# Detection of Some Toxic Biogenic Amines in Some Ready to Eat Meat Products

**Nesreen, Z. H. Eleiwa**
Animal Health Research Institute, Tanta lab.

## Abstract

**Sixty random samples of Shawerma, Kofta and kabab (20 of each) were collected from different restaurants in Gharbia governorate to be analyzed for biogenic amine by using High Performance Liquid Chromatography. The results revealed that the average concentrations of histamine were 12.91 ± 1.9, 8.86 ± 1.3 and 5.24 ± 0.6 mg/100g in the examined samples of shawerma, kofta and kabab, respectively. On the other side, the higher concentration of tyramine was persisted in Kofta (14.47 ± 1.9 mg / 100g), while the lowest one was recorded in Kabab (8.15 ± 1.1 mg / 100g). Meanwhile, Shawerma contained the highest level of Putrescine (17.63 ± 2.8 mg / 100g) followed by Kofta and Kabab (13.65 ± 1.7 and 11 ± 1.5 mg / 100g) respectively. The differences associated with such examined samples were significant at ($p<0.05$). Comparing the obtained results with the permissible limit (20mg/100g) recommended by (7), 25%, 15%, and 35 % of examined shawerma samples were exceeded this limt for histamine, tyramine and Putrescine, respectively. While 10%, 30%and 25% of kofta samples were unacceptable, respectively. Only 5% and 20 %of kabab samples had tyramine and putrescine above such standard limit. Concerning the permissible limit (10mg/100g) adopted by (8), the shawerma samples contained the exaggerated values of histamine and putrescine, however, kofta had the highest level of tyramine. This study discussed the factors affecting production of biogenic amines, public health hazard and control the production of these amines in meat products.**

## Introduction

Biogenic amines are compounds commonly present in living organisms in which they are responsible for many essential functions. They can be naturally present in many kinds of foods such as fruits and vegetables, meat, fish, chocolate and milk, but they can also be produced in high amounts by microorganisms through the activity of amino acids decarboxylases. Excessive consumption of these amines can be of health concern because their not equilibrate assumption in human organism can

generate different degrees of diseases determined by their action on nervous, gastric and intestinal tract and blood pressure **(24)** .

The biogenic amines which are the end products of the decomposition of amino acid or the transformation products of aldehydes and ketones were considered as indicators of food quality **(3)**.

The decarboxylation of the amino acids finally leads to generation of the amines which must be monitored to ensure consumer protection **(5)**.

Several bacterial species posses decarboxylase activity and reduce biogenic amines. Some strains have a rather wide spectrum and are able to decarboxylate many amino acids, where others have only strictly substrate specific decarboxylases. There is also great difference between the rates of amine production by different strains of the same species.

Only a few histamine-positive Gram – negative bacteria posses the ability to decarbxylate tyrosine. By contrast, several Gram-positive bacteria produce histamine and tyramine. For biogenic amines other than histamine and tyramine, bacterial species reported to be capable of putrescine production include ***Escherichia Coli***, Enterobacteraerogenes, ***Klebsiella pneumonia*** and ***Schewanella putrefaciens.*** The amine-postive bacteria are introduced to food mainly with raw materials **(13).**

The production of histamine, tyramine and putrescine has been related to the presence of lysine, histidine and lysine decarboxylase enzyme which can be synthesized by auto-enzyme or bacteria **(14 and 21)**.

The mono amine oxidase inhibitors (MAOI) have facilitated absorption of both tyramine and histamine from the intestine and potentiated their action **(20)**. Also, tyramine has been proved as a cause of adverse reactions with anti- depressant drugs **(10).**

The objective of this study was to assess the concentrations of biogenic amines (histamines, tyramine and putrescine) in some meat products (Shawerma, Kofta and Kabab) using High Performance Liquid Chromatography (HPLC).

