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OCCURRENCE OF HISTAMINE IN SAUSAGE PRODUCED OF TWO MAJOR MANUFACTURERS IN KHUZESTAN PROVINCE BY HPLC METHOD

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Abstract

Introduction: Histamine 2-(4-imidazolyl) ethylamine is a primary amine that produces from decarboxylation of the amino acid, L-histidine. This substance is being produced in fermentational nutritional products. The aim of this study was checking of histamine concentration in sausages produced of Khuzestan and karine factories at Khuzestan province using HPLC method. **Material and method**: Mobile phase consist 85% of buffer solution and 15% acetonitrile. For preparing buffer solution 1.7 gr KH_2PO_4, 2.85 gr $K_2HPO_4{\cdot}3H_2O$ and 0.49 $C_{10}H_{21}O_3SNa$ in 1 l deionized water were solved and the pH of the solution reached 6.9. 20 ml of perchloric acid 1M added to 5gr homogenized sausage sample and then 1 minuets shaked and 15 minutes this suspension was in ultrasonic bathroom and centrifuged. Supernatant liquid was removed and then all this process applicated for second time. Both of two supernatant liquids were mixed and aliquot of 1,1- dimethyle biguanide dihydrochloride (inner standard) added to obtain 5 ppm concentration, was brought to volume 50 ml with deionized water. Finally 20 µl of filtrate injected into HPLC instrument with C18 column and UV detector in wavelength 214 nm at flow rate of 0.7 ml/min. **Results**: All reviewed samples for both factories were contaminated by histamine. On average the amount of histamine in Khuzestan and Karin factories in order were 0.056 and 0.24 ppm. **Conclusion**: Because the amount of histamine was low and it was under the allowed amount (50 ppm) determined by FDA , the use of producing sausages of this factories in consumer is not toxic and allergic.

Keywords: histamine, sausage, HPLC, Histidine, 1,1-dimethyl bigunide dihydrochloride..

1. Introduction

The method of fermentation is a traditional preservation process that produce relatively stable products with typical sensorial characteristics. One of this fermented products is sausage that made from mixture of minced meat, several ingredients, such as salt, oil, starch, sugar, spices and curing agents is stuffed into a casing(1). For the first time about two hundred years ago, first sausage in commercial scale was produced by George Lehner (2). nowadays heating meat products especially all kinds of sausage have become more common and taken into consideration in human societies(3).

1.1. Boogenic amines

The basic nitrogenous compounds in foods such as wine, beer, fermented meat (sausages), fish products, cheese, and fermented vegetables are biogenic amines. These compounds are produced by microbial activity of some species of genera *Pseudomonas, Enterococcus, Lactobacillus*, and of the genera of Enterobacteriaceae family.

The importance of biogenic amines in foods have two reason:

1.as a indicator quality in some foods
2.biogenic amines are hazardous for human health due to their toxic effects(4).

1.2. Histamine

Histamine (1H-imidazole-4-ethanamine, Him) is one of the biogenic amines which causes an allergies like food poisoning. It will be produce by decarboxylation of L-histidine in foods when it is contaminated with microorganisms that has strong histidine decarboxylase (EC.4.4.4.22) or (Ec 4.1.1.26) activity (Figure 1) (5). in body histamine mainly presence in mast cells and basophiles and affects by its interaction with one of at least three subclasses of specific histamine receptors, H1, H2, and H3 that are G protein coupled receptors. These reactions causes effects such as rhinitis, pruritus, mucus secretion, gastric acid secretion, smooth muscle and etc(6).

1.2.1. Histamine poisoning

For the first time Japanese reaserchers reported histamine poisoning in 1950 years. In this term histamine poisoning was the greatest reason of the food illness in Japan (7, 8). it can be cause of ingestion of food containing unusually high level of histamine. 500 mg/kg (ppm) caused histamine food poisoning histamine poisoning is an allergen-type reaction and makes symptoms such as itching difficulty in breathing, vomiting, rash, fever, and hypertension (4).

Since all over the world histamine poisoning as a result of sausage consumption have been reported. This study was designed in order to understand histamine condition in sausages which producing in khouzestan province companies in iran.

