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Research Article

BACTERIOLOGICAL QUALITY AND BIOGENIC AMINES DETERMINATION BY HPLC IN BASSA FISH IMPORTED TO SAUDI ARABIA

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ABSTRACT

Collected fifteen samples of bassa fish for human consumption Ui Tnam purchased from retail markets in Qassim region, Saudi Arabia, were analyzed for their bacteriological quality, biogenic amines were determined by HPLC . It was found that, there were variations between samples regarding aerobic plate counts. Putrescine and histamine were detected particularly in all collected bassa fish samples in all governorate in Qassim (Buraydah, Unayzah, Al-Ras, Al- Badaye and Al- Bukayriyah). At the same time, tryptamine , and tyramine were observed as the lowest two, detected in fish samples collected from Al- Bukayriyah and Unayzah governorates respectively. Concerning the concentration of biogenic amines in the tested bassa fish samples, histamine and putrescine showed the higher concentration in Buraydah governorate and it was found to be 35.9 mg /kg and 17.33 mg/kg , respectively.

Keywords: Bacteriological quality, Biogenic amines, Histamine, Tyramine, Fish, HPLC.

INTRODCTION

Biogenic amines are low molecular weight organic bases, they can be formed and degraded as a result of normal metabolic activity in animals, plants and microorganisms, in the latter case, biogenic amines may be used as an indicator of food spoilage. These amines are usually produced in foods by decarboxylation of amino acids as the result of the decaroxylase activity of bacteria present in the food, or that contaminate the food during transporting, handling, processing and marketing (1-3). Excessive oral intake of biogenic amines may exert vasoactive or psychoactive effects. Histamine poisoning, which results from ingestion of foods that contain considerable amounts of histamine, has been reported to be one of the major illnesses among foodbrone diseases. Other amines such as putrescine, cadaverine, tyramine, β-phenylethylamine, spermine and spermidine have been described as a potentiators that enhance the toxicity of histamine. In addition, putrescine, cadaverine, spermine and spermidine may produce carcinogenic nitrosamines in the presence of nitrites (4-8). The threshold levels for intoxication in humans by biogenic amines are very difficult to establish, because they depend on individual responses and the presence of other amines (9-10). Low levels of biogenic amines in food are not considered a serious risk. However, when consumed in excessive amounts, they may cause distinctive pharmacological, physiological and toxic effects. It is worthy to mention that there is also evidence of linkaging elevated biogenic amine levels and cancer (11). It has been reported that, 40 mg of biogenic amines per meal can be considered potentially toxic. The determination of biogenic amines in foods is of great interest not only due to their possible toxicity, but also can be used as indicators for quality of freshness or spoilage of foods (12-13). Biogenic amines, especially histamine, putrescine and cadaverine have been suggested as indicators of spoilage of some foods, such as fresh fish, meat and vegetables (14). The amount and type of biogenic amines formed is strongly influenced by the food composition, microbial flora and by other parameters which allow bacterial growth during food processing and storage (e.g., food treatment prior to storage, food additives, temperature, moisture, ripening and packaging) (15-16).

It had been reported that, there were high polyamine concentrations in breast and colon cancer cells (17). Several analytical methods for the determination of biogenic amines in foods have been described. These include thin layer chromatography(TLC) (18), biosensors (15,19), and reversed phase high performance liquid chromatography (HPLC) (20-21).

The present study was executed to quantify biogenic amine levels in bassa fish imported to Saudi Arabia

MATERIALS AND METHODS

a) Sampling

A total of 15 bassa fish samples were collected from retail markets in region of Qassim included four governorates and one city (Unayzah, Al -Ras, Al Badaye, Al- Bukayriyah and Buraydah City, Saudi Arabia in August 2009. Samples were transported frozen to the laboratory for analysis.

b) Biogenic amines determination

Six biogenic amines included histamine, tyramine, tryptamine, cadaverine, putrescine and β-phenylethylamine were extracted and determined in all tested samples according to (22) as follows:

c) Reagents

1) Dansyl chloride (5- { Dimethylamino} naphtalene -1- sulfonyl chloride) (Sigma Co. Louis, Mo 63178 U.S.A.) Dansyl chloride solution: 500 mg of dansyl chloride were dissolved in 100 ml acetone.