## Material and Methods

A total of 60 random samples of ready-to-eat meat products represented by Shawerma, Kofta and Kabab (20 of each) were collected

from different restaurants in Gharbia Governorate to determine their contents for biogenic amines. Therefore, histamine, tyramine and putrescine were estimated by High Performance Liquid Chromatography (HPLC) according to **(16)**.

1-**Amine extraction** :

Accurately, 25 g of homogenized meat product samples mixed with 125ml of 5 % trichloroacetic acid (TCA) for 3 minutes using a warning blender, and then filtration was achieved using filter paper Whatman (No.1). Moreover, 10 ml of the extract was transferred into a suitable culture tube with 4 g Nacl and 1 ml of 50 % NaOH, then shacked and extracted 3 times by 5 ml n- butanol chloform (1: 1 V/V), stoppered and shacked vigorously for 2 min., followed by centrifugation for 5 min. at 3000 rpm and the upper layer was transferred to 50 ml separating funnel using disposable pasture pipette. To combine organic extracts (upper layer), 15 ml of n- heptanes was added and extracted 3 times with 1.0 ml portions of 0.2 NHCL, then NHCL layer was collected in a glass stopper tube. Solution was evaporated just to dryness using water bath at 95 °C with air of air currents.

2-**Derivatives formation** (Dansyl amines):

200 ml of each stock standard solution (or sample extract) were transferred to a culture tube and dried under vacuum. About 0.5 ml of saturated $NaHCO_3$ solution was added to the residue of the sample extract (or the standard).The tube stopppered and carefully mixed to prevent loss duo to spattering. Carefully, 1.0 ml dansyl chloride solution was added and mixed thoroughly using Vortex mixer. The mixture was kept in a water bath at 70 °C for 10 min. then, the extraction of dansylated biogenic amines was carried out using 3 times of 5.0 ml portions of diethyl-ether , and the ether layers were collected in a culture tube using disposable pasture pipette . The combined ether extracts were carefully evaporated at 35 °C in dry film and dissolved in 1 ml methanol, then 10 micro liters injected in HPLC (**19**).

3-**Interpretation of HPLC (16)** :

The most common technique for amine analysis is HPLC using derivatization before detection. Accordingly, 5-dimethylamine -1-naphalene sulphonyl chloride was used as derivatization reagent which characterized by the reaction with both primary and secondary amine groups. The

chromatographic separation was carried out to separate the three dansyl amines. Furthermore, 10, 20, 30, 40, and 50, microliter of dansyl amine standard as well as 10 micro liter of each dansylated sample extract were used. However, the chromatogram was examined under long wave of ultraviolet (254 nm) to establish weather or not the asyl amines of interest are present in the examined sample.

Finally, the concentration of each biogenic amine in the samples was recorded as mg/10g according to the following formula:

Amine concentration (mg/100g) = CV / V

Where,

C: concentration of amine standard (mg / g).

V: final dilution of sample extracts (ml).

W: weigh of the sample in the final extract (g).

**Statistical analysis:**

The obtained results were statistically evaluated by using analysis of Variance (ANOVA) test according to (**9**)

## Results

**Table (1): Average concentrations of histamine (mg / 100g) in the examined samples of ready-to-eat meat products (n = 20).**

| Products | Min. | Max. | Mean ± S.E |
|---|---|---|---|
| Shawerma | 3.1 | 31.6 | 12.91 ± 1.9* |
| Kofta | 2.7 | 23.6 | 8.86 ± 1.3 |
| Kabab | 1.2 | 12.1 | 5.24 ± 0.6 |

*Significant differences ($p<0.05$).

**Table (2): Average concentrations of Tyramine (mg / 100g) in the examined samples of ready –to-eat meat products (n = 20).**

| Products | Min. | Max. | Mean ± S.E |
|---|---|---|---|
| Shawerma | 4.3 | 28.5 | 11.17 ± 1.6* |
| Kofta | 6.0 | 36.8 | 14.47 ± 1.9 |
| Kabab | 1.7 | 21.6 | 8.15 ± 1.1 |

*Significant differences ($p<0.05$).

