Effect of microbial load on the level of histamine for some types of domestic and imported red meat during the storage period.

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Abstract:

Background: Histamine is one of the biogenic amines that belong among the naturally occurring substances. It can be formed in food as a result of metabolic processes of microorganisms .If the concentration of histamine is above a normal level (5mg/100g) this could possibly due to bacterial contamination of food harmful affect may occur.

Objective: The purpose of this study was to detect histamine forming bacteria and quantification of histamine in fresh meat product available in local meat markets in Baghdad.

Methods: Histamine level determination in some red meats product was performed by known colorimetric methods. This method enables a rapid and precise determination of histamine in many samples, in this study, total number of ten red meat were collected from Baghdad local market. Meats were analyzed for histamine. Extraction and determination of histamine in all meat samples were made by colorimetric method. One gram of red meat samples were added to 99ml of sterile peptone water to make dilution 10^{-1}, further dilution was made. 0.1 ml was put to inoculate selective culture media, incubated at 37C for 24 hours. Microbes which have been diagnosed were a Staphylococcus spp. and *Pseudomonas* spp. by conventional methods.

Results: In this study histamine was detected in all red meat samples at concentration ranging from 0.87 to 17.30 mg/100g.Relatively, the histamine level was higher in amber, followed by Al-Hana and Al-murad. Histamine contents were established according to (WHO) regulation. Tested samples contain *Pseudomonas* spp. and *Staphylococcus* spp.

Conclusions: Regarding the presence of biogenic amines in meat, it also would be useful the study of their role in formation of certain compound in meat and meat products. Contamination of red meat samples with *Pseudomonas* spp. May be attributed to cross –contamination.

Keywords: Histamine, red meat, colorimetric methods.

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Introduction:

Histamine is one of the biogenic amines that belong among the naturally occurring substance. Biogenic are nitrogene compounds of biological importance in foods, vegetables, and microbial and animal cells (**1**).vMeats are among those high- protein containing foodstuffs in which enzymatic and microbial activities cause the formation of amino acids and biogenic amines (**2**). The interest in amine determination is due to their ability to have a direct or indirect effect, on human vascular and nervous systems. In deed large amount of the biogenic amines can cause rash, headache, nausea, hypo- or hypertension, cardiac palpitation, intracerebal hemorrhage (**3**). Histamine (2-4-imadazoyl-ethylamine) is a biogenic amines produced by some species of bacteria such as *Morganellamorgais,Proteus* spp. ,*Pseudomonas* spp. and *Staphylococcus* spp. occurring to various degree in many foods (**4**). The determination of major biogenic amines in food products are putrescine, histamine, tryamine,tryptamine,spermine,agmatine is of great interest not only due to their possible toxicity, but also due to acting as potential indicators to determine the quality or freshness or spoilage of food products (**5**). The aim of this study was to determine levels of histamine in ten meat samples consumed in Baghdad city.

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Materials and Methods:

A total number of 10red meat samples were collected from the supermarkets of Baghdad. All samples were analyzed before their expiry date. All samples were immediately transferred to the microbiology laboratory at Market Research and Consumer Protection Center, Baghdad University, and stored at -18C° in the deep freezer until use (Table1). The media used were in a dehydrated form and prepared according to the manufacturer's instructions. One grams of red meat were added to 99ml of sterile distilled peptone water in a flask and shaken well to make 10^{-1} dilution. Further dilutions were prepared in sterile distilled peptone water. Prepared samples were serially diluted (10^{-6}) in sterile water and used to enumeratedbacteriain specific culture medium. This was carried out according to the methods described by (**6**and**7)**, which include the following methods: Total plate count were enumerated by pour plate method using standard plate count .Diluted samples were cultured on plate count Agar by

using one ml of each dilution (10^{-6}), which added to petri-dish and incubated at 37C° for 24 hours, colonies were counted. For the enumeration of coagulase positive *Staphylococcus* (*Staphylococcus aureus*), the mannitol salt agar was used for confirmation and incubated at 37C° for 24 - 48 hours, developed colonies were counted. For the enumeration of *Pseudomonas* spp., skimmed meat samples from the dorsal half of the meat were used for the analysis. Twenty five g of the meat sample were homogenized in 225 ml peptone water, and then, serial decimal dilutions were prepared. Amount of 0.1 ml of each dilution was spread on *Pseudomonas* cetrimide, nalidixic acid (CN) agar; Pseudomonas agar base contain 10 ml/l glycerol and selectivity made by inclusion of cetyltrimethyl ammonium bromide (cetrimide; 200 mg/l) and nalidixic acid sodium salt (15 mg/l) and were incubated at 25 °C for 48 h. All colonies that developed on the medium were counted and confirmed their identity as *Pseudomonas* spp. by oxidase testing (8). Quantification of histamine was carried out using colorimetric method reported by Patange *et al* (2005)(9). In this method, 1 ml of the muscle extract was taken into a glass-stoppered test tube anddiluted to 2 ml with saline and 0.5g of salt mixture containing 6.25g of anhydrous sodium sulfate to 1 g trisodium phosphatemonohydrate was added. The tubes were stoppered and shaken thoroughly. 2 ml of n-butanol was then added and the tubes shakenvigorously for 1 min and allowed to stand for 2 min and thenshaken briefly to break the protein gel. The tubes were further shaken vigorously for few seconds and then centrifuged at 3100rpm for 10 min. The upper butanol layer (only1ml) was transferred into a clean and dry test tube and evaporated todryness in a stream of nitrogen. The residue was dissolved in 1 mlof distilled water. In a clean tube 5 ml of 1.1% sodium carbonate solution was taken and 2 ml of the chilled reagent, p-phenyldiazoniumsulfonate was added slowly and mixed. It was 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 at496 nm. The histamine concentration in sample was estimated using the following formula:

Histamine (mg/100 g) =

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

where A is the value of histamine obtained in l g/ml from thestandard curve.