2) Histamine-2HCl, tyramine - HCl, cadaverine - 2 HCl, putrescine -2 HCl, tryptamine - 2 HCl, and B-phenylethylamine were purchased from (Sigma- Co. Louis, Mo 63178 U.S.A).

3) Standard solutions: Stock standard solutions of the tested amines: 25 mg of each standard pure amines histamine-2HCl, tyramine - HCl, cadaverine - 2 HCl, putrescine -2 HCl, tryptamine - 2 HCl, and B-phenylethylamine were dissolved in 50 ml distilled water individually.

4) Working standard solutions

Two milliliters of each stock standard solution were pipetted into 100 ml volumetric flask and diluted to volume with 5% trichloroacetic acid (TCA). This solution is prepared freshly (weakly) and stored in a refrigerator.

d) Apparatus

High-performance liquid chromatograph (HPLC, Agilent 1100 series) equipped with a photodiode array DAD (G 1315 B) analysis was carried out with a liquid chromatograph equipped with solvent delivery systems (Agilent Technologies, Inc.200 Regency Forest Drive, Suite 330 Cary, NC 27511 USA) system containing a G1322A Vacuum Degasser, a G1312A Binary and a reverse-phase analytical column packed with C_{18} material (Agilent ZORBA, X DB- 5 µm, 150 mm × 4.6 mm) .

Mobile phase solvents

Solvent A : Acetonitrile : 0.02 N acetic acid (1:9), Solvent B : 0.02 N acetic acid : acetonitrile : methanol (1 : 9 : 9).

Solvents A and B was used in gradient elution program as follow:

Time min.	Flow rate Ml/min	Solvent A%	Solvent B%	Curve
0	1	25	75	-
10	1	10	90	6
20	1	5	95	6
25	1	25	75	6

Separation was performed at ambient temperature at a flow rate of 1.0 ml/min; the injection volume was 50 µl for both standard solutions and sample extracts by auto sampler (G1329A). The Dad detector was operated at 254 nm .

HPLC column : Reverse phase C_{18} column 250 x 4 mm, 10 µm packing. The detection was performed using DAD detector (Aligant 486) at 254 nm wavelength, using linear program of 25 min period and 1 ml / min constant solvent flow rate.

d- f. Extraction, formation of dansylamines , calculation steps were carried out according to (22).

g-h. Preparation of samples, aerobic plate counts (APC) surface spread plate technique were carried out according to the technique recommended by (23).

RESULTS AND DISCUSSION

A total of 15 bassa fish samples were analysed for this survey. Samples of bassa fish of this study were collected from the retail market. Fish samples were purchased from Vietnam. Results of biogenic amines contents in the imported frozen bassa fish to Saudi Arabia are shown in Table (1). It was found that, there were variations between bassa fish samples regarding aerobic plate counts. It was found also that, biogenic amines levels were varied in bassa fish putrescine, tryptamine, histamine, tyramine were found in some samples; Meanwhile cadaverine and β-phenylethylamine were not detected in all samples of bassa fish, collected from location Qassim in Saudi Arabia.

Table 1: Aerobic plate counts and biogenic amine contents (mg/kg) of bassa fish samples