**Table (3): Average concentrations of Putrescine (mg / 100g) in the examined samples of ready –to eat meat products (n = 20).**

| Products | Min. | Max. | Mean ± S.E |
|---|---|---|---|
| Shawerma | 5.7 | 49.9 | 17.63 ± 2.8* |
| Kofta | 4.8 | 31.0 | 13.65 ± 1.7 |
| Kabab | 2.7 | 24.7 | 11 ± 1.5 |

* Significant differences ($p<0.05$).

**Table (4): Acceptability of the examined samples of ready-to-eat meat products (n= 20) based on their contents of biogenic amines (7).**

| Products / Biogenic amines | Maximum Permissible Limit ( mg / 100g ) | Shawerma | | Kofta | | Kabab | |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % |
| Histamine | 20 | 5 | 25 | 2 | 10 | 0 | 0 |
| Tyramine | 20 | 3 | 15 | 6 | 30 | 1 | 5 |
| Putrescine | 20 | 7 | 35 | 5 | 25 | 4 | 20 |

**Table (5): Acceptability of the examined samples of ready-to-eat meat products (n= 20) based on their contents of biogenic amines (8).**

| Products / Biogenic amines | Maximum Permissible Limit ( mg / 100g ) | Shawerma | | Kofta | | Kabab | |
|---|---|---|---|---|---|---|---|
| | | No. | % | No. | % | No. | % |
| Histamine | 10 | 10 | 50 | 6 | 30 | 1 | 5 |
| Tyramine | 10 | 9 | 45 | 10 | 50 | 5 | 25 |
| Putrescine | 10 | 12 | 60 | 10 | 50 | 8 | 40 |

## Discussion

It has been recognized for some time that biogenic amines occur in wide range of foods, among them meat and meat products. Meat is an important component of the diet in developed countries. The presence of these amines in food is of interest for two reasons: firstly, for toxicological reasons, in the sense that high levels of dietary biogenic amines can be toxic for certain consumers and secondly, for their role as possible quality indicators.

Table (1) showed that the mean concentration of histamine in examined samples were 12.91 ± 1.9, 8.86 ± 1.3 and 5.24 ± 0.6 in shawerma, Kofta and Kabab, respectively. Significant differences ($p<0.05$) were reported between these examined samples as a result of their contents of histamine. The high level of histamine in shawerma may be attributed to the unfavorable temperature and the presence of additives such as pepper, tomatoes and other spices which play an important role in growth and multiplication of histamine forming microorganisms **(10).** Meanwhile, the lowest concentration of histamine was in Kabab and this may be attributed to the large slices of meat used which may constitute a protective line from the superficial m.o. to penetrate the meat and cause degradation of the amino acids. These results were lower than that recorded by **(17)** who found

that the concentration of histamine in smoked turkey breast fillets was 11.9 mg / kg.

Histamine poisoning is a chemical intoxication, following the ingestion of foods that contain high levels of histamine. The incubation period is very short (few minutes to few hours) and duration of illness is short (few hours). The exact toxic threshold of biogenic amines is difficult to determine due to its independence on the efficiency of detoxification mechanisms of individuals. However, according to **(4)** and **(11)**, 10 mg of histamine in 100 g of sample can cause histamine poisoning. The most common symptoms are facial flushing, urticaria, edema, nausea, vomiting and diarrhea as well as, neurological involvement, tingling and burning sensation in the mouth. Symptoms may be more sever in patient taking certain medications that show the breakdown of histamine by their liver, such as isoniazid and doxycycline **(25)**.

Regarding to table (2) Kofta samples represented the higher concentrations of tyramine followed by Shawerma and Kabab (14.47 ± 1.9, 11.17 ± 1.6 and 8.15 ± 1.1), respectively. The differences between the examined samples were significant at ($p<0.05$). These results may be arised due to the higher temperature favoured proteolytic and decarboxylase activity of microorganisms resulting in increased amine concentrations **(2)**. Also, addition of acidic materials to the minced meat used for Kofta as onion Juice lowering the pH of the product which activate the acidic bacteria to form biogenic amine. These results are supported by the theory that the formation of biogenic amines is a protective mechanism of bacteria against acidic environments **(6 and 12)**.

