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Determination of histamine levels by LC-MS/MS in various fish species available in the local markets of Punjab, Pakistan

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Abstract

Sixty apparently fresh and properly iced samples of fish species were randomly collected from five major markets of Punjab, Pakistan. These samples were transported to the Fish Quality Control Laboratory, FR&TI, Lahore kept in ice boxes and temperatures below -1 °C. The samples were examined for Physical Parameters including Appearance, Glazing, Texture, Odour, Presence of Black Spots and Chemical Parameter including determination of Histamine levels by LCMS/MS. All Physico-Chemical Parameters were found within normal range. Histamine was either not detected or found within permissible limits as compared with International Standards for human consumption. In conclusion, significant decomposition and histamine formation can be avoided by good fish handling practices including icing or rapid immersion of the catch in chilled water followed by uninterrupted frozen storage.

Keywords: Histamine levels; LCMS/MS; fish species; fish market

1. Introduction

Fish is an important and good quality source of protein, vitamins and minerals but it deteriorates fastly when decarboxylase enzyme is formed by bacterial growth causing generation of bioactive amine including histamine [1]. Histamine poisoning, also known as scombroid poisoning, is a type of food poisoning caused by elevated level of histamine being present in fish. Its formation is typically associated with decomposition of the fish and fish products. The sensory analysis cannot always ensure the presence or absence of histamine in a decomposed product (determined organoleptically), however, this can reliably be quantitatively chemical analyzed down to 5 ppm [2].

Histamine is a heterocyclic biologically active primary amine formed by post-mortem in fish muscle rich in free histidine by the action of certain bacteria in addition to the histamine released during decomposition and spoilage by proteolysis [3]. Its level produced in fish is mainly exogenous and serves as an indicator of spoilage [4]. It is heat stable and once formed cannot be destroyed by cooking, canning, smoking or freezing [5]. Histamine poisoning basically results from consumption of spoiled fish containing its high levels, resulting from growth and action of certain bacteria aided due to incorrect storage of fish [6]. Such fish consumption can cause food born disease / intoxication and the toxicity occur from several minutes to several hours after ingestion of toxic fish and the illness typically lasts few hours but may continue for several days which include cutaneous rash, urticaria, burning itching, edema, gastrointestinal inflammation, nausea, vomiting, diarrhea, hemodynamic, hypotension and neurological headache [7]. Histamine consumed through spoiled fish is more toxic than pure histamine taken orally due to imidazole compound derived from histidine consequently increasing the histamine poisoning problem [8]. Bodmer *et al.* [9] worked on critical steps of histamine formation resulting from microbial contamination during foods and beverages production processes. They established technology based methods for reduction of histamine levels.

The tropical condition of Pakistan with an average temperature ranging between 25-40 °C is 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.) can have profound effect on histamine formation. Thus this project was planned

to check the histamine levels/loads in various fish species procured from the commercial fish markets; to ascertain their safety for human consumption and to compare this with International Standards.

The aim of the research was to study the histamine contamination levels in various fish species available in the five major markets of Punjab, Pakistan to ensure the food safety levels.

2. Materials and Methods

2.1 Sample Collection

Fish samples of various species were collected using the sterile aseptic bags, from various commercial Fish markets in Punjab viz. Lahore, Faisalabad, Gujranwala, Multan, Rawalpindi including a Lahore Super Store (Fig 1). The net weight, size and date of expiry of each fish were noted from the information provided on the packing label. Each individual sample was thoroughly identified, placed separately in plastic bag kept in ice box and transported to the Fish Quality Control Laboratory, FR&TI, Lahore.