Materials and Methods

2.1.Chemicals

Methanol, acetonitrile (solvents used for HPLC), KH_2PO_4, $K_2HPO_4{\cdot}3H_2O$, perchloric acide (analytical grade) and HCL 0.1N (for preparation of histamine stock solution) all of these were supplied by Merck. The reference standard histamine dichlorohydrate (99% pure), chromatographic reference standard 1,1-dimethylbiguanide and 1-decanesulfonic acid sodium salt ($C_{10}H_{21}O_3SNa$) (98% pure) were supplied by sigma Aldrich.

2.2.Preparation of standard solutions

The histamine stock solution containing 2000 mg/l was provide by dissolving 10 mg in 5 ml of a solution of HCl 0.1 N. The histamine stock solution was stable for 2 month at +4 ℃ .the stock solution of 1,1-dimethylbiguanide hydrochloride (inner solution) was provide by dissolving 128.2 mg in 100 ml of solution of HCl 0.1N. the inner standard stock solution was stable for 6 month at +4 ℃. the solutions which were used in working at a concentration of 0.25-0.5-1-2-5-10-20-50-100 mg/l were provide by suitably diluting the histamine stock solution HCl 0.1N. for the achievement to a final concentration of about 5mg/l 1,1-dimethylbiguanide was added to all the working solution.

2.3.HPLC eluents

For the preparing of buffer solution was addeds 1.7g of KH_2PO_4, 2.85g of $K_2HPO_4{\cdot}3H_2O$ and 0.49g of decanesulfonic acid sodium ($C_{10}H_{21}O_3SNa$) was added to 1 ml water, obtaining to final pH of 6.9. Eluent A is containing 85% of buffer solution and 15% of methanol and Eluent B is containing of acetonitrile.

2.4.Equipment and conditions

Mobile phase was made from the combination of 85% Eluent A and 15% Eluent B using Luna C18 (2) column, 250 mm × i.d. incidentally the separations were applied under isocratic conditions. The flow rate was 1ml/min and the injection volume 20 µl and the detector was at a wavelength of 214nm.

2.5.Sample preparation

5 grams of previously homogenized sausage were disported and 20ml of 1mo/l $HCLO_4$ added to It. This mixture was vortex for 1min and then was putted in ultrasound bath for 15min. centrifuged at 4160 RCF×g at +4 ℃ for 15min. the supernatant fluid was removed and. This process was repeated twice. The extracts were combined then 1,1-dimethyle biguanide dihydrochloride (internal standard) added to It for achievement to concentration of about 5mg/l and was brought to volume 50 ml with deionized water. Finally 20 µl of filtrate injected into HPLC instrument.

Results and Discussion

Calibration curve of histamine standard and 1,1-dimethylbiguanide standard while intra-day and inter-days (n=10) (Figure 2), excellent linear correlation coefficient were R^2=0.9994 and R^2=0.99862 respectively, observed above the range of 0.25 to 100 ppm (Figure 3-11). retention times for histamine and 1,1-dimethylbiguanide standard were (7/81±0/1)and (8/91±0/1) min, respectively. The average recoveries and relative standard deviation (RSD %) for intra-day and inter-days of the applied analytical methods for has been shown (Table 1). All recoveries were more than 78% (Table 2), indicating the high accuracy of the method (Figure 12). The limit of detection (LOD) and limit quantification (LOQ) for histamine determined were 0.3 and 1 mg/kg. The mean of histamine concentration in Khuzestan and karine sausages were 0.056 and 0.24 ppm respectively.

Table 1.Descriptive statistics of Data of Investigated sausages samples

Descriptive Statistics	N	Mean	Std. Deviation	Minimum	Maximum	Percentiles		
						25th	50th (Median)	75th
Histamine Concentration (ppm)	54	0.15008767	0.195224171	0.000969	0.747544	0.03452048	0.06635873	0.15145395
Type of sausage	54	3.50	1.724	1	6	2.00	3.50	5.00

Table 2.Recoveries for histamine in sausage samples

Spiked level (ppm)	Added volume amount to samples	Obtained histamine con (ppm)	Average recovery (%)
0.25	125 µl	38.4	77
50	2.5 ml	43.6	88
100	5 ml	45	90

