Dept. of Pharmacology.
Faculty of Med. Assiut University.
Head of Dept. Prof. Dr. F. Hussein.

HISTAMINE CONTENT IN TWO TYPES OF FRESH WATER FISH UNDER DIFFERENT STORAGE CONDITIONS

(With 6 Tables)

By

A.ASSI; A.SH. SEDDEK* and SH. M. FATHI**

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مستوى الهستامين تحت ظروف مختلفه من التخزين فى نوعين من أسماك المياه العذبه

عبد العظيم عاصى ، عبد اللطيف شاكر ، شوكت فتحى

تم فى هذه الدراسة أستخدام طريقتين لقياس الهستامين فى لحوم الأسماك حيث أستخدمت الطريقة الفلومتريه والطبقه الرقيقه الكروماتوجرافيه.

بلغت نسبة الهستامين ما بين ٦ ، ٤٥٦ جزء فى المليون ، وقد كانت أعلى النسب فى الأسماك المخزونه لمدة شهرين على درجة ٤ م وبلغت نسبة الهستامين أقلها فى الأسماك المخزونه تحت -٢٠ م لمدة أسبوع وحدثت زيادة طفيفه غير معنويه فى نسبة الهستامين فى الأسماك المخزونه فى درجة حراره -٢٠ م لمدة شهرين وبالتالى يمكن القول بأن كميات الهستامين يمكن أن تشكل خطوره صحيه على مستهلكى الأسماك الطازجه لزيادتها عن المستوى المسموح به عالميا والمخزونه بطريقة غير جيده مما يعرف الادميين للاخطار الصحيه ، ويمكن القول أيضا أن الهستامين يعتبر أحد الدلائل على سوء تخزين هذه الأطعمه.

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*: Dept. of Forensic Med. & Toxi., Fac. of Vet. Med., Assiut Univ.
**: Dept. of Food Control. Fac. of Vet. Med., Assiut University.

SUMMARY

Histamine content was determined in two types of fresh water fish (Claris lazera and Tilapia nilotica) by means of fluorometric and paper chromatographic methods. Amounts of histamine between 6 and 456 mg/Kg could be detected in fish samples. The highest concentration of histamine was detected in fish stored for two months at 4°C. while the lowest concentration was detected in fish stored at -20°C for only one week. Long periods of storage (2-3 months) at -20°C. produced only a non-significant increase in the histamine contnet. It could be concluded that long periods of storage of fish at temperatures more than 4°C. results in the formation of histamine form the already present histidine.

INTRODUCTION

Histamine is generated in foods, from the amino acid, histidine, via an enzymatic decarboxylation reaction catalysed by the enzyme histidine decarboxylase it leads to an intoxication called scombroid food poisoning, although the illness is not always associated with scombroid fish belonging to the families scombridae and scomberesocidae. A common feature among all these fish being the presence of free histidine *(MACKIE and RILCHIE, 1974).*

Toxicity of histamine is controversial, but ingestion of 70-1000 mg will usually cause clinical intoxication *(HENRY, 1960).*

The hazard action level in food is set al 500 ppm of histamine but only for tuna *(FEDERAL REGISTER SEPT. 4. 1982).*

Histamine is formed in some foods from histidine by various microorganisms through the action of the enzyme histidine decarboxylase *GURAYA and KOEHLER (1991) and FOO (1977).* Histamine is a toxin and the exposure of humanbeings to excessive levels present a health problem.

DALE and RICHARDS (1977) demonstrated that small doses of histamine in anaesthetised cats and dogs cause constriction of certain arteries and veins and general dilatation of small blood vessels. *DALE and LAIDLOW (1977) and DALE and RICHARDS (1977)* demonstrated dose dependent depressor responses to adminsration of histamine in these species with asteep fall in systemic blood pressure, followed shortly by an intermediate and incomplete recovery of arterial pressure, in some cases irreversibly. Despite contraction of large arteries and veins,

vasodepression is the dominant response to large doses of histamine due to marked dilatation of small blood vessels with pooling of blood in micro circulatory beds, escape of plasma from vascular system in the areas of increased permiability, and consequent reduction of effective circulatory fluid volume. Arteriolar dilatation and systemic hypotention also represent the predominant yascular response to intravenous histamine injection in man. In the present study, we evaluated the effect of the storage conditions of fish on its histamine content.

