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DETERMINE THE LEVELS OF HISTAMINE IN CERTAIN TYPES OF LOCALE AND IMPORTED FISH

Raafat A. Abu-ALmaaly
Market Researches and Consumer Protection Center, University of Baghdad/ Baghdad/ Iraq
*Corresponding author e-mail: r12maaly@gmail.com

ABSTRACT
Histamin poisoning occurs when consumption food that containing high levels of histamine**,** fish particularly. Because of fish consumption widely in Iraq, this study aimed to determine the histamine levels in the fish available in local markets, for this purpose 50 samples of fresh, frozen and canned fish were collected from Baghdad city local markets were analyzed for microbiological and histamine value. Extraction and determination of histamine levels in fish samples were made by colorimetric assay. The results of isolation histamine-forming bacteria shows that 14 from 108 isolates were positive belonging to genera, *Clostridium perfringens*, *Proteus spp.*, *Klebsiella spp.*, *Pseudomonas aeruginosa*, *Pseudomonas fluorescens* and *Enterobacter spp.*. Fish samples were recorded the higher count of histamine-forming bacteria, frozen Black Tilapia, frozen Mackerel and fresh carp. The level of histamine in 23 out of the 50 analyzed fish samples was higher than the tolerance limit of histamine contents (50mg/100g) established by EU regulation. Frozen Mackerel, canned tuna and sardine recorded the maximum level of histamine 73.4, 62.5 and 64.6 mg/100g of fish respectively, while the minimum level were in frozen Atlantic croaker and frozen Aurata, 17.9 and 20.2 mg/100g of fish respectively

KEY WORDS: histamine, locale and imported fish.

INTRODUCTION

The fish Classified with Quick corruption food, which is one of the causes of food poisoning, due to wrong habits in fish handling practices, Such as fish displayed on tables as it is offered to the high air temperature and vehicle exhaust, dust, flies and microbes[1]. The meat of some species of fish can cause a sudden disease to humans after a very short period of eaten it called Scombrotoxin fish poisoning (SFP) (often called "histamine poisoning") [2]. Scombroid poisoning is usually a mild illness with a variety of symptoms including rash, urticeria, nausea, vomiting, diarrhea, flushing, tingling and itching of the skin [3]. This disease caused by ingestion of certain species of marine fish that contain high levels of histamine and possibly other biogenic amines. The fish (scombroid fishes) species involved include tuna, mackerel, sardine, herring and anchovy which account for significant global fish production. However non scombroid fishes such as mahi-mahi, blue fish, amberjack and anchovies have also been implicated in histamine fish poisoning [4]. The result of research and studies shows that These fish species contain high levels of free histidine (free amino acid that present in the fish muscles) in their tissue and when conditions are favorable for bacteria to multiply in fish, bacterial decarboxylation of histidine leads to histamine formation, the most commonly reported biogenic amines occurring in foods[5,6,7]. Biogenic amines such as histamine, tyramine, putrescine, and others can be formed in food during processing or the period of storage, primarily due to the release of specific amino acids by the action of decarboxylases produced by microorganisms. Certain bacterial genera, with potential probiotic characteristics, can form biogenic amines [8]. Enterobacteria, a large group of bacteria have been reported to be responsible for the histamine found in fish. Some studies have indicated that the diversity of bacteria with histidine decarboxylase activity observed in scombroids can be attributed to the type of seafood, fish species, duration and temperature storage condition. As well as there are some other factors such as feeding habits, geographical position, fishing gear, season, water temperature and salinity, the way of product handling after harvest and factors that influence on marketing process which can affect composition and type of histamine-forming bacteria in fish[9,10]. In The EU, histamine average must be less than 10 mg / 100 g (100 ppm) to accept tuna and other fish, which the Scomberescoidae family. According to the World Health Organization, the safe limits of histamine must not exceed 20 mg / 100 g of food, while in Egypt safety limits required for the substance histamine are 50 mg/100 g[1]. The presence of other substances if accompanied by the presence of histamine in food increase the toxic effect like some amino compounds such as tyramine compound is produced from the amino acid tyrosine and trepettomain produced from the amino acid tryptophan, and the alcohol and certain medications increase the harmful effect of histamine through inhibition of intestinal enzymes that work on histamine metabolizing material such as diamine oxidase[9]. Factors affecting growth of histamine producing bacteria include type and size of fish, handling techniques and cooling methods [11].
This study aimed to determine the levels of histamine and isolating some types of bacteria which is produced it from fish present in the local markets in Baghdad city, because of the opening of Iraqi market on the various types of local and imported fish, fresh, frozen and canned.