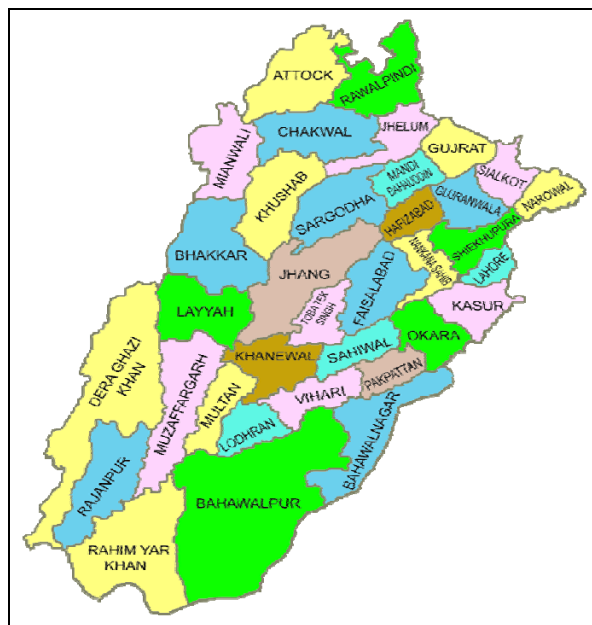


Fig 1: Punjab Map including Five Locations under Study

2.2 Chemicals and Reagents

Methanol (HPLC grade), Formic acid, Deionized water

2.3 Standard Preparation

Histamine stock solution was prepared at concentration 1 mgml⁻¹ in methanol and the working standards were prepared as per requirement from the stock solution.

2.4 Sample Preparation

For the analysis, a representative portion of fish sample (50 g) was chopped into small pieces and finely ground with a blender to homogenize it before extraction. A total of 5gm of fish sample (in triplicate) was taken in 100 mL volumetric flask and volume was make up with 70% methanol solution. The sample was then treated with ultrasonic radiations for 30 minutes in sonicator. Second dilution was carried out by

taking 1 mL sample in 10 mL methanol and sample was filtered through 0.45 um filter paper following Standard Methods [10]. The determination of Histamine is carried out in triplicate for each sub-sample and the average value for sample was considered.

2.5 Instrumentation

Liquid Chromatography-Mass Spectrometer (LC-MS/MS), LCQ Fleet with Ion Trap Mass Analyzer coupled to Surveyor Plus Pump and Surveyor Plus Degasser of Thermo Scientific was used for the present study. The separation was achieved using Hypersil Gold C-18 column (250x 4.6 mm). APCI in +ve mode was used for present study. The full scan mass range m/z was 50-150 amu and corresponding mass spectra of histamine was at 112 amu (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 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

2.6 Quantification

Quan Browser of Xcalibur (software) was used for quantification of histamine in standards and fish samples.

3. Results

In the present study, sixty fish samples from a variety of twenty-five different fish species were collected from

randomly selected sites within the jurisdiction of the province of Punjab, Pakistan for the determination of histamine levels. These included five major commercial fish markets lying in Lahore, Faisalabad, Gujranwala, Multan, Rawalpindi and Hyper star Super Store situated in Lahore. The common and scientific names of the fish species, weight and length are recorded in Table 2.

Table 2: The Common and Scientific Names along with Weight and Length of the Fish Species in the Present Study