No of samples	Aerobic plate counts CFU/g	Biogenic amine contents concentration (mg/kg) Putrescine Mean ±SD	Tryptamine Mean ±SD	Histamine Mean ±SD	Tyramine Mean ±SD	Total of biogenic amines
1	7.1±0.28	15.5 ±0.32[a]	Nd	32.5±0.28	Nd	48.00
2	9.5±0.40	19.0 ±0.57	Nd	35.2 ±0.44	Nd	54.20
3	9.2±0.20	17.50 ±0.28	Nd	40.1 ±0.44	Nd	57.60
4	3.2±0.16	0.12 ± 0.01	Nd	8.4 ±0.26	0.08±0.003	8.60
5	4.0±0.14	0.61 ±0.06	Nd	7.2 ± 0.14	0.12 ±0.001	7.93
6	2.0±0.10	0.35 ±0.02	Nd	10.2 ±0.18	0.22 ±0.001	10.77
7	2.9±0.90	7.33 ±0.19	Nd	8.4 ±0.16	Nd	15.37
8	3.0±0.23	8.33 ±0.08	Nd	9.3 ±0.14	Nd	17.63
9	3.9±0.40	11.2 ±0.15	Nd	8.66 ±0.08	Nd	19.86
10	4.8±0.20	12.75 ±0.11	Nd	11.0 ±1.0	Nd	23.75
11	5.3±0.10	14.4 ±0.24	Nd	13.0 ±1.15	Nd	27.40
12	3.2±0.15	15.1 ± 0.23	Nd	2.63 ± 0.2	Nd	17.73
13	2.0±0.50	10.50 ±0.09	43.7 ±0.14	1.71 ±0.10	Nd	55.91
14	9.7±0.30	11.3 ±0.08	40.3 ±0.20	2.3 ±0.14	Nd	53.90
15	6.1±0.20	13.4 ±0.14	36.4 ±0.53	3.23 ±0.15	Nd	53.03

Nd: not detected. [a] Mean ± S.D. for triplicate determinations.

Table 2: Mean concentration of biogenic amines in bassa fish collected from Qassim region in Saudi Arabia.

Region	Putrescine Mean (mg/kg)	Tryptamine Mean (mg/kg)	Histamine Mean (mg/kg)	Tyramine Mean (mg/kg)
Buraydah	17.33	Nd	35.9	Nd
Unayzah	0.36	Nd	8.6	0.14
Al-Ras	8.9	Nd	8.78	Nd
Al –Badaye	14.08	Nd	2.6	Nd
Al- Bukayriyah	11.7	40.13	2.08	Nd

Nd: not detected.

Fish is one of the most perishable foods, mainly due to the action of microorganisms occurring on the surface of the newly caught fish. Microbiological quality determination has a very important role in maintaining the high quality of the final fishery products. From Table (1) it could be seen that, the APC of raw imported bassa fish ranged from 2.00 to 9.70 Log CFU/g in all samples collected from Qassim. Aerobic plate counts (APC) have been used to assess the microbial load, sanitary quality, organoleptic properties acceptability, safety and utility of various food by definition of the total number of microorganisms without reference to specific types, spoilage of fish and meat products is mainly related to the extent of the bacterial load density, thereupon, determination of the total bacterial count by plate count method is perhaps the most obvious way for determining the spoilage status of meat product (24).

Biogenic amine contents in 15 bassa fish samples are shown in Table (1). The result showed that, bassa fish contained at least four biogenic amines and the content of biogenic amines was quite different in the tested samples. Histamine was detected in all samples (100% of the bassa fish). From the above Tables, it could be noticed that putrescine and histamine were detected particularly in all collected bassa fish samples, at least in 100 %,in all governorates and City in Qassim region (Buraydah city , Unayzah, Al-Ras, Al-Badaye and Al- Bukayriyah). At the same time, tryptamine , and tyramine were observed as the lowest two, detected in fish samples collected from Al- Bukayriyah and Unayzah governorates respectively. Concerning the concentration of biogenic amines in the tested bassa fish samples, histamine and putrescine showed the higher concentration in Buraydah city and it was found to be 35.9