MATERIAL and METHODS

Histamine was determined in fish samples by fluorometric method employing the o-phthalaldhyde condensation products (1, 2, 5, 7, 8, 9). The simultaneous determination of histidine and histamine in fish samples was performed by using a simple paper chromatographic method based on the formation of pink complex of cadmium-ninhydrin reagent with histidine and histamine as described by *FOO (1977).*

Apparatus :

a. Chromatographic paper. Whatman No. 3MM.
b. Ultrasonic bath. Cole - Parmer Instrument Co., Chicago, IL Model 8845-3.
c. Spectrophotometer, Beckman Model 24.
d. Chromatographic tube: 200 x 7 mm polypropylene tube (Chromaflex. Kontes Glass Co., No. K-420160) fitted with Kontes No. K-422372 Kel-F Hubs and CA 45 cm Teflon tubing.
e. Photofluorometer-Perkin-Elmer Model 204 with medium pressure Hg lamb. American Instrument Co. No. 4-7125 with GEF4T4/BL lamp.
f. Histamine standard solution: 10 mg histamine. 2Hcl (Sigma) were dissolved in 10 ml water.

Reagents :

All reagents and solvents. except chromatographic solvents, were analytical grade.

a. Anion exchange resin (Bio-Rad Agi-X8).
b. Cadmium stock solution: 0.50 g cadmium acetate (Merck) was dissolved in 60 ml with acetone.
c. Spray solution: prepared fresh by dissolving 0.20 g ninhydrine (Merck) in 100 ml cadmium stock solution.
d. Histidine standard solution: 10 mg histidine. Hcl. H20 (Sigma) were dissolved in 10 ml water.
e. Histamine standard solution: 10 mg histamine. 2Hcl (Sigma) were dissolved in 10 ml water.

I- Paper Chromatographic Determination Of Histamine In Fish Samples:

A) *Preparation of Fish Samples:*

10 ml of 5% trichloroacetic acid solution were added to 5 g of previously ground fish, mixed in ultrasonic bath for 15 min and centrifuged for 5 min at 3000 xg. The liquid was decanted through glass wool. The extraction procedure was repeated with two 10 ml portions of 5% trichloroacetic acid solution: first breaking up the solid before mixing it in ultrasonic bath for 5 min, and centrifuged as before. The extracts were combined and after addition of 1 ml 0.1 N H_2SO_4, the resulting solution was washed with three 20 ml portions of ether. The aqueous solution was concentrated to small volume under pressure, the residue was dissolved in methanol-water (4:1), and diluted to 10 ml. This solution was used directly for paper chromatography.

B) *Experimental Design:*

Aliquotes of extracts were applied at 5 ul with microliter syringe. The chromatograms were developed by ascending chromatography using 2-methyl-propan-2-ol + methyl ethyl ketone + 30% ammonia solution + water (50 : 30 : 10 : 10) as the developing solvent. Histamine and histidine spots were located by applying pure solutions of histamine and histidine on the same chromatogram. Standard curves were prepared by applying varying volumes of standard solutions to the paper and developing chromatogram as for extracts.

C) *Development of Colour:*

The developed chromatograms were lightly sprayed with spray reagent and dried Ca 10 min at 40°C. The chromatograms were sprayed again, but only at regions of developed spots of histamine and histidine until areas are saturated with spray reagent. The chromatograms are reheated for 3 hr. at 40°C to ensure full colour development.

D) *Extraction of Colour Complex:*

The developed spots containing appropriate compounds were cut into strips and placed in 10 ml conical flasks. Methanol (5.0 ml) was added to each flask and placed in ultrasonic bath 3 min. The absorbance of unfiltered solutions was measured at 500 nm in 1 cm cells against blank similarly prepared.

II- Fluorometric Determination Of Histamine In Fish Samples:

The AOAC official method of analysis of histamine in fish *(Fluorometric method, Staruszkiewicz, 1977)* was used. The method specified a rapid ion exchange step to remove interfering compounds from a methanol extract of fish. Histamine was reacted with o-phthalaldehyde and the fluorescence of the derivative was used to quantitate the amine.

