Histamine as a quality parameter in marine fish

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ABSTRACT

Histamine is a member of a group of compounds known as biogenic amines; Biogenic amines are biologically active compounds normally produced by decarboxylation of free amino acids and are present in a variety of foods, eg fish, fish products, meat, cheese and fermented foods. The presence of biogenic amines in these foods is an indicator of food spoilage. Histamine content is an essential quality parameter in sea food quality. In the present study a total of 90 random samples of fresh fishes represented by Pagrus, Barboni and Sardine (30 of each) were collected at different times from various fish markets in Gharbia governorate, Egypt, and examined for the presence of histamine by ELISA. The results revealed that 36.7%were acceptable and 63.3% non-acceptable for Sardine, 53.3% were acceptable while 46.7% non-acceptable for Barboni and 66.7% were acceptable and 33.3% non-acceptable for Pagrus.

Keywords: Histamine, ELISA, Marine fish.

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1. INTRODUCTION

Fish is a great source of protein, vitamins, minerals, omega-3 fatty acids and a key nutrient for brain development. A well-balanced diet that includes a variety of fish and shellfish can contribute to heart health and children's proper growth and development (Jaclyn et al., 2010). Seafood may harbor a number of biological, chemical, and physical hazards, the most prevalent of which are biogenic amines (BAs) and bio toxins (chemical), pathogenic bacteria and viruses (biological), and metal inclusion (physical). 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). Consumption of histamine can result in toxicological effects to consumers such as hypertension, headache, diarrhea, rash, and localized inflammation when ingested in extreme amounts, cardiac palpitation and even death in very severe cases (Rawles et al., 1996). Assessing biogenic amine presence is important not only from a toxicological point of view, but because these substances can be used as indicators of food degree of freshness or spoilage (Silva et al., 2013). The most important BAs, histamine, tyramine, tryptamine, putrescine, and cadaverine, are formed from free amino acids. They added that BAs accumulation in food requires the presence of microorganisms with amino acid decarboxylases and favorable conditions for their growth and decarboxylation activity. The consumption of high amount of BAs, above all histamine, can result in food borne poisoning which is a worldwide problem (Zarei et al., 2011). The most common fish associated with histamine fish poisoning are scombroid fish poisoning, the scombroid fish including tuna, mackerel, and non scombroid fish include sardine herring and anchovy (Flick et al., 2001).

The symptoms of HFP ranged from mild urticaria and oral allergy-like syndrome to life-threatening cardiovascular reactions that can be mistaken for seafood allergy (Lionte, 2010). Histamine poisoning may not be caused to all the people consuming contaminated fish Thus, even if the same histamine-containing fish is ingested, some consumers may be poisoned and some may not (Tao et al., 2009). Further, the accumulation of biogenic amines in fish is involved in nitrosamine formation (known as carcinogens) (Yurchenko and Molder, 2006).

2. MATERIAL AND METHODS

2.1. Collection of samples:

A total of 90 random samples of fresh fishes represented by Pagrus, Barboni and Sardine (30 of each) were collected at different times from various fish markets in Gharbia governorate, Egypt.

Each sample was kept in a separated sterile plastic bag and preserved in an ice box then transferred to the laboratory under complete aseptic conditions without undue delay. The collected samples were subjected to the chemical examinations to evaluate their freshness through determination of proteolytic and lipolytic indices as well as their histamine contents.

2.2. Determination of histamine by ELISA:

2.2.1. Sample preparation and acylation:

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. Then add 25 µL of Acylation Buffer to all wells; add 25 µL of Acylation Reagent to all wells after that incubate for 45 min at RT (20-25°C) on a shaker (approx. 600 rpm); add 200 µL of distilled water to all wells. Then 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.

2.2.2. Histamine ELISA:

Pipette 25 µL of the acylated standards, controls, and samples into the appropriate wells of the Histamine Microtiter Strips. And pipette 100 µL of the Histamine Antiserum into all wells and cover plate with Adhesive Foil. 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. Then 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. Pipette 100 µL of the Enzyme Conjugate into all wells. Incubate for 30 min at RT (20-25°C) on a shaker (approx. 600 rpm). Then 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. Pipette 100 µL of the Substrate into all wells and incubate for 30 min at RT (20-25°C) on a shaker (600 rpm). Avoid exposure to direct sunlight. Add 100 µL of the Stop Solution to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution. 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.