MATERIALS & METHODS

Total 50 samples of fresh fish (pomfrete, carp and mullet), frozen fish (Rohu, Aurata, Black Tilapia, Atlantic croaker and mackerel) and canned fish (tuna and sardine) was collected from the markets of Baghdad city, transferred directly into the refrigerated, clean and sealed containers to the laboratories of market researches and consumer protection center, University of Baghdad as carried out by the microbiological and histamine colorimetric analysis immediately within an hour.

For microbiological analysis, since the tissues of gill and gut are considered as the major source of histamine-forming bacteria in fish[12], 5g of muscle near the gills were collected aseptically, taken in sterile conditions, then diluting 1:10 (wt/vol) with sterile 0.1% peptone water, homogenized using sterile high speed blender, then used immediatelyfor microbiological analyzes. All Preparations and isolate methods were according to[11].

Fish composite samples were serially diluted in sterile 0.1% peptone water, 0.1 ml from every dilute were spread plated in duplicate on tryptic soya agar (TSA) (HiMedia, India), incubated at 37˚C for 48 h. The Enterobacteriaceae family was enumerated in violet red bile dextrose agar (VRBA) after incubation at 37°C for 24h. Representative isolates were selected from TSA plate. Isolates were purified on TSA plates and incubation at 37˚C for 48 h. The pure cultures were transferred to TSA slants containing 2% NaCl and incubated at 37˚C for 24 h. Each isolate taken from each slant was plated on Niven's agar medium and incubated at 37˚C for 48 h. Niven's positive isolates with purple halo around Niven's medium were enumerated. These positive colonies were aseptically isolated and streaked on trypticase soy agar slants supplemented with 0.1% L-histidine-HCl with pH= 6.0 and incubated at 37°C for 24h to screen for histamine production. Isolates were stored at a temperature of 2°C until used for bacterial species identification. All isolates were further identified using gram stained and examined under oil immersion and biochemical tests.

For histamine confirmation Gram positive and Gram negative isolates were streaked in triplicates on TSA plates containing 2% NaCl supplemented with 2% histidine and incubated at 37˚C for 24 h. A representative colony from each of the plate was inoculated into 9 ml of tryptic soy broth supplemented with 2% NaCl, 2% histidine and 0.0005% pyridoxal-HCl (pH 5.8) [TSB+] and incubated at 37˚C for 24h. A 1 ml sample of each TSB+ suspension was transferred into a tube of fresh TSB+ media and incubated at 37˚C for 48 h. A 3 ml subsample of this final culture was transferred into polypropylene centrifuge tubes and centrifuged at 1170 rpm for 20 min. Supernatants were diluted 1 to 10 in saline and histamine concentrations were determined with colorimetric assay of histamine [13].