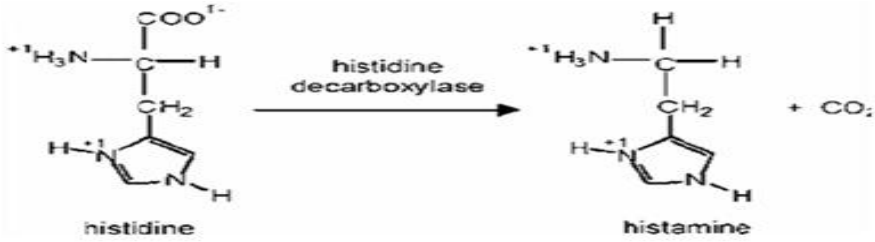

Figure 1. conversion of histidine to histamine by histidine decarboxylase

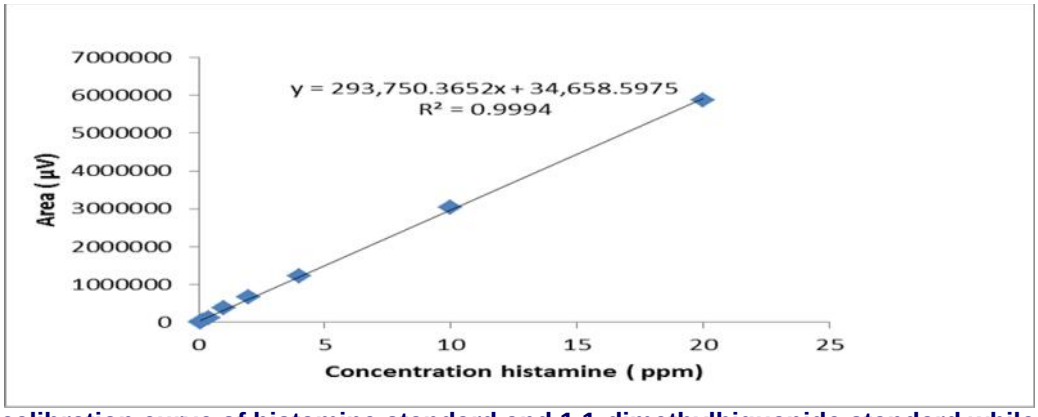

Figure 2. calibration curve of histamine standard and 1,1-dimethylbiguanide standard while intra-day monitored at 214 nm; condition: ODS (C18)-H-OL 5-36060 Column, 250mm × 4.6mm ID, particle diameter 5 µl, eluents A-B (85:15,v/v) at a flow-rate of 0.7 ml/min, injection loop 20µl

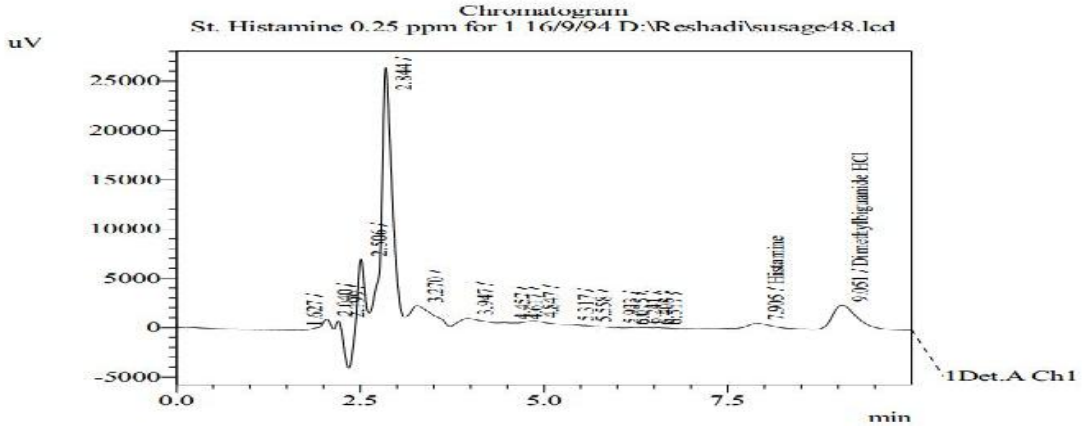

Figure 3. HPLC chromatograms of Histamine standard 0.25 ppm and 1,1-dimethylbiguanide standard 5mg/l

