations in fresh fish and shellfish; in addition, their formation is frequently associated with bacterial spoilage (Nurullah *et al*., 2007; Rezaei*etal*., 2007 and Kim *et al*., 2009).The consumption of fish and shell fish could be considered as one of the major sources of human exposure to bacterial infections and environmental contaminants (Chong *et al*., 2011).Moreover, high amount of amines can be produced by bacteria during amino acids decarboxylation and have been identified as one of the important agent causing seafood intoxication (Kim *et al*., 2009). Histamine represents the major and the main cause of scombroid (histamine) poisoning, and other biogenic amine, such as tyramine, cadaverine, and putrescine, acts as potentiates of histamine toxicity (Al Bulushi *et al*., 2009; Joshi and Bhoir, 2011). Therefore, the present study was conducted to evaluate the contamination levels of fish and shellfish with biogenic amines and their ability of human consumption.

2. MATERIAL AND METHODS:

2.1. Collection of samples

A total of 90 random samples of fresh fish (*Clarias lazera, Oreochromis niloticus* and *Mugil cephalus*, 15 of each) and shellfish (Oyster, Shrimp and Crab, 15 of each) (weighted 25 gm) were collected from different fish markets in Kalyobia governorate, Egypt, for determination of histamine and cadaverine levels.

2.2. Determination of biogenic amines

All collected samples were examined for determination of two biogenic amines (histamine and cadaverine) levels on the basis of wet weight (mg/Kg) according to the protocol recommended by Krause *et al*. (1995) and Pinho *et al*. (2001).

2.2.1. Reagents preparation:

- Dansyl chloride solution: 500mg of dansyl chloride were dissolved in 100 ml acetone.
- Standard solutions: Stock standard solutions of the tested amines were prepared as the following: 25 mg of each standard pure amine (histamine-2HCl, tyramine-2HCl Standard solutions were added: Stock standard solutions of the tested amines were prepared as the following: add 25 mg of each standard pure amine (histamine-2HCl, tyramine-2HCl Standard solutions: Stock standard solutions of the tested amines were prepared as the following: add 25 mg of each standard pure amine (histamine-2HCl and tyramine-2HCl) were dissolved in 25 ml distilled water individually.

2.2.2. Extraction of samples and formation of dansylamines (Armagan, 2006):

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 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 High performance liquid chromatography (HPLC), data were integrated and recorded using Chemstation Software program.

3. RESULTS:

The obtained results in Table (1), revealed that, the minimum and the maximum histamine concentrations "mg %" in the examined samples of fish (C. *gariepinus, O. niloticus* and *M. cephalus*) ranged from 2.44 to 39.72; 1.85 to 30.94 and 1.27 to 23.50 respectively, with a mean value of 21.59 ± 1.72; 18.31 ± 1.45 and 11.64 ± 1.19, respectively. Meanwhile, for shellfish samples (oyster, shrimp and crab) they ranged from 4.82 to 73.26; 3.12 to 61.51 and 1.06 to 34.87 respectively, with a mean value of 41.75 ± 3.26; 33.08 ± 2.57 and 19.92 ± 2.02, respectively. Moreover, 33samples, 6C. *gariepinus* (40%)*;* 3 *O. niloticus* (20%)*;* 2 *M. cephalus* (13.3%)*;* 10oyster (66.7%)*;* 8shrimp (53.3%) and 4 crabs (26.7%), were unaccepted, as they were exceeded the maximum permissible limit of histamine in fish and shellfish that should not exceed 20 mg % (EOS, 2010).

The results in Table (2) showed that, the minimum and the maximum cadaverine concentrations "mg %" in the examined samples of fish (C. *gariepinus, O. niloticus* and *M. cephalus*)ranged from 1.92 to 31.06 ; 1.59 to 28.32 and 0.84 to 20.19 respectively, with a mean value of 17.86 ± 1.40 ; 16.57 ± 1.24 and 8.94 ± 0.76 , respectively. Meanwhile, for shellfish samples (oyster, shrimp and crab) they ranged from 2.50 to 44.12; 2.01 to 35.77 and 1.14 to 21.28 respectively, with a mean value of 29.16 ± 2.05;21.83 ± 1.61 and 13.09 ± 1.14, respectively. In addition;18samples, 4C. *gariepinus* (26.7%)*;* 2 *O. niloticus* (13.3%)*;* 1 *M. cephalus* (6.7%)*;* 5oyster (33.3%)*;* 4shrimp (26.7%) and 2 crabs (13.3%), were unaccepted, as they exceeded the maximum permissible limit of cadaverine in fish and shellfish that should not exceed 20 mg % (EOS, 2010).

Table (1): Analytical results of histamine concentrations "mg/Kg" in the examined samples of fish and shellfish (n=15).