Table1: Collected redmeatsamples from Baghdad markets

No	Sample Name	origin	Date of production	Date of expiry	Volume /gram
1	Al-Baraka	Iraq	2016/01/13	2017/1/12	300
2	Al-murad	Iraq	2016/2/27	2016/6/26	250
3	Al-Fakher	India	2016/3/2	2017/2/29	1000
4	Halal	India	2015/12/29	2016/12/28	300
5	Al-Ahmed	India	2016/1/20	2017/1/19	250
6	Al-Anwar	India	2016/2/15	2017/2/14	600
7	Kirat-karbala	Iraq	2016/2/10	2017/2/9	700
8	Al-Hanaa	India	2016/1/15	2017/1/14	300
9	Al-wasam	India	2015/12/7	2016/12/6	100
10	Amber	India	2015/10/11	2016/10/10	100

Statistical Analysis: Statistical significance was assessed by using least significant differences – LSD (T-test) P – value < 0.05 was considered significance.

Result:

The results concerning total viable count are presented in table (2) Table (2) shows the total microbial count in ten red meat.Higher counts was recorded amongAmber(45×10^3 CFU/g). Followed by Al-murad (34×10^3CFU/g). , Al-Ahmed (28×10^3CFU/g.) and Al-Anwar(20×10^3CFU/g.) Also higher bacterial contamination was recorded amongAmber which recorded *Staphylococci*(22×10^3 CFU/g.) followed by*Pseudomonas* spp. which recorded (14×10^3 CFU/g.)(Table 2). Thisstudy wasconcerned the most important biogenic amines namely histamine that can be found in various kinds of meat. Table (3) shows the variation of histamine in ten red meat samples obtained from local markets of Baghdad city. Higher level concentration of histamine were recorded among Amber (17.3 mg/100g) followed by Al-Hanaa (16.2 mg/100g) and Al-murad (14.5 mg/100g) .

Table (2): Isolation of microbial species identified in the red meat samples.

No	Trade Mark of meat samples	Total Count Bacteria CFu/g	*Staphylococci* CFu/g	*Pseudomonas* spp. CFu/g
1	Al-Baraka	11×10^2	6×10^1	12×10^1
2	Al-murad	34×10^3	1×10^3	8×10^4
3	Al-Fakher	7×10^1	10×10^2	2×10^1
4	Halal	15×10^3	9×10^2	7×10^4
5	Al-Ahmed	28×10^3	25×10^2	19×10^3
6	Al-Anwar	20×10^3	14×10^1	8×10^1
7	Kirat-karbala	12×10^2	6×10^1	5×10^1
8	Al-Hanaa	32×10^1	11×10^2	13×10^3
9	Al-wasam	9×10^2	13×10^2	2.6×10^2
10	Amber	45×10^3	22×10^3	14×10^3

Table (3): Histamine variation in red meat (Mg/100g).

No	Trade Mark of meat samples	Histamine mg/100 g
1	Al-Baraka	0.96
2	Al-murad	14.5
3	Al-Fakher	9.8
4	Halal	10.1
5	Al-Ahmed	11.6
6	Al-Anwar	10.2
7	Kirat-karbala	0.87
8	Al-Hanaa	16.2
9	Al-wasam	6.8
10	Amber	17.3

Discussion:

In this study *Pseudomonas* spp. was found in the types of red meatsfrom Amber and Al-Ahmed while *Staphylococcus* spp. was found in Al- Ahmed and Amber at higher concentration. Also initial load of *Pseudomonas* spp. was< 100 CFU/g on meat products under aerobic condition (10,11). This study was in - agreement with others (12and 13). who stated that *Pseudomonas* spp. isolated from chicken meat and sardine fish respectively. More research is needed to evaluate the impact of the factors regarded biogenic amine formation in food and should some light on how toxic compounds of this kind could affect the consumers (14). The importance of using measures focused on the hygienic quality of both raw materials and processing units to avoid the development of aminogenic contaminant bacteria and in turn, to reduce biogenic amines content, is well known. However, proper hygiene may not be enough to avoid some biogenic amines formation and other technological measures must be applied (15). Generally, biogenic amines in all kinds of food can be controlled by strict use of good hygiene in both raw materials and manufacturing environments with corresponding inhibition of spoiling microorganisms .A better understanding of the mechanisms by which biogenic amines are being produced is necessary to prevent their formation of toxic amines (16).

Conclusion:

The red meats had a lot of microbial contamination and this mean that it lead to higher concentration of biogenic amines namely histamine. Contamination of red meat with *Pseudomonas* spp. May be attributed to cross-contamination.

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