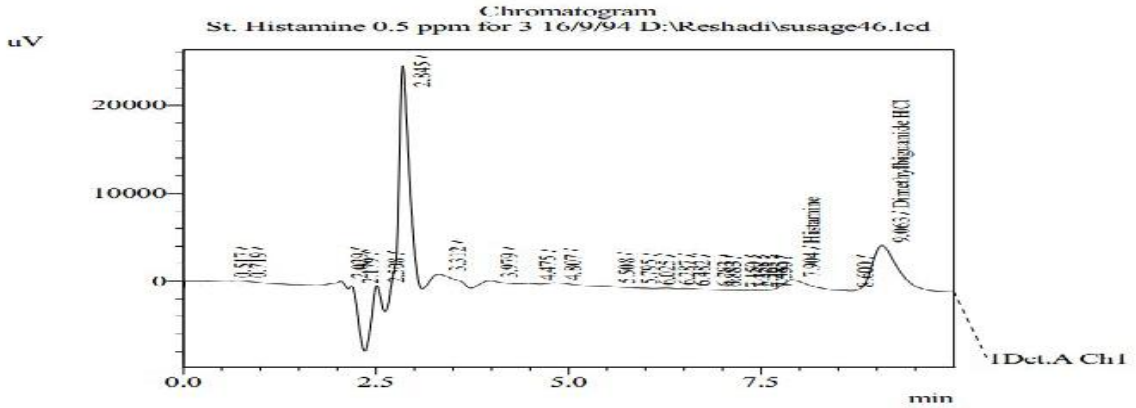

Figure 4. HPLC chromatograms of Histamine standard 0.5 ppm and 1,1-dimethylbiguanide standard 5mg/l

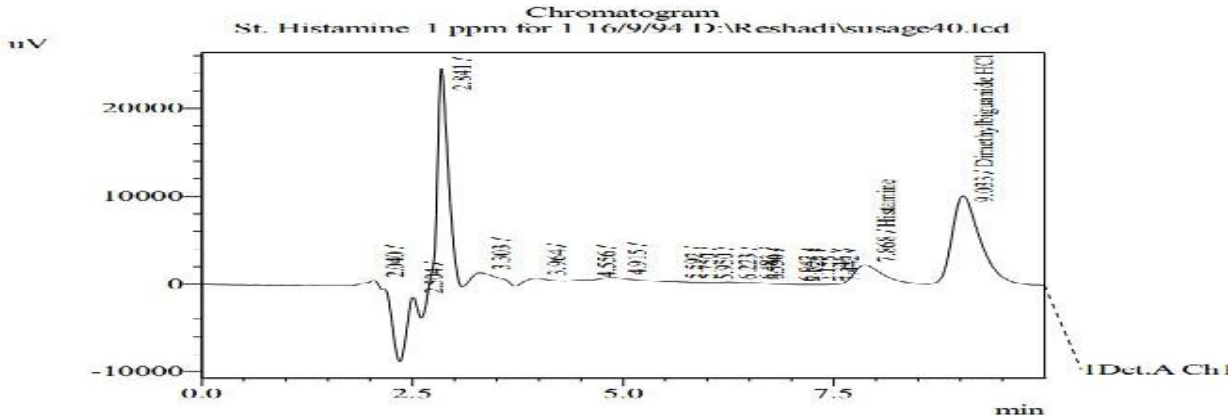

Figure 5. HPLC chromatograms of Histamine standard 1 ppm and 1,1-dimethylbiguanide standard 5mg/l