3. RESULTS

It is evident from the results recorded in table (1) that the Histamine values in examined fish samples were varied from 5.4 to 65.5mg% with an average of 36.87± 1.53 mg% for Sardine; 3.9 to 61.5 mg% with an average 30.14±1.38 mg% for Barboni and 2.2 to47.8 mg% with an average 18.36±0.95 for Pagrus. The differences between the examined samples of different fish species were high significant (P<0.01). Table (2) revealed that 36.7%, 53.3% and 66.7% of the examined samples of sardine, Barboni and Pagrus were accepted, however, 63.3%, 46.7% and 33.3% of such samples were unaccepted, respectively.

4. DISCUSSION

Results were nearly similar to those obtained by (Humaid and Mamdoh, 2014) detected histamine formation in three fish species, Indian mackerel (*Rastrelliger kanagurta*),Goldstripe sardinella (*Sardinella gibbosa*) and Pink ear emperor (*Lethrinus lentjan*) were monitored during storage at 4ºC for 144 hours and 25ºC for 24 hours. Throughout the storage periods, Pink ear emperor had the lowest levels of histamine, 1.3±0.2ppm and 3.4±0.4 ppm respectively, while in Indian mackerel and Gold stripe sardinella reached to13.0±2.6 ppm, 7.0±0.2 ppm at 4ºC and 175.0±2.6 ppm, 122.9±3.5 ppm at 25ºC, respectively. While, higher results were obtained by Rajapaksha and Jayakody (2008) who found that fish at the landing sites had the lowest histamine level in a study done at landing sites in Sri Lanka (range = 0.223-9.4 ppm). Lower than those obtained by (Kim et al., 2009) who measured the histamine levels of Saury, Mackerel, Spanish mackerel and Amberjack stored at 25ºC for 24 hours, were 2123.9, 1776.7, 189.9 and 36.6ppm, respectively. Fish commonly implicated in histamine fish poisoning include both scombroid (mackerel, tuna and saury) and non- scombroid (sardine, anchovies, blue fish, as they contain large amount of free histadine (Lehane and Olley, 2000).

The hazardous level of histamine for human health has been suggested as 50 mg%, although, low levels as 5mg%have been reported in histamine poisoning (Huss et al., 2003). The content of biogenic amines differs according to species for example Scombridae family, such as tuna and bonito and Clupeidae family, such as

Table (1): Statistical analytical results of histamine (mg %) as quality index of spoilage in the examined samples of fish (n=30).

	Positive samples				
Fish species	No.	%	Min	Max	Mean ± S.E*
Sardine	30	100	5.4	65.5	36.87 ± 1.53++
Barboni	28	93.3	3.9	61.5	30.14 ± 1.38
Pagrus	27	90.0	2.2	47.8	18.63 ± 0.95

S.E* = standard error of mean. ++ = High significant differences (P<0.01)

Table (2): Acceptability of the examined samples of fish based on their levels of histamine (n=30).

	MRL	Accepted samples		Unaccepted samples	
Fish Products	(mg %)*	No.	%	No.	%
Sardine	20	11	36.7	19	63.3
Barboni	20	16	53.3	14	46.7
Pagrus	20	20	66.7	10	33.3

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

sardines characterized by the presence of high levels of free histamine in their muscle, also according to the season of the year, genetics, environment, food, sex, physiological stage, storage period and sampled tissue (Lee et al., 2012). High levels of biogenic amines can be prevented by the application of good hygiene practices and proper temperature during handling, delivery and storage (Visciano et al., 2012). Preventive measures of freezing and cooling directly after death will prevent rapid development of enzyme histidine decarboxylase, because the hazard control is no possible after formation of enzyme (Anon, 2001). Biogenic amines can be used as quality index, once they are formed by bacterial activity and are resistant to thermal treatment, thus reflecting the quality of the raw material and hygienic conditions of food processing (Sagratini et al., 2012). Acceptability of examined fish samples based on their levels of histamine according to EOS (2005) the results revealed that 36.7%were acceptable and 63.3% non-acceptable for Sardine, 53.3% were acceptable while 46.7% non-acceptable for Barboni and 66.7% were acceptable and 33.3% non-acceptable for Pagrus. Unfortunately, unhygienic practices, in sufficient refrigeration cause increase susceptible to contamination by BAs-producing microorganisms and other spoilage bacteria (proteolytic and lipolytic) leading to rapid spoilage and outbreaks of fish poisoning.

As conclusion, histamine is the main marker for the evaluation of quality and safety of fish. Also, the application of good hygiene practices and proper temperatures during handling, delivery and storage reduce the bacterial growth and multiplication with further undesirable changes.

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