and 17.33 mg/kg , respectively. It is worth to mention that, histamine ingestion with spoiled fish is much more toxic than histamine ingestion alone in an aqueous solution, that may be due to the presence of histamine toxicity potentiators in spoiled fish, including cadaverine, putrescine, tyramine, tryptamine and β-phenylethylamine (6, 25- 26). Moreover, some biogenic amines, namely tyramine, tryptamine and β-phenylethylamine are included in the precursor amine group which are vasoactive amines causing a rise in blood pressure. Also tyramine and β-phenylethylamine have been implicated in the onset of migraine headaches as reported by (27). The production of biogenic amines is an extremely complex phenomenon, depending on several variables such as raw materials, processing conditions, growth kinetics of microorganisms, and their proteolytic and decarboxylase activities, which interact with each others (28). Protein content had a limited effect here, as proteolysis and peptidolysis to give free amino acids are necessary for biogenic amine production. Quality loss and histamine accumulation often occur after frozen fish of the above mentioned species are thawed and kept for long periods of time at room temperature before further processing. Since histamine is heat resistant, it can remain intact in canned or cooked fish products (29).

Table (3): The levels of biogenic amines in the bassa fish collected from Qassim region Buraydah city.

Biogenic amines	No of samples	Mean	Minimum	Maximum
Put	3	17.33	15.50±0.32	19.00±0.57
His	3	35.90	32.50±0.28	40.10±0.44
Trypt	3	Nd	Nd	Nd
Tyramine	3	Nd	Nd	Nd

Unayzah governorate

Biogenic amines	No of samples	Mean	Minimum	Maximum
Put	3	0.36	0.12 ± 0.01	0.61 ±0.06
His	3	8.60	7.20± 0.14	10.20±0.18
Trypt	3	Nd	Nd	Nd
Tyramine	3	0.14	0.08±0.003	0.22 ±0.001

Al-Ras governorate

Biogenic amines	No of samples	Mean	Minimum	Maximum
Put	3	8.90	7.33 ±0.19	11.20±0.15
His	3	8.78	8.40±0.16	9.30±0.14
Trypt	3	Nd	Nd	Nd
Tyramine	3	Nd	Nd	Nd

Al- Badaye governorate

Biogenic amines	No of samples	Mean	Minimum	Maximum
Put	3	14.08	12.75 ±0.11	15.10± 0.23
His	3	2.60	1.94 ± 0.03	3.40±0.06
Trypt	3	Nd	Nd	Nd
Tyramine	3	Nd	Nd	Nd

Al- Bukayriyah governorate

Biogenic amines	No of samples	Mean	Minimum	Maximum
Put	3	11.70	10.50 ±0.09	13.40±0.14
His	3	2.08	1.71 ±0.10	3.23 ±0.15
Trypt	3	40.13	36.40±0.53	43.70±0.14
Tyramine	3	Nd	Nd	Nd

Put= putrescine, His= histamine and Trypt= tryptamine

Mean ± S.D. (mg/kg) for triplicate determinations.

Data in Table(3) , found that, the mean of histamine levels in Bassa fish samples collected from Saudi Arabia in the locations (Buraydah, Unayzah, Al-Ras, Al- Badaye and Al- Bukayriyah Cities) 35.9, 8.60, 8.78, 2.60 and 2.08 mg/kg respectively. (30-31) indicated that, Histamine formation by some bacteria appears to be maximal at the middle of the exponential growth phase. Others have indicated that, histamine is formed during the stationary phase (8, 10). These differences were probably due to the type of bacteria and the growth conditions. On the other hand, histamine induction by many other bacteria at low pH has been documented by several authors (10, 31, 32). In general, bacterial amino acid decarboxylases such as lysine, arginine and glutamine decarboxylases are induced at acidic pH and /or under anaerobic conditions (33-34) and it is now clear that these amino acid decarboxylase systems play important roles in bacterial survival under acidic conditions (34). (35) indicated that histamine at a concentration of 500 mg/kg in food to be hazardous for human health. On the other hand, (9) reported that 100–800 mg/kg of tyramine in foods are toxic; while, (12) suggested that more than 1000 mg/kg (total amines in food) was dangerous for health. As shown in Table (1, 2 and 3) the concentrations of histamine in all of bassa fish samples tested were less than values considered as dangerous for health.