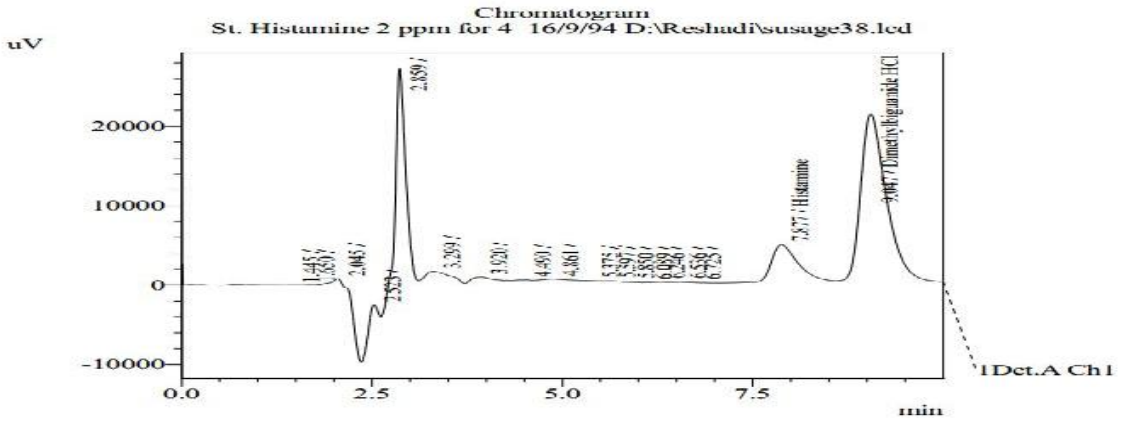

Figure 6. HPLC chromatograms of Histamine standard 2 ppm and 1,1-dimethylbiguanide standard 5mg/l

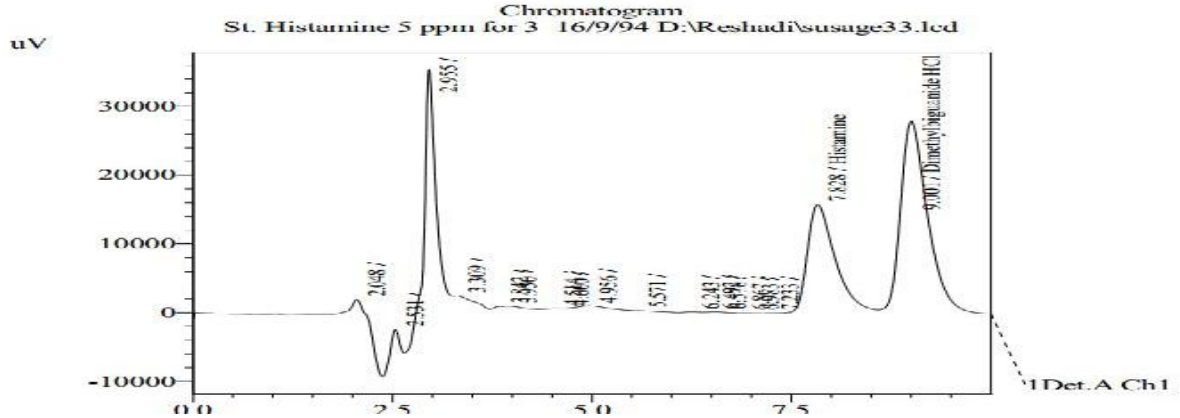

Figure 7. HPLC chromatograms of Histamine standard 5 ppm and 1,1-dimethylbiguanide standard 5mg/l

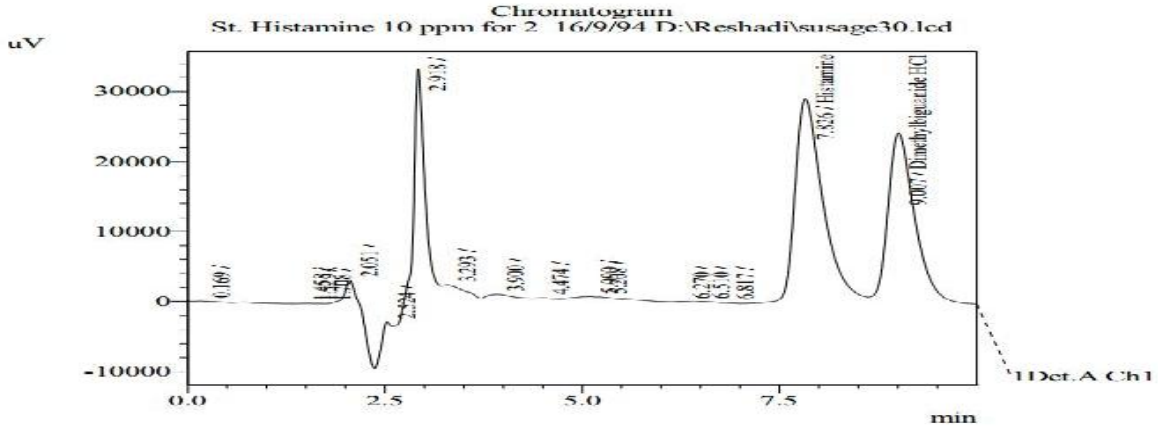

Figure 8. HPLC chromatograms of Histamine standard 10 ppm and 1,1-dimethylbiguanide standard 5mg/l

