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Demonstration of histamine in Nile fish

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ABSTRACT

The aim of this study is to determine histamine concentration in three types of fish (*Oreochromis niloticus*, *Clarias lazera* and *Mormyrus niloticus*). 30 of each collected from fish markets of El Qualiobeya governorate, Egypt. The results revealed that the mean value of histamine in the examined samples were 18.23 ± 1.56 mg/kg: 14.90 ± 1.21 mg /kg and 12.65 ± 1.07 mg /kg for *Oreochromis niloticus*, *Clarias lazera* and Mormyrus niloticus respectively. According to Egyptian Organization of Standardization which recommended that the critical limits for histamine should not be more than 20mg/100g in fish, the un-accepted samples represented as 26.67 %, 16.67 % and 13.33 % in Oreochromis niloticus, Clarias lazera and Mormyrus niloticus, respectively. So, all samples are acceptable. Although biogenic amines (Bas) formation is the result of bacterial growth, the presence of these undesirable compounds, especially histamine, is not always noticed by consumers. Thus, histamine is the main marker for the evaluation of quality and safety of fish.

1. INTRODUCTION

Fish is one of the most vital foodstuffs, easily digested and of high palatability. It is also known to have a higher food conversion rate than other meat type animals, with further advantages of shorter production cycle and low cost of rearing and feeding (AbdEl Ghany, 2003). Furthermore, Fish oil represents a good source of calories and provides many important vitamins as B group, A and D, beside calcium, phosphorus and iodine (Feldhusen, 2000).

Fish meat is sharing to solve 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 (FAO, 1997). Histamine builds up as a result of growth of histidine decarboxylase positive bacteria that found in fish flesh under favorable conditions for synthesis and activity. Presence of high histamine level in fish muscles indicate spoilage. Histidine is an amino acid that exists naturally in many types of food, including fish. At temperatures above 16 °C/60 °F, histidine is converted to the biogenic amine histamine via the enzyme histidine decarboxylase.

Histamine is the main natural chemical responsible for true allergic reactions and a variety of serious symptoms (Otwell, 2015; Feng, 2016). So, the current study is performed for studying of histamine residues in the examined three types of fish as one of the most harmful biogenic amines that affected harmfully on human health.

2. MATERIAL AND METHODS

2.1. Collection of samples:

A total of 90 random samples of Nile fish represented by *Oreochromis niloticus*, *Clarias lazera* and *Mormyrus niloticus* (30 of each) were collected from the different fish markets located in Benha city, Qualiobeya governorate, Egypt. Each sample was kept in a separated plastic bag and preserved in an ice box then transferred to the laboratory without undue delay and examined as quickly as possible.

2.2. Determination of histamine by ELISA (Leszczynskai et al., 2004):

2.2.1. Intended use and principle of the test:

This enzyme immunoassay is for the quantitative determination of histamine in plasma and urine as well as different tissues of the body. In combination with supplementary kit (available for purchase separately, cat. no. BA E-1100), the assay is performed for the determination of histamine release in heparinized whole blood and tissues of the body. First, histamine is quantitively acylated. The subsequent competitive ELISA kit uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated standards controls, samples, and the solid phase bound analyte compete for a fixed number of antiserum binding sites. After the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing.

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The antibody bound to the solid phase is detected by an anti-rabbit IgG- peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm. Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations.

2.2.2. Test procedure:
All reagents and samples are allowed to reach room temperature prior to use. Measurement in duplicates is recommended.
2.2.3. Preparation of reagents:
2.2.3.1. Wash Buffer
Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1,000 ml.
Storage: up to 6 months at 4 – 8°C.

2.2.3.2. Acylation Diluent
The Acylation Diluent has a freezing point of 18.5°C. To ensure that the Acylation Diluent is liquid when being used, it must be ensured that the Acylation Diluent has reached room temperature and forms a homogeneous, crystal-free solution before being used. Alternative the Acylation Diluent can be stored at room temperature (20–25°C) separate from the other kit components.