Tyramie has been proved as a cause of adverse reactions involving headache, hypertensive crisis and interactions with anti-depressive drugs **(10)**. 10-80 mg of tyramine can cause cheese reaction (6 mg if patient receiving MAOI **(4 and 11)** .Minced meat can be regarded as matrice potentially contaminated by biogenic amines if provides with both micro-organisms and free amino acids required for amine formation , together with environmental factors favouring bacterial growth and decarboxylase activity **( 23 ; 19 and 1)** .

Table (3) revealed the average concentration of Putrescine in the examined samples which appeared to be higher in Shawerma followed by Kofta then Kabab (17.63 ± 2.8, 13.65 ± 1.7 and 11 ± 1.5) respectively. Significant differences appeared between these examined samples ($p<0.05$). These limits of Putrescine agree with those recorded by **(2)** and **(22),** who found the mean value of Putrescine processed meat was (23 mg / kg).The high concentration of Putrescine in Shawerma may be regarded to the quality of the raw material in minced meat which appears to be one of the main factors affecting biogenic amines formation whose levels increase in conjunction with microbial spoilage under favourable conditions **(5, and 15)**.

Putrescine persist an important interest because it can react with nitrates to form carcinogenic nitrosamine **(18)**.

Comparing the obtained results with the Standard recommended by **(7)** as showed in table (4) histamine not exceeded the permissible limits in Kabab samples but exceeded in Shawerma and Kofta (25 % and 10 %), respectively, while tyramine in the examined Shawerma, Kofta and Kabab samples were exceeded the limits (15%, 30%and 5 %), respectively, meanwhile, the exceeded percentage for Putrescine were (35%, 25% and 20 %), respectively for the same samples.

On the other side, histamine, tyramine and Putrescine were exceeded the recommended permissible limits of **(8)** as shown in table (5) by (50%, 45% and 60 %) for Shawerma ; (30%, 50%and 50 %) for Kofta and (5%, 25% and 40 %) for Kabab, respectively.

Finally, it is concluded that the high biogenic amine levels found in the examined samples indicate poor handling and / or processing of these products, therefore, the greatest emphasis in the preventing of the formation of biogenic amines should be placed on good quality of raw meat used and maintaining hygienic standards during manufacturing process.

## References

1- **Allen, G. ; Green, D. ; Bolton, G. ; Jaykus, L. and Cope , G. (2004) :** Detection and identification of histamine producing bacteria associated with harvesting and primary processing of Tuna in North Carolina, Journal of Food Production , Vol. 67 , sup. A, 79 - 81.

2- **Bover-Cid, S.; Izquierdo-Pulido, M. and Vidal-Carou, M.C. (2000):** Influence of hygienic quality of raw materials on biogenic amine production during ripening and storage of dry fermented sausage. J. Food Prot., 63 (11): 1544-50.

3- **Brink, B.; Damimk, C.; Joosten, H. M. L. J. and Huis in' t Veld, J.H.J. (1990):** Int.J.Food Microbiol.11:73-84.

4- **Chang, S.F.; Ayres, J.W. and Sandine, W.F. (1985):** Analysis of cheese for histamine, tyramine, histidine, tyrosine and tryptophane. J. Dairy Sci., 68 (11): 2840-6.

5- **Edwards, S.T. and Sandine, W.E. (1981):** Public health significance of amines in cheese. J. Dairy Sci., 64: 2431.

6- **Eitenmiller, R. R.; Koehler, P.E. and Reagan, P.E. (1981):** Tyramine in fermented sausage: Factors affecting formation of tyrosine and tyrosine decarboxylase. J. Food Sci., 43, 689-693.

7- **E O S (1996):** Egyptian Organization for Standardization and Quality Control, Detection of poisons and control, report No. 1796.