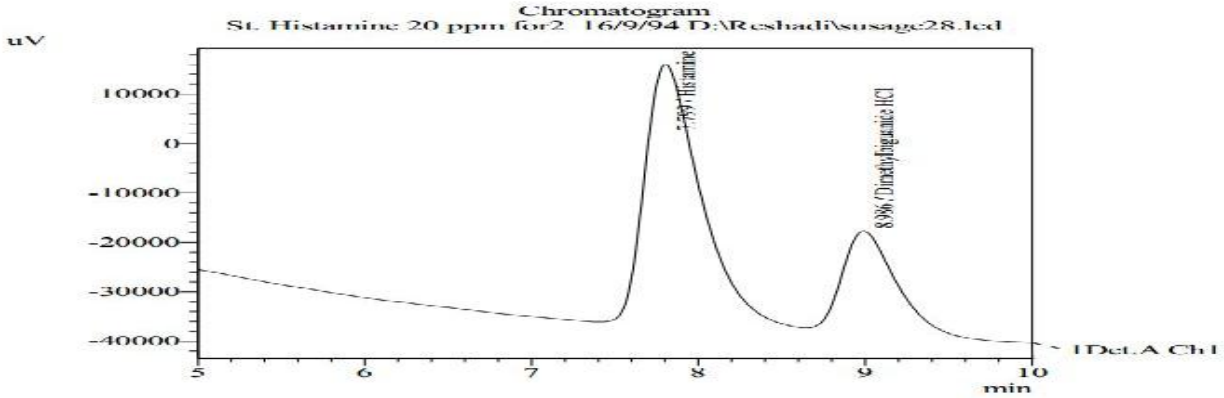

Figure 9. HPLC chromatograms of Histamine standard 20 ppm and 1,1-dimethylbiguanide standard 5mg/l

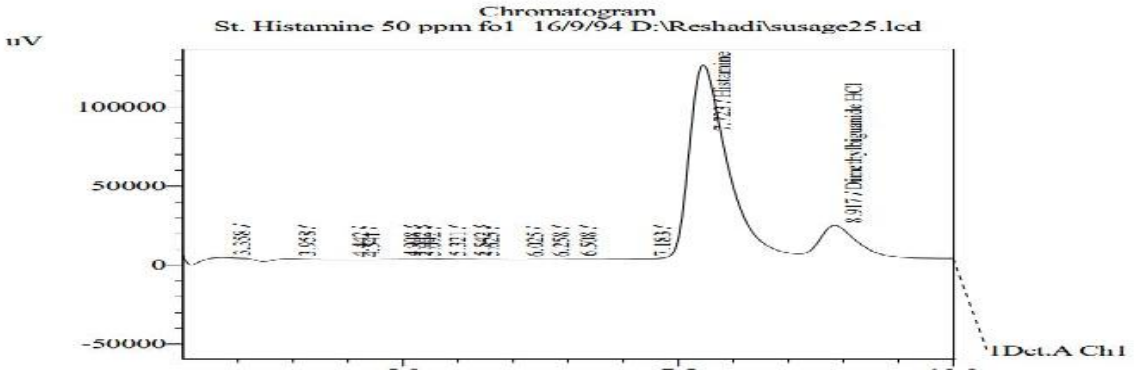

Figure 10. HPLC chromatograms of Histamine standard 50 ppm and 1,1-dimethylbiguanide standard 5mg/l