2.2.3.3. Acylation Reagent
Reconstitute each vial with 1.25 mL Acylation Diluent. The Acylation Reagent has to be newly prepared prior to the assay (not longer than 1 hour in advance). If more than 1.25 mL is needed, pool the contents of 2 or 3 vials and mix thoroughly.

2.4. Sample preparation and acylation:
2.4.1. Pipette 25 µL of standards, 25 µL of controls, 25 µL of plasma samples, 10 µL of urine samples, or 50 µL of supernatant from the release test* into the respective wells of the Reaction Plate.
2.4.2. Add 25 µL of Acylation Buffer to all wells.
2.4.3. Add 25 µL of Acylation Reagent to all wells.
2.4.4. Incubate for 45 min at RT (20-25°C) on a shaker (approx. 600 rpm).
2.4.5. Add 200 µL of distilled water to all wells.
2.4.6. Incubate for 15 min. at RT (20-25°C) on a shaker (approx. 600 rpm).
Take 25 µL of the prepared standards, controls, and samples for the Histamine ELISA.
* For the release test the Histamine Release supplementary kit (available for purchase separately, cat. no. BA E-1100) has to be used.

2.5. Histamine ELISA:
2.5.1. Pipette 25 µL of the acylated standards, controls, and samples into the appropriate wells of the Histamine Microtiter Strips.
2.5.2. Pipette 100 µL of the Histamine Antiserum into all wells and cover plate with Adhesive Foil.
2.5.3. Incubate for 3 hours at RT (20-25°C) on a shaker (approx. 600 rpm).
Alternatively, shake the Histamine Microtiter Strips briefly by hand and incubate for 15 – 20 hours at 2 – 8°C.
2.5.4. Remove the foil. Discard or aspirate the contents of the wells and wash each well 4 times thoroughly with 300 µL Wash Buffer. Blot dry by tapping the inverted plate on absorbent material.
2.5.5. Pipette 100 µL of the Enzyme Conjugate into all wells.
2.5.6. Incubate for 30 min at RT (20-25°C) on a shaker (approx. 600 rpm).
2.5.7. Discard or aspirate the contents of the wells and wash each well 4 times thoroughly with 300 µL Wash Buffer. Blot dry by tapping the inverted plate on absorbent material.
2.5.8. Pipette 100 µL of the Substrate into all wells and incubate for 20-30 min at RT (20-25°C) on a shaker (600 rpm). Avoid exposure to direct sunlight.
2.5.9. Add 100 µL of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
2.5.10. Read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 450 nm with a reference wavelength between 620 nm and 650 nm.

2.6. Calculation of results:

Concentration of the standards					
Standard	B	C	D	E	F
Histamine (ng/mL = µg/L)	0.5	1.5	5	15	50
Histamine (nmol/L)	4.5	13.5	45	135	450
Conversion:	Histamine (ng/mL) x 9 = Histamine (nmol/L)				

2.6.1. The calibration curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x- axis).
2.6.2. Use a non-linear regression for curve fitting (e.g., spline, 4- parameter, akima).
2.6.3. The concentrations of the plasma samples and the controls can be read directly from the standard curve.

2.7. Quality control:
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit controls, or other commercially available controls, should fall within established confidence limits. The confidence limits of the kit controls are printed on the QC- Report.
2.7.1. Calibration:
The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. The extinction values also depend on the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20-25°C. In cases of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm.

2.4. Statistical Analysis:
Analysis of Variance (ANOVA) test was applied for statistical evaluation of the obtained results for each parameter according to Feldman et al. (2003).

3. RESULTS

As shown in Table (1) and figure (1) results indicated that the concentration of histamine in the examined samples of the three types of fish were varied from 2.75 to 34.06 mg/kg with average of 18.23 ± 1.56 mg/kg in *Oreochromis niloticus*; 2.11 to 29.52 mg /kg with an average of 14.90 ± 1.21 mg /kg for *Clarias lazera* and 1.87 to 22.31 mg /kg with an average of 12.65 ± 1.07 mg/kg for *Mormyrus niloticus*.