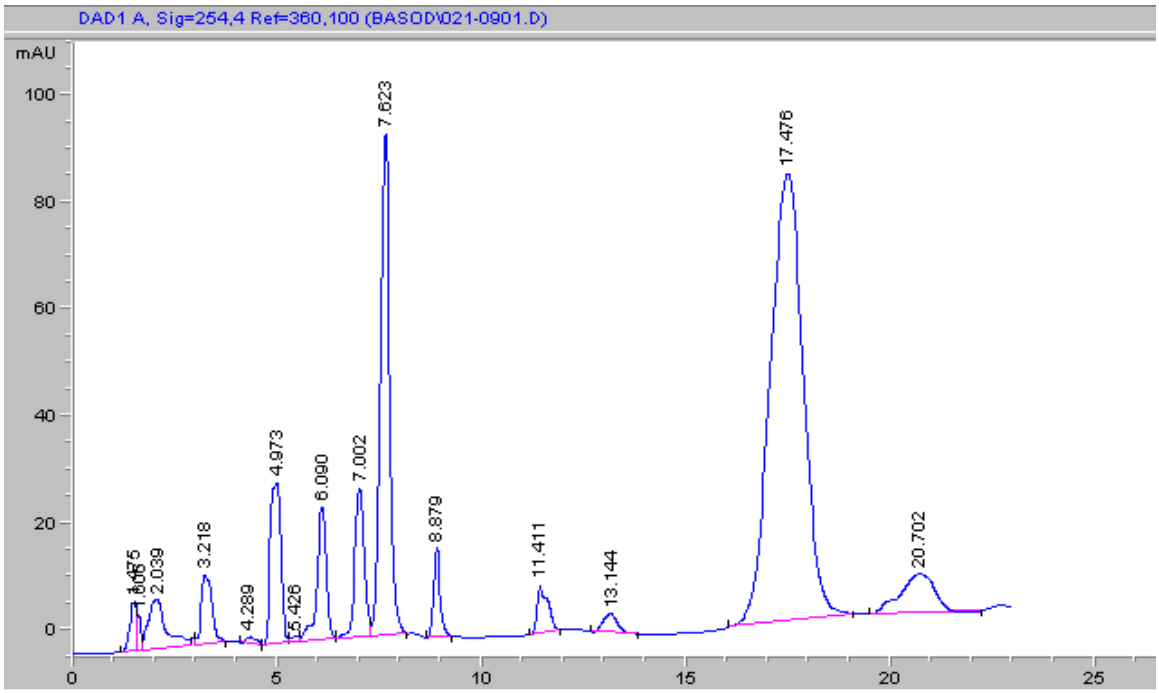

Fig. 1: HPLC chromatogram of standard biogenic amines

Trypt= Tryptamine Rt:6.0, B-Ph= B-Phenylethylamine Rt:7.002 , Put= Putrescine, Rt:7.6 Cad= Cadaverine Rt:8.8 , His= Histamine, Rt:11.4 Tyr= Tyramine,Rt:17.4 , BA= biogenic amines and Rt=Retention time.