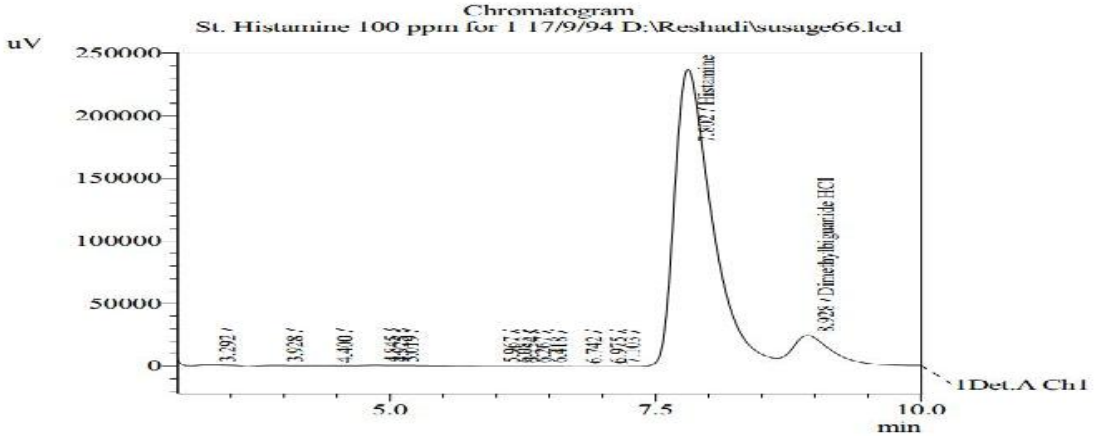

Figure 11. HPLC chromatograms of Histamine standard 100 ppm and 1,1-dimethylbiguanide standard 5mg/l

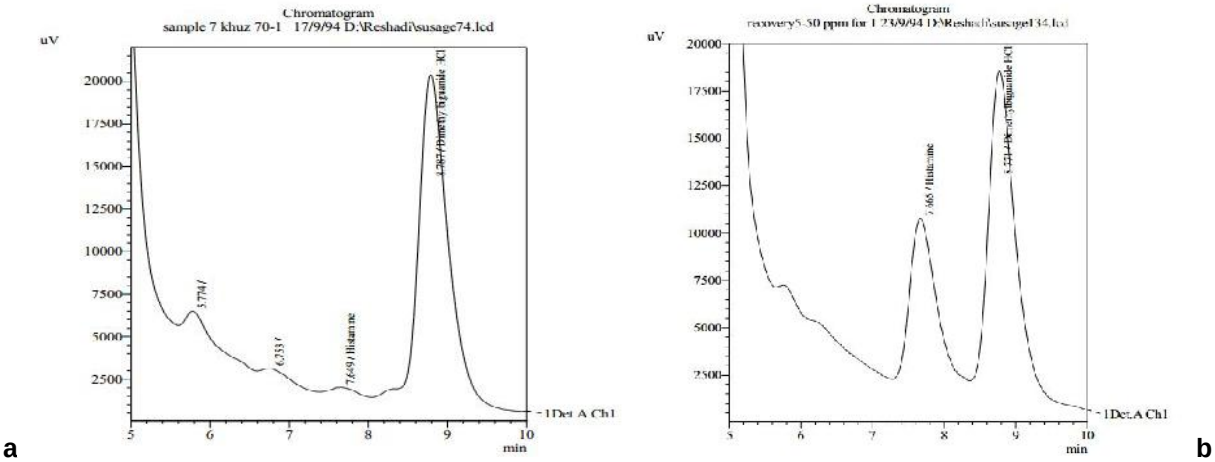

Figure 12. HPLC chromatograms of sausages:(a) blank, (b) recovery

Discussion

In body histamine is well produced as a product of basophils and mast cells and release in allergic qualifications from these sources that caused allergic symptoms(9). its In fermented foods like sausages, histamine formed by enzymatic decarboxylation of L-histidine. Therefore consumers of these products are at risk of histamine food poisoning. Histamine food poisoning is one of the most prevalent type of food intoxication reported(10).

As a general rule, 500 mg/kg (ppm) caused histamine food poisoning(4). the FDA require that histamine levels not exceed 50mg/kg(11).

In the currently study, the histamine content was measured in sausage and then it compared with FDA standards.

Mean concentration of histamine in two brands (Khuzestan and Karin) sausage samples were significantly lower than maximum tolerable level of histamine set by the FDA. In the present study, histamine was detected in all samples inconsequential (lower than 1ppm). The maximum level of histamine belonged to Karin sausage in 0.747mg/kg. generally level of histamine in Karin samples were detected more than Khuzestan.