5 g of fish muscle tissue has been taking from three locations (head, belly and tail) to prepare the fish samples for histamin analysis, put in a sterile high speed blender then 50mL of 0.85% NaCl solution added and homogenized for 2 min and centrifuged at 4000 rpm for 10 min. Preparation and quantification of histamine was carried out using colorimetric method reported by [13]. 1 ml of the muscle extract was taken into a glass test tube, diluted to 2 ml with saline, and then 0.5 g of salt mixture containing 6.25g of anhydrous sodium sulfate to 1g trisodium phosphate monohydrate was added. The tubes were stoppered and shaken thoroughly. 2 ml of n-butanol was then added and the tubes shaken vigorously for 1 min and allowed to stand for 2 min. In order to break the protein gel, the tubes were shaken briefly, and were further shaken vigorously for few seconds, the tubes centrifuged at 3100 rpm for 10 min. Only 1 ml from the upper butanol layer was transferred into a clean and dry test tube, evaporated to dryness in a stream of nitrogen. The residue was dissolved in 1 ml of distilled water. 5 ml of 1.1% sodium carbonate solution was taken in a clean tube and 2 ml of the chilled reagent, phenyldiazonium sulfonate was added slowly and mixed, then added to the tube containing 1 ml solution of the residue collected in the extraction process. The absorbance of the color produced was measured immediately after 5 min at 496 nm. The concentration of histamine in sample was obtained from the standard curve for the corresponding absorbance measured at 496 nm. The histamine concentration in sample was estimated using the following formula:

$$\text{Histamine (mg/100 g)} = A \times 2 \times 25 \times 100 / 5 \times 1000$$
$$= A \text{ mg/100 g}$$

Where A is the value of histamine obtained in l g/ml from the standard curve.

RESULTS & DISCUSSION

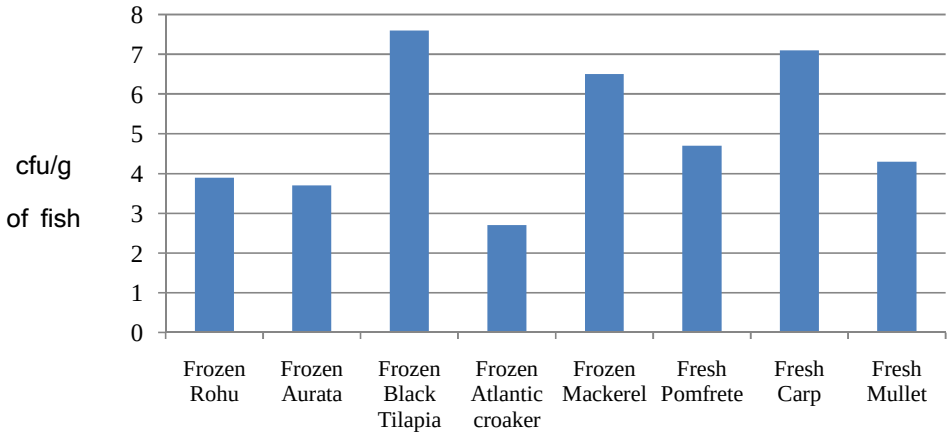
Microbiological analysis of fresh and frozen fish samples shows that 108 tentative histamine-forming bacteria with purple halo around Niven's medium agar were isolated (Table, 1), belong to several bacterial genera. Just 14 isolates was confirmed histamine-forming bacteria when streaked on trypticase soy agar slants supplemented with 0.1% L-histidine -Hcl, belonging to the genera, (3) *Clostridium perfringens*, (3) *Proteus spp.*, (1) *Klebsiella spp.*, (4) *Pseudomonas aeruginosa*, (1) *Pseudomonas fluorescens* and (2) *Enterobacter spp.* These results approached with founding of [11] when he explained that 7 from 40 isolates had ability to forming histamine by histidine-decarboxylase in fresh mackerel and sardine from the retail shops at Kalyan, India. In study of Valiollah, et al [14], they found that 8 from 40 strains with histidine decarboxylase activity showed positive results to produce histamine in frozen skipjack tuna in Iran. The isolated bacterial genera in two previous studies are similar with this study. Figure (1) shows the total counts of histamine-forming bacteria record the higher counts 7.6 log cfu/g and 7.1 log cfu/g in frozen Black Tilapia and fresh carp respectively, while the less counts was 3.7 log cfu/g and 3.9 log cfu/g in frozen Aurata and Rohu respectively, the results of this study was similar with [14, 15] when they found the counts of histamine-forming bacteria in fish samples was 7.4 and 8.6 log cfu/g respectively, amongst these genera, *Clostridium perfringens*, *Proteus* spp, *Klebsiella* spp. and *Enterobacter* spp.