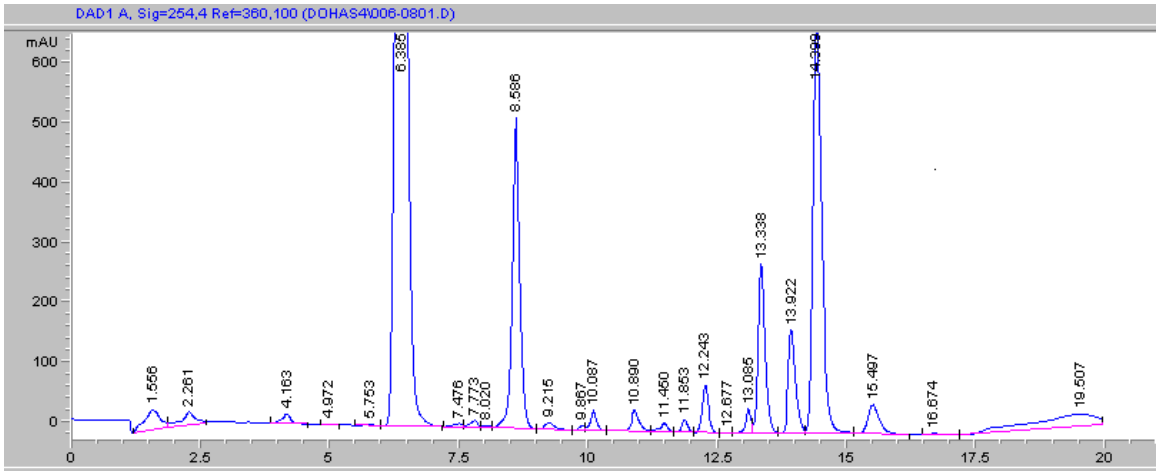

Fig. 2: HPLC chromatogram of positive sample biogenic amines in bassa fish

Figures 1 and 2 shows the HPLC chromatograms of biogenic amines separation of both standards and a positive bassa fish sample, respectively.

REFERENCES

1. Stratton J E, Hutkins R W and Taylor S L. Biogenic amines in cheese and other fermented foods: A review .Journal of Food Protection 1991; 54 (6):460-470.
2. Wendakoon C N and Sakaguchi M . Combined effects of sodium chloride and clove on growth and biogenic amine formation of *Enterobacter aerogenes* in mackerel muscle extract .Journal of food protection 1993; 56 (5): 410-413.
3. Heranandez-Jover T, Izquierdo-Pulido M, Veciana- Nogues M T and Vidal – Carou MC. Ion-Pair High-Performance Liquid chromatographic determination of biogenic amines in meat and meat products. J. Agric. Food Chem 1996; 44: 2710-2715.
4. Vandekerckove P. Amine in dry fermented sausage.Journal of Food Science 1977; 42: 283 – 285.
5. Pfannhauser W and pechanek U: Biogenic amines in food. formation, occurrence, analysis and toxicalogical evaluation. Z. Ges. Hyg. 1984: 66.
6. Taylor S L, Stratton J E and Nordlee J A. Histamine posioning (Scombroid fish poisoning): an allergy-like intoxication. Clin, Toxicol. 1989; 27:225-240.
7. Morrow J D, Margolies G R, Rowland J and Roberts L J. Evidence that histamine is the causative toxin of scombroid-fish poisoning. New Engl J Med. 1991; 324: 16-20.
8. Kim S H , Gigry B, Barros B and Price R G. Histamine and biogenic amines production by *M. morganii* isolated from temperature - abused albacore. J. Food prot. 2000; 63: 244-251.
9. Ten Brink B Damink C Joosten H M L J and Huis in't veld J H J. Occurrence and formation of biologically active amine in foods. Inter. J. of Food Micro 1990; 11: 73-84.

