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Determination of Histamine in Various Marine Fish Species Available in Major Markets of Punjab, Pakistan by LC-MS/MS

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Abstract

Fish samples from selected five (5) major markets of Punjab (Lahore, Faisalabad, Multan, Rawalpindi and Gujranwala) were collected randomly consisting of nine (9) marine fish species. LCQ Fleet with ion trap mass analyzer coupled with Surveyorplus pump and Surveyorplus degasser of ThermoScientific was used for histamine determination in present study. The histamine detected in different nine (9) samples from different markets was within safe limits. The fishes were also examined for appearance, texture, odour and presence of black spots. All the physical parameters were within normal range. It was concluded that the marine fish species being sold in various fish markets of Punjab province are fresh with good physical conditions and having histamine in safe limits.

Keywords: Histamine, Marine Fish, Punjab, Pakistan, LCMS, Markets, FQCL.

INTRODUCTION

Histamine is a primary amine formed in the muscle of fish having free histidine by the action of specific bacteria Eitenmiller and Desouza (1984). The histamine development is temperature and pH dependent as produces in the result of histidine deamination followed by bacterial activity. According to Joshi & Vishal (2011) the histamine producing factors include type and size of fish, handling techniques and cooling method.

The level of histadine in fish is exogenous and indicates the spoilage of fish. Histamine is heat stable and cannot be destroyed by cooking, canning, smoking and freezing Becker *et al.* (2001). Spoiled fish containing high levels of histamine produced by specific bacteria due to incorrect storage results histamine poisoning Sabroe and Kobza (1998).

Histamine poisoning caused by elevated level of histamine being present in fish is a type of food poisoning. Fish is an important source of protein, minerals and vitamins readily deteriorates when decarbolase enzyme is farmed by bacterial growth causing generation of bioactive histamine Moreno and Torres (2001). The food born diseases and intoxication occur from several minutes to several hours after ingestion of fish with high levels of histamine. The illness includes burning, itching, edema, cutaneous rash, nausea, urticarial, gastrointestinal inflammation, hypotension, vomiting, diarrhea, haemodynamic, and neurological headache may continuous for several days Hall (2003).

Histamine taken orally is less toxic than histamine consumed through spoiled fish due to missing factor immdazole compound. The compound derived from histidine consequently increase the histamine poisoning problem Lehane and Olley (2000). The sensory analysis can not determine the presence or absence of histamine as decomposed product (determined organoleptically) does not always produce histamine and the presence of histamine does not always occur in decomposed products. Histamine could reliably be quantified by chemical analysis down to 5 ppm FDA (2013).

The tropical conditions of Pakistan are suitable for proliferation of histamine forming bacteria in fish and fish products. Various stages of fish handling (harvest, procurement, retail marketing) and processing (drying, salting, freezing etc.) have profound effect on histamine formation. Thus the present study was formulated by systematical random sampling of 9 marine fish species from the five major cities (Lahore, Multan, Gujranwala, Faisalabad and Rawalpindi) to check the level of histamine by using the LC-MS/MS.

MATERIALS AND METHODS

Sample Collection

Marine fish samples of nine species were collected from major markets of Punjab (Lahore, Faisalabad, Multan, Rawalpindi and Gujranwala). Each individual sample was placed separately in aseptic plastic bag on ice, thoroughly identified and delivered to the lab.

Chemicals and Reagents

Methanol (HPLC grade), Formic acid, Histamine and Deionized water.

Standard Preparation

Histamine stock solution was prepared at concentration of 1mg ml^{-1} in methanol. The working standards were prepared as per requirement from the stock solution.

Sample Preparation

For the analysis, 50 gm of fish sample was chopped into small pieces and finely ground with a blender to homogenize it. A total of 5gm of fish sample was taken in 100 mL volumetric flask and volume was made with 70% methanol solution. Then the sample was treated with ultrasonic radiations for 30 minutes in sonicator. Second dilution was made by taking 1 mL of sonicated mixture in 10 mL 70% methanol and sample was filtered through 0.45 μm filter paper following the method to determine Histamine AOAC (2012).

Instrumentation

In the present study histamine analysis was carried out by LC-MS/MS in all freshly prepared samples with following given conditions of instrument in table 1.

Table 1. Instrumental parameters

LC System:	Thermo Fisher Surveyor LC Pump plus serial no. 500294
Column:	Hypersil Gold C ₁₈ , Dim (mm) 250 x 4.6
Mobile Phase:	Isocratic mobile phase composition of methanol: 0.1% formic acid (30:70 v/v). The mobile phase components were degassed with Super Sonic WUC-A06H Sonicator before use.
Flow Rate:	250 μL
Injection Vol.:	25 μL
MS:	LCQ Fleet mass spectrometer system, serial no. LCF 10334
Ion Source:	APCI
Polarity:	Positive
Mode:	SRM
Precursor ion	112 amu

Quantification

Quantification of histamine in standards and fish samples. Data was analyzed statistically using SPSS software.