TABLE 1: Total of tentative histamine forming bacteria in fish samples

Bacterial species	No. of tentative histamine-forming bacteria	Confirmed histamine-forming bacteria	No. of isolates histamine-forming bacteria	Frequency (%)
Clostridium perfringens	20	+	3	21.42
Proteus sp.	18	+	3	21.42
Klebsiella sp	4	+	1	7.15
Escherichia coli	11	-		
Pseudomonas aeruginosa	16	+	4	28.57
Pseudomonas fluorescens	3	+	1	7.15
Enterobacter sp.	12	+	2	14.29
Staphylococcus sp.	16	-		
Aeromonas sp.	5	-		
Not identified	3	-		
total	108		14	

The most important factors that affecting on growth of histamine producing bacteria includes type and size of fish, handling techniques and cooling methods [2]. In this study, most of the frozen fish in the markets were kept outside the ice vessels or placed in unstable freezer temperature, due to instability power, as well as the length of time between fishing and marketing operations until it reaches to the consumer, for fresh fish, that gives a long time which results and affect by increase temperature of fish and this gives opportunity for histamine producing bacteria to growth and proliferate.

FIGURE 1: Total counts of histamine forming bacteria in fish samples



In this study, histamine was detected by colorimetric assay method in all samples analyzed. The level of histamine detected ranged from 17.9 to 73.4 mg/100g of fish. Frozen Mackerel recorded the maximum level of histamine73.4 mg/100g of fish. While Frozen Atlantic croaker was the least value between the analyzed samples 17.9 mg/100g of fish. Table (2) presents the levels of histamine in positive fish samples as determined by colorimetric assay method in analyzed fish samples.

TABLE 2: Levels of histamine in positive fish samples as determined by colorimetric assay method

No.	fish samples	levels of histamine mg/100g
1	Frozen Rohu	55.9
2	Frozen Aurata	20.2
3	Frozen Black Tilapia	53.7
4	Frozen Atlantic croaker	17.9
5	Frozen Mackerel	73.4
6	Fresh Pomfrete	50.7
7	Fresh Carp	57.4
8	Fresh Mullet	35.3
9	Canned tuna	62.5
10	Canned sardine	64.6

The level of histamine in 23 out of the 50 analyzed fish samples was higher than the tolerance limit of histamine contents (50mg/100g) established by EU regulation [17], according to this standard 3 fish samples (frozen Atlantic croaker, frozen Aurata and fresh Mullet) in levels 17.9, 20.2 and 35.3 mg/100g of fish respectively, were within normal range, while the FDA recommends that histamine amount in fresh or frozen raw fish should not exceed 20 mg/100 g and in tinned fish should not exceed 50 mg/100 g [18], so just frozen Atlantic croaker and frozen Aurata

samples was within normal range of FDA 17.9 and 20.2 mg/100g of fish respectively, (as frozen fish). It was clear in this study that the scombroid fish samples (Mackerel, tuna and sardine) recorded the highest levels of histamine 73.4, 62.5 and 64.6 mg/100g of fish respectively. Previous studies reported that the histamine levels were higher than the standard limit in scombroid fish samples [4, 5, 10, 11].

REFERENCES

[1]. Hassan, S.F., Abd Almalik, A.M. (2012) Scombroid Food Poisoning. Assiut Journal for Environmental Researches 15(36): 85-90.

[2]. WHO (2012) World Health Organization Call for data and experts on public health risk of histamine and other biogenic amines from fish and fishery products : 23 – 27, July 2012 FAO Headquarters, Rome, Italy.

[3]. Smela, D., Pechova, P., Komprda, T., Klejdus, B. and Kuban, V. (2003) Liquid Chromatographic Deter-mination of Biogenic Amines in a Meat Product during Fermentation and Long-term Storage. Czech J. Food Sci. 21(5): 167–175.