S. No.	Common Names	Scientific Names	No. of Fish	Average Weight gm	Average Length cm	Origin
1.	Rohu	<i>Labeo rohita</i>	5	515.00	33.40	Fresh water
2.	Mori	<i>Cirrhinaus mrigala</i>	4	507.25	34.03	Fresh water
3.	Thaila	<i>Catla catla</i>	4	527.38	31.88	Fresh water
4.	Silver carp	<i>Hypophthalmichthys molitrix</i>	2	690.50	38.75	Fresh water
5.	Grass carp	<i>Ctenopharyngodon idella</i>	3	457.10	33.90	Fresh water
6.	Indian Mackerel	<i>Rastrelliger kanagurta</i>	3	177.90	9.77	Marine
7.	Pangasius	<i>Pangasius pangaius</i>	3	287.13	Fillets	Marine
8.	Sea Soul	<i>Solea solea</i>	3	110.77	Fillets	Marine
9.	Reedfish (Read Bagti)	<i>Erpetoichthys calabaricus</i>	3	157.63	Fillets	Marine
10.	Sea Bam	<i>Mastacembelus armatus</i>	3	178.10	Fillets	Marine
11.	Khagga	<i>Arius maculatus</i>	3	355.33	31.00	Fresh water
12.	Kulbans	<i>Labeo calbasu</i>	1	490.00	32.00	Fresh water
13.	Mallai	<i>Wallago attu</i>	1	390.00	28.00	Fresh water
14.	Singhari	<i>Sperata sarwari / Aorichthys aor sarwari</i>	1	350.00	33.00	Fresh water
15.	Buchwa	<i>Clupisoma garua</i>	1	607.00	40.00	Fresh water
16.	Red Snappers	<i>Lutjanus campechanus</i>	2	128.00	Fillets	Marine
17.	Pangasius	<i>Pangasius pangaius</i>	2	209.00	Fillets	Fresh water
18.	Round Sol	<i>Cynoglossus lingua</i>	2	133.00	28.00	Marine
19.	White Dandia	<i>Rasbora dandia</i>	2	252.00	27.50	Marine
17.	Indian Mackerel	<i>Rastrelliger kanagurta</i>	2	164.00	23.00	Marine
18.	Sea Squid	<i>Loligo vulgaris</i>	2	158.50	Fillets	Marine
19.	White Pomfret	<i>Pampus argenteus</i>	2	100.00	21.00	Marine
20.	Lady Fish	<i>Elope saurus</i>	2	121.00	16.00	Marine
21.	Chakori	<i>Polydactylus plebeius</i>	2	145.50	20.00	Marine
22.	Mushka	<i>Sciaenops ocellatus</i>	2	252.50	34.50	Marine

The summarized results given in Table-3 showed the observed physical parameters i.e. appearance, glazing, texture, odour, presence of black spot and recorded histamine levels for fish samples from above cited markets and also

from Hyper star Super Store, Lahore (Table-4). All the physico-chemical parameters were found in normal range for all of the fish samples from all the sites under consideration.

Table 3: Physical Parameters and Histamine Concentration in Collected Fish Species

S. No.	Fish Markets	Fish Species	Parameters studied					
			Appearance	Glazing	Texture	Odour	Presence of black spots	(ppm)
1.	Lahore	Rohu	Fresh	Yes	Soft	Odourless	No	ND
2.		Thaila	Fresh	Yes	Soft	Odourless	No	ND
3.		Silver carp	Fresh	Yes	Soft	Odourless	No	ND
4.		Grass carp	Fresh	Yes	Soft	Odourless	No	ND
5.		Indian Mackerel	Fresh	Yes	Soft	Odourless	No	ND
6.		Pangasius	Fresh	Yes	Soft	Odourless	No	ND
7.		Sea Soul	Fresh	No	Soft	Odourless	No	31.72±1.78
8.		Read Bagti	Fresh	Yes	Soft	Odourless	No	ND
9.		Sea Bam	Fresh	No	Soft	Odourless	No	41.12±1.56
10.		Khagga	Fresh	Yes	Soft	Odourless	No	ND
11.	Faisalabad	Kulbans	Fresh	Yes	Soft	Odourless	No	ND
12.		Rohu	Fresh	Yes	Soft	Odourless	No	ND
13.		Mori	Fresh	Yes	Soft	Odourless	No	ND
14.		Khagga	Fresh	Yes	Soft	Odourless	No	ND
15.		Mallai	Fresh	Yes	Soft	Odourless	No	ND
16.		Shanghri	Fresh	Yes	Soft	Odourless	No	ND
17.	Gujranwala	Thaila	Fresh	Yes	Soft	Odourless	No	ND
18.		Mori	Fresh	Yes	Soft	Odourless	No	ND
19.		Rohu	Fresh	Yes	Soft	Odourless	No	ND
20.		Khagga	Fresh	Yes	Soft	Odourless	No	ND
21.		Buchwa	Fresh	Yes	Soft	Odourless	No	ND