RESULTS AND DISCUSSION

The samples description i.e. common and scientific name, No. of fish samples collected from each cited city, and origin are shown in Table # 2. All the observed physical parameters i.e. appearance, odor, texture, presence of Black spot are recorded in the Table # 3. A Total of eighty seven samples were analyzed and the outcomes found within the permissible limits given in Table 4.

The histamine is not detected in Red Snapper, Round Sol, White dandia, Pangasius, White Pomfret, lady fish and

Mushka procure from the selected areas as shown in Table# 4. However, the amount of histamine is within the permissible limits detected in Sea Bam and Indian Mackerel sampled from all cited cities. The histamine value in Sea Bam collected from Lahore, Gujranwala, Faisalabad, Rawalpindi and Multan were 33.27 ± 1.02 , 42.92 ± 1.50 , 55.24 ± 0.98 , 26.23 ± 0.98 , and 87.45 ± 0.78 mg/kg, respectively. While the corresponding analyzed values in the Indian Mackerel were 20.08 ± 0.51 , $.27 \pm 1.04$, 38.41 ± 1.01 and 14.16 ± 0.85 mg/kg, which are under the permissible limit (200 mg/kg) defined by the FAO/WHO, 2012. The histamine was not detected in samples of Indian Mackerel collected from Gujranwala. In this way, histamine was detected only in nine samples among all.

Table 2. The Common and Scientific Names of the Fish Species

Sr#	Common Names	Scientific Names	No. of Fish	Origin
1	Indian Mackerel	<i>Rastrelliger kanagurta</i>	15	Marine
2	Lady Fish	<i>Elope saurus</i>	3	Marine
3	Mushka	<i>Sciaenops ocellatus</i>	6	Marine
4	Pangasius	<i>Pangasius pangaius</i>	15	Marine
5	Red Snappers	<i>Lutjanus campechanus</i>	6	Marine
6	Round Sol	<i>Cynoglossus lingua</i>	9	Marine
7	Sea Bam	<i>Mastacembelus armatus</i>	15	Marine
8	White Dandia	<i>Rasbora dandia</i>	6	Marine
9	White Pomfret	<i>Pampus argenteus</i>	12	Marine

Table 3. Results of Physical Parameters

Sr#	Common Names	No. of Fish	Appearance	Texture	Odour	Presence of Black Spot
1	Indian Mackerel	15	Fresh	Soft	Odourless	No
2	Lady Fish	3	Fresh	Soft	Odourless	No
3	Mushka	6	Fresh	Soft	Odourless	No
4	Pangasius	15	Fresh	Soft	Odourless	No
5	Red Snappers	6	Fresh	Soft	Odourless	No
6	Round Sol	9	Fresh	Soft	Odourless	No
7	Sea Bam	15	Fresh	Soft	Odourless	No
8	White Dandia	6	Fresh	Soft	Odourless	No
9	White Pomfret	12	Fresh	Soft	Odourless	No

The values of histamine determined in the Indian Mackerel were 20.08 ± 0.51 , $.27 \pm 1.04$, 38.41 ± 1.01 and 14.16 ± 0.85 which are for less than the values of histamine determined in fresh mackerel samples by Joshi and Vishal (2011). They have found a value around 20 mg/100 gm

which is much above the defect action level given by FDA indicating potential risk for histamine poisoning. The results of present study are also in line with the study of Ali *et al.* (2016) where the histamine level in indian mackerel was found to be 144.72 mg/kg.

Table 4. Results of Histamine

Sr#	Fish Markets	Fish Species	No. of Fish	Histamine (ppm)
1	Lahore	Indian Mackerel	3	20.08 ± 0.51
2		Red Snappers	3	ND
3		Round Sol	3	ND
4		White Dandia	3	ND
5		Pangasius	3	ND
6		White Pomfret	3	ND
7		Lady Fish	3	ND
8		Mushka	3	ND
9		Sea Bam	3	33.27±1.02
1	Faisalabad	Indian Mackerel	3	65.27±1.04
2		Pangasius	3	ND
3		Red Snappers	3	ND
4		Mushka	3	ND
5		Sea Bam	3	55.24 ±0.98
1	Gujranawala	Sea Bam	3	42.92 ±1.50
2		Indian Mackerel	3	ND
3		Pangasius	3	ND
4		White Pomfret	3	ND
5		Round Sol	3	ND
6		White Dandia	3	ND
1	Rawalpindi	Sea Bam	3	26.23±0.98
2		Indian Mackerel	3	38.41±1.01
3		Pangasius	3	ND
4		White Pomfret	3	ND
5		Round Sol	3	ND
1	Multan	Sea Bam	3	87.45±0.78
2		Pangasius	3	ND
3		Indian Mackerel	3	14.16±0.85
4		White Pomfret	3	ND