Fish and shellfish species	Min.	Max.	Mean ± SEM*	Unaccepted Samples Maximum Residual Limit (mg %) **	
				No.	%
Fish:					
Claris gariepinus	2.44	39.72	21.59 ± 1.72	6	40
Oreochromis niloticus	1.85	30.94	18.31 ± 1.45	3	20
Mugil cephalus	1.27	23.50	11.64 ± 1.19	2	13.3
Shellfish:					
Oyster	4.82	73.26	41.75 ± 3.26	10	66.7

Shrimp	3.12	61.51	33.08 ± 2.57	8	53.3
Crab	1.06	34.87	19.92 ± 2.02	4	26.7

SEM* = standard error of mean

** Maximum Residual Limit of histamine (20 mg %) stipulated by Egyptian Organization of Standardization "EOS" (2010).

Table (2): Analytical results of cadaverine concentrations "mg/Kg" in the examined samples of fish and shellfish (n=15).

Fish and shellfish species	Min.	Max.	Mean ± SEM.*	Unaccepted Samples Maximum Residual Limit (mg%) ** No.	%
Fish:					
Claris gariepinus	1.92	31.06	17.86 ± 1.40	4	26.7
Oreochromis niloticus	1.59	28.32	16.57 ± 1.24	2	13.3
Mugil cephalus	0.84	20.19	8.94 ± 0.76	1	6.7
Shellfish:					
Oyster	2.50	44.12	29.16 ± 2.05	5	33.3
Shrimp	2.01	35.77	21.83 ± 1.61	4	26.7
Crab	1.14	21.28	13.09 ± 1.14	2	13.3

S.E.M* = standard error of mean

** Maximum Residual Limit of cadaverine (20 mg %) stipulated by Egyptian Organization of Standardization "EOS" (2010).

4. DISCUSSION:

Biogenic amines accumulation in foods requires the presence of microorganisms with amino acid decarboxylases and favorable conditions for their growth and decarboxylation activity (Zarei *et al*., 2011).

The high level of histamine in some investigated samples is probably related to bacterial decarboxylase activity due to quality of raw material, miss handling or other causes during their shelf- life (Koutsoumanis *et al.*, 1999). So, when human eat fish have high level of histamine lead to acute illness called scombroid fish poisoning which characterized by facial flushing, sweating, rash, diarrhea and abdominal cramps that usually resolve after several hours without medical intervention. But severe symptoms are respiratory distress, swelling of the tongue and blurred vision that need medical treatment (CDC, 2007). In addition, scombroid poisoning is unique among the seafood toxins since it results from product mishandling rather than contamination from other trophic levels (Hungerford, 2010). The recorded results for histamine concentrations revealed that, the highest histamine

contamination was in oyster followed by shrimp followed by *Claris lazera* then crab then *Oreochromis niloticus* finally *Mugil cephalus*. The results for histamine concentrations in fish samples were nearly similar to those obtained by Ekici and Alisarli (2008); Ayesh *et al.* (2012) and Kulawik *et al.* (2016). But they were disagreed with those of Lapa-Guimarães and Pickova (2004); Auerswald *et al. (*2006); Thaw *et al.* (2004); Ayesh *et al.* (2012); Mostafa- Azza and Salem-Rabab (2015); who detected histamine in fish samples with lower concentrations and with those of Tsai *et al.* (2006); Tao *et al.* (2010); Visciano *et al.* (2012) and El-Sayed, Huda (2014) who detected higher levels. Meanwhile, for shellfish samples, the results came in accordance with those obtained by Lin *et al.* (2012). But, they were disagreed with those of Yang *et al.* (2012) and Lago *et al.* (2015) who detected histamine in shellfish samples with lower concentrations; with those of Moon *et al.* (2011) who detected higher levels and with those of Rigg (1997) who failed to detect it in examined samples of shellfish.

The concentration of cadaverine is a good indicator of spoilage and it significantly related to post processing handling of fish products or post-harvest handling of fresh fish (Flick *et al.*, 2001). The presence of cadaverine potentiates the toxicity of histamine. It united with nitrite to form heterocyclic carcinogenic nitrosamines, nitrosopyrrolidine and nitrosopiperidine. Also, it has a vasoactive activity that may reach to concentrations being dangerous for the most sensitive consumers (Maijala *et al.* 1993).The obtained results for cadaverine concentrations cleared that, the highest cadaverine contamination were in oyster followed by shrimp followed by *Claris lazera* then *Oreochromis niloticus* then crab finally *Mugil cephalus* .The results for cadaverine concentrations in the examined fish samples were nearly similar to those recorded by Lapa-Guimarães and Pickova (2004); Ayesh *et al.* (2012) and Mostafa- Azza and Salem-Rabab (2015). These results were disagreed with those of Thaw *et al.* (2004) and El-Sayed, Huda (2014) who detected cadaverine in fish samples with lower concentrations. Meanwhile; for shellfish samples, the results were disagreed with those of Yang *et al.* (2012) and Lago *et al.* (2015) who detected cadaverine in shellfish samples with lower concentrations. The obtained results declared that the cadaverine was detected in all examined samples of fish and shellfish. This declared as they are particularly good sources of free lysine (Usydus *et al.*, 2009) and the decarboxylation of lysine leads to the formation of cadaverine that has been associated with Enterobacteriaceae count Hong *et al.* (2013).

Finally, the present study proved that, fish and shellfish have public health importance as the levels of biogenic amines (histamine and cadaverine) in their tissues, might be exceeded the recommended safe permissible limits stipulated by Egyptian Organization for Standardization (EOS, 2010) and must be controlled to prevent or minimize them and improve the sanitary status of fish and shellfish.

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