In both paper chromatographic and fluorometric methods, two types of fish (Claris lazera and Tilapia nilotica) stored at different temperatures were used. One group was stored at 4°C for two months and a second group was stored at -20 for the same period of time. Fish samples (stored for one week, two weeks, one months and two months at the above mentioned temperatures) were taken and its histamine and histidine contents were determined.

RESULTS

Are presented in Tables 1 - 6.

DISCUSSION

The mean value from 10 replicate determinations of 3 different concentrations of histidine Hcl are summarised in Table 1. The mean absorbance when plotted against the concentration gives a linear relationship which passes through the origin.

Table 1: Absorbances at 500 nm of 10 replicate determinations of 3 different quantities of histidine. Hcl after paper chromatographic separation.

Histidine. Hcl (ug)	Mean + SD	Cpeff. of Var %
5	0.091+0.003	5.2
10	0.176+0.006	3.9
15	0.286+0.005	2.1

The results indicating the recovered histamine and histidine from fish samples stored at 4°C for one week and two months after paper chromatographic separation are shown in Tables 2 & 3 respectively.

Table 2: The recovered histamine and histidine (histamine. 2Hcl: histidine. Hcl.H20. mg/Kg fish) from fish stored at 4°C for one week by using paper chromatographic method. The data represent the mean of the values obtained from Claris lazera and Tilapia nilotica fish samples.

Sample No	Histidine found		Histamine found	
	C. lazera	T. nilotica	C. lazera	T. nilotica
1	4.9	5.2	0.5	0.3
2	5.8	6.2	0.6	0.5
3	6.1	6.4	0.4	0.6
4	6.3	5.2	0.5	0.8
5	5.7	6.4	0.5	0.4
6	7.3	5.7	0.4	0.5
7	6.2	6.5	0.7	0.8
8	6.3	5.3	0.9	1.2
9	5.9	6.2	1.0	0.9
10	7.2	6.3	0.6	0.8

Table 3: The recovered histamine and histidine (histamine. 2Hcl: histidine. Hcl. H20.mg/Kg fish) from fish stored at -20°C for two months, after paper chromatographic separation. The data represent the mean of the values obtained from Claris lazera and Tilapia nilotica fish samples.

Sample No.	Histidine found		Histamine found	
	C. lazera	T. nilotica	C. lazera	T. nilotica
1	4.0	5.0	0.8	0.6
2	4.4	5.2	0.8	0.7
3	5.9	5.2	0.6	0.9
4	6.1	4.3	0.7	0.7
5	4.5	4.4	0.8	0.7
6	5.7	5.3	0.6	0.8
7	5.9	5.2	0.7	0.7
8	4.5	5.0	0.6	0.9
9	5.1	3.9	0.8	0.7
10	4.4	5.8	0.7	0.9

The cadmium-ninhydrin reagent offers a simple and reliable method for determining histidine and histamine. According to

the work of *FOO (1977)*, using paper chromatography, if the developed chromatograms were left standing 24 hr. or more, it becomes difficult to extract the colour, particularly with high levels of histidine. By drying at 40°C. the period of colour development was shortened, and the use of the ultrasonic bath will hasten the dissolution of the ninhydrin complex. The paper chromatographic method is efficient and the chemical procedure is simple because quantitation is made from the chromatograms. Because the spots are visible, it is possible to check their resolution from other spots and quantitive transfer is simple.

The results of the fluorometric method for the determination of histamine in fish are summarized in Tables 4 and 5. The AOAC fluorometric method is based on the coupling of histamine with ophthalaldehyde (OPT) at a highly alkaline pH to form a fluorescent product which is rearranged on acidification

Table 4: Histamine content (mg/Kg fish sample), as determined by the fluorometric method of analysis (using ion exchange resin. AG1-X8, Bio-Rad-Laboratories). (using ion exchange resin. AG1-X8, Bio-Rad-Laboratories), of fish stored at 4°C for one week.