10. Halasz A, Barath A, Simon-Sakardi L and Holzapfel W. Biogenic amines and their production by microorganisms in food. Trends Food Sci. Technol. 1994; 5: 42–49.
11. Tassoni A, Germana M A and Bagni N. Free and conjugated polyamine content in Citrus sinensis Osbeck, cultivar brasilino N.L. 92, a Navel orange, at different maturation stages. Food Chemistry 2004; 87: 537–541.
12. Silla Santos M H. Biogenic amines. Their importance in foods. International Journal of Food Microbiology 1996; 29: 213–231.
13. Awan M A , Fleet I and Thomas C L P. Determination of biogenic diamines with a vaporization derivatisation approach using solid-phase microextraction gas chromatography-mass spectrometry. Food Chemistry 2008; 111: 462–468.
14. Riebroy S, Benjakul S, Visessanguan W, Kijrongrojana K, and Tanaka M.. Some characteristics of commercial Som-fug produced in Thailand. Food Chemistry 2004; 88:527–535.
15. Carelli D Centonze D Palermo C Quinto M and Rotunno T. An interference free amperometric biosensor for the detection of biogenic amines in food products. Biosensors and Bioelectronics 2007; 23: 640–647.
16. Draisci R Volpe G, Lucentini L, Cecilia A, Federico, R, and Palleschi G. Determination of biogenic amines with an electrochemical biosensor and its application to salted anchovies. Food Chemistry 1998; 62: 225–232.
17. Paproski R E, Roy K I and Lucy C A . Selective fluorometric detection of polyamines using micellar electrokinetic chromatography with laser-induced fluorescence detection. Journal of Chromatography A 2002; 946: 265–273.
18. Lapa-Guimaraes J and Pickova J. New solvent systems for thin-layer chromatographic determination of nine biogenic amines in fish and squid. Journal of Chromatography A 2004; 1045: 223–232.
19. Keow C M , Bakar F A, Salleh A B, Heng L Y, Wagiran R and Bean L S. An amperometric biosensor for the rapid assessment of histamine level in tiger prawn (*Panaeus monodon*) spoilage. Food Chemistry 2007; 105: 1636–1641.
20. Moret S, Smela D , Populin T and Conte L S. A survey on free biogenic amine content of fresh and preserved vegetables. Food Chemistry 2005; 89: 355–361.
21. Oguri S, Enami M and Soga, N.. Selective analysis of histamine in food by means of solid-phase extraction cleanup and chromatographic separation.Journal of Chromatography A 2007; 1139: 70–74.
22. Deabes, M. M.. Studies on some biogenic amines in some meat and fish products with respect to other quality attributes. M.Sc. Thesis, Food Science &Technology Dept., Faculty of Agriculture, Al-Azhar Univ., Cairo, Egypt, 2000.
23. ICMSF. Microorganisms in food, 2. Sampling for microbiological analysis: principals and specifications for foods 1978.
24. Varkont I J. Microbiological quality of quick frozen meat rolls. Hutoiper, 1980*;* 27:105.
25. Hui J Y and Taylor S L. Inhibition of *in vivo* histamine metabolism in rats by fooborne and pharmacologic inhibitors of diamine oxidase, histamine N-methyltransferase, and monoamine oxidase. Toxicol. and App. Pharm 1985; 81:241.
26. Taylor S L and Speckhard M W. Inhibition of bacteria,l histamine productiono by sorbate and other antimicrobial agents J. Food Prot. 1984; 47:508-511.
27. Koehler P E and Eitenmiller R R. High pressure Liquid chromatographic analysis of tyramine, phenylethylamine and tryptamine in sausage, cheese and chocolate. Journal of Food science 1978; 43: 1245.
28. Gardini F, Martuscelli M, Caruso M C, Galgano F, Crudele M A, Favati F, Guerzoni M E, Suzzi G. Effects of pH, temperature and NaCl concentration on the growth kinetics, proteolytic activity and biogenic amine production of Enterococcus faecalis. International Journal of Food Microbiology 2001; 64:105– 117.
29. Lopez- sabater E I, Rodriguz- Jerz J J, Roig-sagues A X and Mora-Ventura M A T. Bacteriological quality of tuna Fish *(Thunus Thynnus)* destined for canning: effect of tuna handling on presence of histidine decarboxylase bacteria and histamine level. Journal of food Protection 1994; 57(4):318- 323.
30. Yoshinaga D H Frank H A. Histamine-producing bacteria in decomposing skipjack tuna *Katsuwonus pelamia*. Appl. Environ. Microbiol. 1982; 44: 447–452.
31. Allison C, MacFarlane G T. Influence of pH, nutrient availability, and growth rate on amine production by Bacteroides fragilis and Clostridium perfringens. Appl. Environ.Microbiol. 1989; 55: 2894–2898.
32. Chander H Batish V K Babu S and Singh R S. Factors affecting amine production by selected strain of Lactobacillus bulgaricus. J. Food Sci. 1989; 54: 940–942.
33. Olson E R. Influence of pH on bacterial gene expression. Mol. Microbiol 1993; 8: 5–14.
34. Bearson S Bearson B and Foster J W. Acid stress responses in enterobacteria. FEMS Microbiol. Lett. 1997; 147: 173–180.
35. Askar A and Treptow H. Amines. In Encyclopedia of food science, food technology and nutrition. New York: Academic Press. 1993: 141-146.