Table (2) showed the analysis of Variance (ANOVA) for histamine. It was revealed that there is a high significant difference among fish species (P<0.01).

Table 1 Prevalence and concentrations of histamine (mg %) in the examined samples of Nile fishes (n=30).

Nile fishes	+ve samples		Min	Max	Mean ± S.E
	No	%			
Oreochromis niloticus	21	70	2.75	34.06	18.23 ± 1.56
Clarias Lazera	16	53.33	2.11	29.52	14.90 ± 1.21
Mormyrus niloticus	10	33.33	1.87	22.31	12.65 ± 1.07

Figure 1 Average concentrations of histamine (mg %) in the examined Nile fish samples.

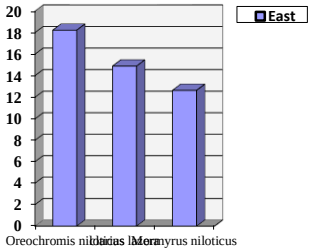

Table 2 Analysis of variance (ANOVA) of histamine levels in the examined Nile fish samples.

Source of variance	D.F	S.S	M.S	F. value
Total	89	657.4557		
Between Products (P)	2	117.4376	58.7189	9.46^{++}
Error	87	540.0181	6.2071	

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

According to Egyptian Organization of Standardization EOS (2010) which recommended that the maximal permissible limits for histamine is (20 mg/100g) in fish, in table (3) and figure (2), the number of un accepted samples were 8, 5 and 4 represented as 26.67 %, 16.67 % and 13.33% in *Oreochromis niloticus*, *Clarias lazera* and *Mormyrus niloticus*, respectively.

Table 3 Acceptability of the examined samples of Nile fishes on their histamine contents according to EOS (2010) (n=30)

Nile fishes	MRL (mg %) *	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Oreochromis niloticus	20	22	73.33	8	26.67
Clarias Lazera	20	25	83.33	5	16.67
Mormyrus niloticus	20	26	86.67	4	13.33
Total (90)		73	81.11	17	18.89

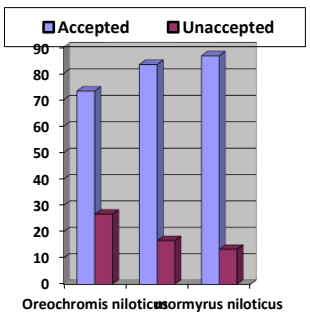

Figure 2 Acceptability of Nile fish samples on their histamine contents.

4. DISCUSSION

These results partially agree with those recorded by Pacheco *et al*. (1998) and Badran and Hussein (2000). While, higher results were reported by Soares and Gloria (1994) and Galarini *et al*. (1996) and lower than those recorded by Samaha *et al.* (1997). It was found that the human tolerance limit for histamine is 10 mg per 100 gm (Hastein *et al*., 2006).

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).

Certain bacteria produce the enzyme histidine decarboxylase during growth. This enzyme reacts with free histidine, a naturally occurring chemical that is present in large quantities in some fish than in others. The result is the formation of histamine. 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).

5. CONCULOSION

The obtained results in the current study allow to conclude that most of fish exposed for consumption were contaminated with different chemical residues such as histamine. Although biogenic amines formation is the result of bacterial growth, the presence of these undesirable compounds, especially histamine, is not always noticed by consumers. Thus, histamine is the main marker for the evaluation of quality and safety of fish. *Oreochromis niloticus* should be consumed with caution due to high levels of histamine which exceed the permissible limits in about 26.67 % of examined samples. *Mormyrus niloticus* is the lowest fish samples in histamine level which does not exceed the permissible limits in 86.67% of examined samples.

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