22.	Multan	Rohu	Fresh	Yes	Soft	Odourless	No	ND
23.		Mori	Fresh	Yes	Soft	Odourless	No	ND
24.		Thaila	Fresh	Yes	Soft	Odourless	No	ND
25.		Grass carp	Fresh	Yes	Soft	Odourless	No	ND
26.	Rawalpindi	Rohu	Fresh	Yes	Soft	Odourless	No	ND
27.		Mori	Fresh	Yes	Soft	Odourless	No	ND
28.		Thaila	Fresh	Yes	Soft	Odourless	No	ND
29.		Grass carp	Fresh	Yes	Soft	Odourless	No	ND
30.		Silver carp	Fresh	Yes	Soft	Odourless	No	ND

ND- Not Detected; All values are average values of three determinations.

Table 4: Physical Parameters and Histamine Conc. in Collected Fish Species from Hyper Star Super Store, Lahore

S. No.	Fish Markets	Name of Fish Species	Parameters studied					Histamine (ppm)
			Appearance	Glazing	Texture	Odour	Presence of black spots	
1.	Hyper Star Super Store, Lahore	Red Snappers	Fresh	No	Soft	Odourless	No	47.73±2.09
2.		Pangasius	Fresh	Yes	Soft	Odourless	No	ND
3.		Round Sol	Fresh	Yes	Soft	Odourless	No	ND
4.		White Dandia	Fresh	Yes	Soft	Odourless	No	ND
5.		Indian Mackerel	Fresh	No	Soft	Odourless	No	144.72±2.47
6.		Sea Squid	Fresh	Yes	Soft	Odourless	No	ND
7.		White Pomfret	Fresh	Yes	Soft	Odourless	No	ND
8.		Lady Fish	Fresh	Yes	Soft	Odourless	No	ND
9.		Chakori Fish	Fresh	Yes	Soft	Odourless	No	ND
10.		Mushka Fish	Fresh	Yes	Soft	Odourless	No	ND

ND- Not Detected; all values are average values of three determinations.

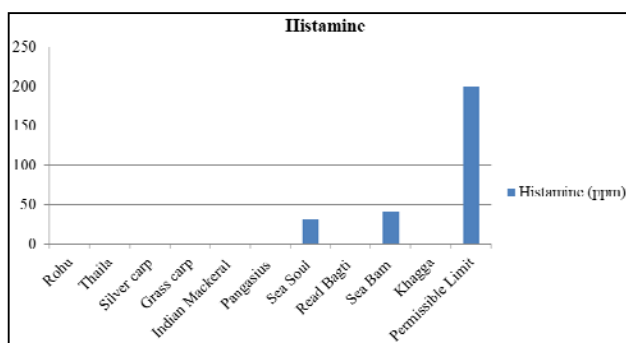


Fig 2: Histamine Concentration in Marine Fish Species of Lahore Fish Market

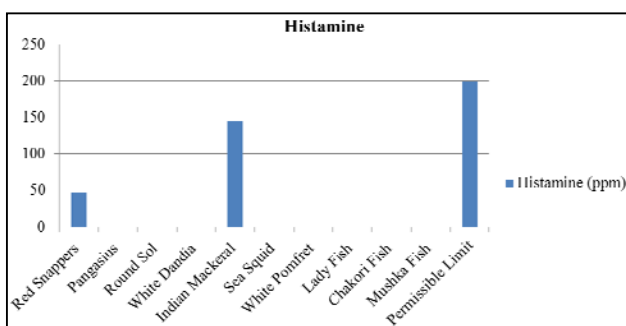


Fig 3: Histamine Concentration in Marine Fish Species of Hyper Star Super Store, Lahore

4. Discussion

The histamine analysis was performed by LC-MS/MS following procedures of AOAC, 2012 [10] in all prepared fish samples. It can be depicted from Table-3 that the histamine contents were not detected in Rohu, Mori, Thaila, Grass Carp, Silver Carp, Indian Mackerel, Pangasius, Read Bagti, Khagga, Kulbans, Mallai, Shangri and Buchwa obtained from all the five source markets in Punjab under consideration. However, the histamine was detected in Sea Soul and Sea Bam samples

(Fig 2) collected from Lahore fish market. The histamine values in Sea Soul were 31.72±1.78 mgkg⁻¹ and in Sea Bam were 41.12±1.56 ppm which was within permissible limits i.e. below 200 ppm [11].