In 2004 a view in turkey The histamine contents of Turkish style fermented sausages were determined on 46 samples of five different brands obtained from retail stores in Van in Turkey. Histamine was found in all samples in the range 19.64–87.47 mg/kg (mean 32.13 mg/kg). Histamine levels in the brands differed significantly (P<0.01). The results suggest that the histamine levels in the sausages were not hazardous in terms of public health although they may be a potential risk to sensitive individuals(12). an else study in turkey was accomplished on sausage In this study, 5. Turkish fermented sausage (sucuk) samples from different producers were examined with respect to their biogenic-amine contents, including histamine, tryptamine, and tyramine. Histamine and tyramine were found in all samples in the range of 6. 72 to 362. 22 mg/kg. So in this view level of histamine in all samples was more than the US FDA allowable limit of 50mg/kg(13). A survey was conducted to determine the biogenic amine contents of Egyptian dry sausage. Histamine was found in 46% of the tested samples with an average of 5.25 mg/kg(14). Histamine detected in finish dry sausage in the range 1-200mg/kg(15). in 2012 during a study Twenty-five tuna sausage products were purchased from retail markets in Taiwan. The rates of occurrence of biogenic amines, histamine-forming bacteria, and adulteration by pork and poultry were determined. The average content of various biogenic amines in all tested samples was less than 2.0 mg/100 g (<0.05 to 1.85 mg/100 g) lower than the FDA allowable limit(16).

References

1. Latorre-Moratalla M, Veciana-Nogués T, Bover-Cid S, Garriga M, Aymerich T, Zanardi E, et al. Biogenic amines in traditional fermented sausages produced in selected European countries. Food Chemistry. 2008;107(2):912-21.
2. Bem, Z. and H. Hechelmann.. problems with manufactured of small canned sausage .J. Fliescherei 1994;45(10):55-59.
3. Soriano A, Cruz B, Gómez L, Mariscal C, Ruiz AG. Proteolysis, physicochemical characteristics and free fatty acid composition of dry sausages made with deer (*Cervus elaphus*) or wild boar (*Sus scrofa*) meat: A preliminary study. Food Chemistry. 2006;96(2):173-84.
4. Ercan S, Bozkurt H, Soysal Ç. Significance of biogenic amines in foods and their reduction methods. J Food Sci Eng. 2013;3:395-410.

Ten Brink B, Damink C, Joosten H, In't Veld JH. Occurrence and formation of biologically active amines in foods. International Journal of Food Microbiology. 1990;11(1):73-84.
6. Bachert C. The role of histamine in allergic disease: re-appraisal of its inflammatory potential. Allergy. 2002;57(4):287-96.
7. Middlebrooks B, Toom PM, Douglas WL, Harrison RE, McDowell S. Effects of Storage Time and Temperature on the Microflora and Amine-Development in Spanish Mackerel (*Scomberomorus maculatus*). Journal of Food Science. 1988;53(4):1024-9.
8. Bean NH, Griffin PM. Foodborne disease outbreaks in the United States, 1973-1987: pathogens, vehicles, and trends. Journal of Food Protection®. 1990;53(9):804-17.
9. Malone MH, Metcalfe DD, editors. Histamine in foods: its possible role in non-allergic adverse reactions to ingestants. Allergy and Asthma Proceedings; 1986: OceanSide Publications, Inc.
10. Slorach S. Histamine in food. Histamine and Histamine Antagonists: Springer; 1991. p. 511-20.
11. Tsai Y-H, Kung H-F, Lee T-M, Chen H-C, Chou S-S, Wei C-I, et al. Determination of histamine in canned mackerel implicated in a food borne poisoning. Food Control. 2005;16(7):579-85.
12. Ekıcı K, Şekeroğlu R, Sancak YC, Noyan T. A note on histamine levels in Turkish style fermented sausages. Meat Science. 2004;68(1):123-5.
13. Şenöz B, Işikli N, Çoksöyler N. Biogenic amines in Turkish sausages (sucuks). Journal of Food Science. 2000;65(5):764-7.
14. Shalaby A. Survey on biogenic amines in Egyptian foods: sausage. Journal of the Science of Food and Agriculture. 1993;62(3):291-3.
15. Eerola H, Sagués AR, Hirvi T. Biogenic amines in Finnish dry sausages. Journal of Food Safety. 1998;18(2):127-38.
16. Kung H-F, Tsai Y-H, Chang S-C, Hong T-Y. Biogenic amine content, histamine-forming bacteria, and adulteration of pork in tuna sausage products. Journal of Food Protection®. 2012;75(10):1814-22.

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