Type of fish	N	X	S	Minimal value	Maximal value
laris Lazera	15	23	102	7	32
Tilapia nilotica	15	26	123	9	35

Table 5: Histamine content (mg/Kg fish sample) as determined by fluorometric method of analysis (using ion exchange resin AG1-X8. Bio-Rad-Laboratories) of fish stored at 4°C for 2 months.

Type of fish	N	X	S	Minimal value	Maximal value
Claris Lazera	15	157	149	6	439
Tilapia nilotica	15	161	144	14	456

Table 6: Histamine content (mg/Kg fish sample) as determined by fluorometric method of analysis (using ion exchange resin AG1-X8, Bio-Rad-Laboratories) of fish stored at -20°C for 2 months.

Type of fish	N	X	S	Minimal value	Maximal value
Claris Lazera	15	32	113	11	37
Tilapia nilotica	15	35	109	14	40

N = Number of samples X = Mean of values S= Standard error.

to form a highly fluorescent and stable fluorophore *(SHORE, 1971)*. The fluorescence was recorded at 350 nm excitation and 440 nm emission wavelength. The fluorescence was recorded within 30 min. since a gradual decrease in fluorescence was noticed with time. The OPT reaction and the necessary preliminary cleanup steps were designed to detect histamine without interferece of the amino acid histidine, which is present in large amount in fish. The experimental route chosen was to adsorb amino acids from fish extracts onto an anion exchange resin without retention of histamine and to use detection conditions that would favour fluorescence of the histamine derivative over those of other compounds. Anion exchange resins have been used previously to remove interferences from extracts of biological samples containing histamine. One type of anion exchange resin (Bio-Rad AGI-X8) was used in the experiments. Standard practices were used to prepare and elute the columns. Resins were slurried in water and added to the columns so as to exclude air bubbles. Care was exercised to minimize dead volumes when glass wocl was used as a bed support. The liquid level was always kept above the top of the resin and flow rates of approximately 3 ml/min were used. The resins were convereted to the hydroxide form with 1 N NaOH and excess base was removed by washing with distilled water to a pH of <8.5 Samples were diluted with water before passage through resin columns to permit good ionic exchange with the anion exchange groups. An excess of water was used to wash unretained histamine through the column to obtain maximum recovery. Extracts were nerver allowed to stand in the resin columns but were eluted immediately. No more than 24 extracts were applied to a column of resin and a methanol blank followed each set of 6 extracts through the column.

Excellent sensitivity fo histamine with minimal interference from other compounds (10) was obtained by using a 0.1% OPT soultion and terminating the reaction with phosphoric acid to give a final pH of 1.9. The time of reaction was taken to e between 3.5 and 4 min (9) measured with stopwatch precision. The fluorescence of the derivative was stabilized after dilution of phosphoric acid. The pH was controlled by using a small sample size followed by a large dilution with standard Hcl. Mercury lamps were used to excite the OPT derivatives.

As the result indicated, histamine level was significantly ($P<0.01$) increased upon storage of fish at 4°C for two months. When compared with fish stored for only one week at the same temerature. This increase in the histamine

content of fish with storage at 4°C is probably due to the effect of various microorganisms on the already present histidine converting it to histamine.

The mean values recorded in this study revealed that the proper storage of fish revealed a values of histamine much lower than the recommended levels. In the other hand improper storage result in biological changes resulting in more histamine concentration higher more than that recommended (200 ppm in tuna) it is aduisable that histamine levels could be considered one of the chemical tests which ensure improper handling of raw fish and decay resulting in significant increase in its level in food.

CONCLUSIONS and REOMMENDATIONS

Histamine is the best chemical indicator of decomposition for fish. The paper chromatographic and the fluorometric methods permit the practical use of this indicator by providing a rapid, sensitive and relatively inexpensive means of detection. Minimal effort is required to prepare samples and the procedure can be used successfully with only a short training period because of the few techniques required. The precision and accuracy of the fluorometric method is however, more than that of the paper chromatographic method. We recommend the use of fresh fish instead of the stored one in order to avoid hypersensitivity reactions resulting from the possible release of histamine. Long periods of storage of fish at temperatures more than 4°C is not recommended.

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