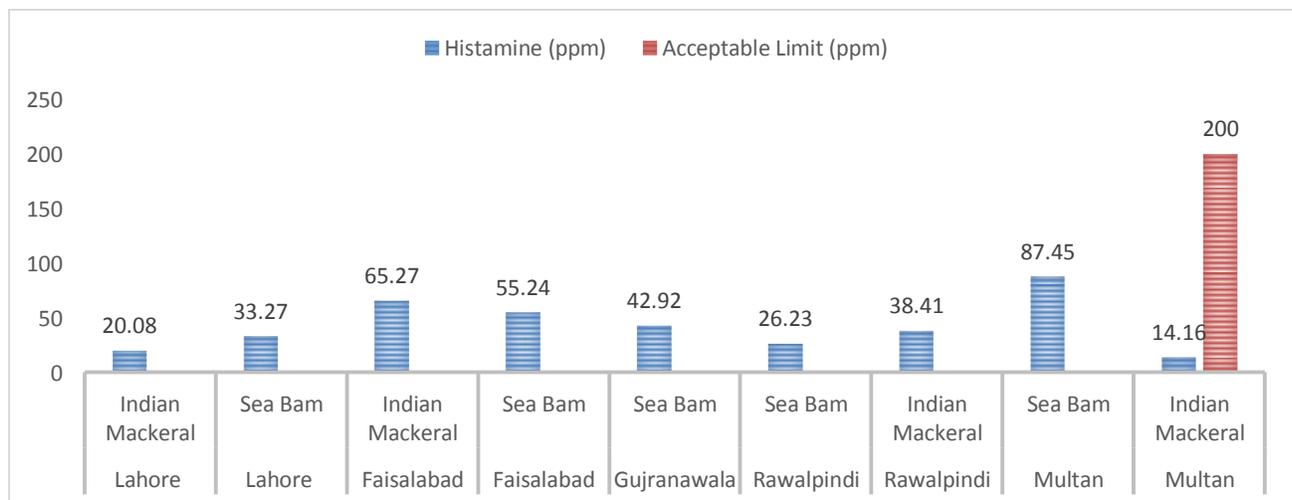


Fig. 1. Graphic representation of determined histamine in different fish species

This study also agree with the Nahla *et al.* (2005) who determined the histamine level using TLC in some local fishes *Euthynnus affinis* “Kawakaw”, *Sardinella gibbosa* and *Mugil cephalus* “Mullet” and found a minimum and maximum level of histamine 18-26, 13-19 and 7-15 mg/100gm, respectively. Similarly they determined the histamine level in some imported fishes *Trachurus* “Atlantic Horse Mackerel”, *Orcynopsis unicolor* “Plain bonito” and *Sardina pilchardus* “European pilchard” and found a minimum and maximum level of histamine 39-50, 22-39 and 18-27 mg/100 gm, respectively. They concluded that significant decomposition and histamine formation can be avoided by good fish handling practices including icing or rapid immersion of the catch in water chilled to (-1°C).

In our study the histamine was either found absent in the marine fish samples or if present are within the safe limit. This situation got agree with the results of Eitenmiller and Desouza (1984) who stated that fresh fish do not contain free histamine.

In the present investigation none of the fish sample contain histamine levels greater than equal to 200 mg/kg. Since, the histamine levels of 200 and 500 mg/kg are considered as spoilage and hazard action levels respectively by the FDA of USA. Our research also agree with the results shown by Chamberlain (2001) who conducted studies on histamine levels on long lined tuna in Fiji and undertake a comparison of histamine production between species samples, locations and temperature. His results revealed that when fish samples were stored at 6 °C then the histamine level remain well below the recommended maximum value.

In this study the fish was well preserved and maintained at low temperature so the histamine was not found in fish samples tested. This is also in line with the study of Bhilng and Taylor (1982) who stated that rapid chilling of fish during postharvest and storage below refrigerated temperature is common used in controlling histamine formation.

CONCLUSION

It is concluded that the marine fish being sold at different markets in Punjab is safe for use as histamine is either absent or where present is within the safe limits for human consumption.

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CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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