[4]. Er, B., Demirhan, B., Bas, S.Y., Yentur, G. and Oktem, A.B. (2014) Determination of Histamine Levels in Canned Tuna Fish. Bulgarian Journal of Agricultural Science, 20 (4): 834-838.

[5]. Abu Zar Fathi, Abde Rahman Pooladgar, Shamsedin Ghaem Maghami and Rahman loni (2013) The Changes Evaluation of Biogenic Amins (histamine) by HPLC, in Shanak Yellow Fin Fish *(Acantho pagrus Latus)* Within 18 Days of Ice Storage. Ind. J. Sci. Res. and Tech. 1(1): 29-34.

[6]. Auerswald, L., Morren, C. and Lopata, A.L. (2006) Histamine levels in seventeen species of fresh and processed South African seafood. *Food Chemistry*, 98: 231–239.

[7]. Kan, K., Ushiyama, H., Shindo, T. and Saito, K. (2005) Survey of histamine content in seafood on the market. *Shokuhin Eiseiqaku Zasshi*, 46 (3): 127–132.

[8]. Sullivan Brian O' B.Sc. (2000) The Analysis of Biogenic Amines by standard and Novel Methods. Thesis. School of Biological Sciences Dublin City University, February, 2000.

[9]. Mercogliano, R., Anastasio, A., Colarusso, G., Marrone, R. & Cortesi, M.L. (2008) Evaluation of histamine profile in Thunnusthynnus processed sea foods. *Veterinary Research Communication* 32, 331-333.

[10]. Koohdar, V.A., Razavilar, V., Motalebi, A.A., Mosakhani F. and Valinassab, T. (2011) Isolation and Identification of Histamine-forming bacteria in frozen Skipjack tuna (*Katsuwonus pelamis*). Iranian Journal of Fisheries Sciences, 10(4) 678-688.

[11]. Joshi, P.A., Vishal, Bhoir, S. (2011) Study of Histamine Forming Bacteria in Commercial fish samples of Kalyan city. Int J Cur Sci Res. 1(2): 39 – 42.

[12]. Lopez-Sabater, E.I., Rodriguez-Jerez, J.J., Hernandez-Herrero, M. and Morana-Ventura, M.T. (1996) Incidence of histamine-forming bacteria and histamine content in Scombroid fish species from retail markets in the Barcelona area. *International Journal of Food Microbiology*, 28(3), 411-418.

[13]. Patange, S., Mukundan, M., Kumar, A. (2005) A simple and rapid method for colorimetric determination of histamine in fish flesh. J. Food Control. 2005; 16: 465-472.

[14]. Valiollah, K. Vadood, R., Abolhassan, K. and Alireza, Sh (2012) Histamine-producing bacteria isolated from frozen longtail tuna (*Thunnus tonggoh*) African Journal of Microbiology Research, 6(4):751-756. DOI: 10.5897/AJMR11.882.

[15]. Sumitha, D., Sundar, K. and HaladyShetty, P. (2014) Occurrence of Histamine Forming Bacteria and Histamine in Dried Fish. Advance Journal of Food Science and Technology 6(4): 428-432.

[16]. Chen, H. Ch., Kung, H.F., Chen, W.Ch, Lin, W.F., Hwang, D.F., Lee, Y. Ch. and Tsai Y.H. (2008) Determination of histamine and histamine-forming bacteria in tuna dumpling implicated in a food-borne poisoning. Food Chemistry 106 (2008) 612–618.

[17]. EC (2005) European Commission Regulation (EC) No 2073/2005 on microbiological criteria for foodstuffs. *Official Journal of the European Union*, 15 November 2005.

[18]. FDA Food and Drug Administration (1995) Decomposition and histamine in raw, frozen tuna & mahi-mahi; canned tuna and related species: availability of guide to Federal Register., 60(149): 39754-39756.