Table-4 showed that the histamine contents were absent in Pangasius, Round Sol, White Dandia, Sea Squid, White Pomfret, Lady Fish, Chakori and Mushka while the histamine contents were present in Red Snapper and Indian Mackerel from Hyperstar Super Store, Lahore. The histamine concentrations found in Red Snapper was 47.73±2.09 ppm and in Indian Mackerel was 144.72±2.47 ppm (Fig 3). However, these values were also within the permissible limits i.e. below 200 ppm, as recommended by the FAO / WHO, 2012 [11]. The results revealed that histamine was found absent in samples of all fresh water fish species while it was present in varying amounts though within limits in marine fish samples. These findings are in agreement with the previous research findings that histamine is developed only in rotten fish from marine sources and is not a problem of fresh water fish. The histamine contents were either found absent in the fish samples; or if present were found within the normal limits, this situation got agreed with the results of Eitenmiller and Desouza [3] who have stated that fresh fish do not contain free histamine. Since, in this study all the fishes in the markets were fresh and properly iced. The fishes transported to the lab in the ice boxes and temperature kept below -1 °C hence there was no opportunity for histamine producing bacteria to develop which was in accordance with the studies of Joshi and Bhoir [12] who explained that the factors affecting the growth of histamine producing bacteria include size and type of fish, handling techniques and cooling method.

In the present investigation, none of the fresh water and marine fish contained histamine levels >500 mg/kg since the histamine levels of 200 and 500 mgkg⁻¹ are considered as spoilage and hazard action levels respectively by the Food and Drug Administration of the USA [5]. Our research agreed the results shown by Chamberlain [13] who conducted studies on histamine levels in long lined tuna in Fiji and undertook a comparison of histamine production between species, sample

location and temperatures. His results revealed that when fish samples were stored at 6 °C, then the histamine levels remain well below the recommended maximum value; this research validated the recommended USDA guidelines.

Our research has also been supported by the results of Nahla *et al.* [14] who worked on histamine and histamine producing bacteria in local / imported fish and their public health significance. They examined 180 apparently healthy fish samples for counting, isolation and identification of total aerobic and histamine producing bacteria and for determination of the histamines level in each sample. The mean value of total aerobic and histamine producing bacteria count of local fish samples were higher than that of imported fish samples while the mean value of histamine levels in the muscle tissue of local fish samples were lower than that of imported fish samples. A cleanup procedure has been introduced by Brillantes and Samosorn [15] using a solid phase extraction technique and High Pressure Liquid Chromatography (HPLC) method for determination of histamine in fish sauce from Thailand. They analysed 549 commercial fish sauce samples showing that the samples contain histamine at various levels with the majority ranging from 200-600 mg/liter.

Robert *et al.* [16] have analyzed eleven biogenic amines, indicator of food freshness, in food / beverages and described the development of an LC/MS/MS Technique and further investigated that the biogenic amines like histamine are produced naturally in food by enzymatic decarboxylation of free amino acids. Joshi and Bhoir [12] have used a simple and rapid colorimetric method for quantification of histamine in fish level in fresh mackerel samples of Kalian city, India which was found to be around 24mg/100 gm, much above the defect action level (5 mg/100 gm) given by Food and Drug Administration [4] indicating potential risk for histamine poisoning.

5. Conclusion

The results of this study proved that histamine is not a problem of the fresh water fish species as none of these contained histamine contents, however, it may cause a problem in the marine fish samples as these may contain the histamine contents. It can also be concluded that the significant decomposition of fish and histamine formation therein can be avoided by good fish handling practices including icing or rapid immersion of the catch in water chilled to -1 °C followed by uninterrupted frozen storage. Moreover, our study suggested that practice of more hygienic and sanitary conditions during handling and processing of fish can minimize the contamination due to histamine production.

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