8- **F D A (2001):** Food and Drug Administration Hazards and Controls. guidance, 3 rd ed., Center of Food Safety and Nutrition, Washington, U.S.A

9- **Feldman, D.; Hoffman, R. and Simpson, J. (2003):** The solution for data analysis and presentation graphics. 2 nd Ed., Abacus Lancripts, Inc., Barkeley, CA, U.S A.

10- **Fonberg-Broczek, M. and Sawilska-Rautenstrauch, D. (1995):** Level of histamine and tyramine in ripening cheese. Rocz Panstw Zakl Hig. 46 (3): 243-6.

11- **Halasz, A.; Barath, A.; Simon-Sarkadi, L. and Holzapfel, W. (1994):** Biogenic amines and their production by microorganisms in food. J. Food Sci., Technol. 5, 42-49.

12- **Maijala, R. (1994):** Formation of biogenic amines in dry sausage with special reference to raw materials, Lactic acid bacteria, pH decrease, Temperature and time. Ph. D. thesis, University of Helsinki, Finland.

13- **Maijala, R. and Eerola, S. (2002):** Biogenic Amines. Veterinary and Fod Research Institute, Helsinki Finland.

14- **Maijala, R.L.; Eerola, S.H. and Nurmi, E. (1993):** Contaminant lactic acid bacteria of dry sausage produce histamine and tyramine. J. Meat Science, 35.

15- **Marino, M.; Maifereni, M.; Moret, S. and Rondini, G. (2000):** The capacity of Enterobacteriaceae species to produce biogenic amines in food. Appl. Microbiol., 31 (2): 169-173.

16- **Moret, S. and Conte, L. (1996):** High performance liqud chromatographic evaluation of biogenic amines in foods. J.Chromatography, 729: 363-369.

**17- Nizimani, A.G. ; Paleologos, E.K. ; Savvaidis, I.N. and Kontominas M.G. ( 2008 )** : Formation of biogenic amines and relation to microbial flora and sensory change in smoked turkey breast fillets stored under various packaging conditions at 4 degrees C. Food Microbiol. 25 (3): 509-17.

**18- Oliveira, C. P.; Gloria, M. B. A.; Barbour, J. F. and Scanlan, R. A. (1995):** Nitrate, nitrite and volatile nitrosamines in whey- containing food products. J. Agric. Food Chem. 43, 976-969.

**19- Ordonez, A. I.; Francisco, C.; Paloma,T . and Yolanda, B. (1997):** Formation of biogenic amines in food products J. Food Protection , 60 ( 11) : 1371-1375.

**20- Rice, S.; Eitenmiller, R. R. and Koehler, P. E. (1976):** Histamine and tyramine content of meat products. J. Milk and Food Technol., 38 (4): 256-258.

**21- Roig-Sagues, A. X.; Hernand- Herrero, M. and Mora-Ventura, M.T. (1997):** Histidine decarboxylase activity during the production of ripened sausage and its influence on the formation of Cadaverine. Journal of Food Protection; 60 (4): 430-432.

**22- Saccani, G.; Tanzi, E.; Pastore. P.; Cavalli, S. and Rey, M. (2005):** Determination of biogenic amines in fresh and processed meat by suppressed ion chromatography-mass spectrometry using a cation-exchange column. J. Chromatogr. A., 1082 (1): 43-50.

**23- Silva-Santos, M. H. (1996):** Biogenic amines, their importance in foods. International Journal of Food Microbiology; 29 (2/3) 213-231.

**24- Suzzi, G. and Gardin, F. (2003:** Biogenic amines in dry fermented sausage: a review. Int. J. Food Microbiol. 88 (1): 41-54.

**25- Taylor, S. L. ; Stratton, J. E. and Nordlee, J. A. ( 1989 ) :** Histamine poisoning ( Scombroid fish poisoning ) : an allergy – like intoxication . J. Toxical. Clin. Toxicol. 27 (4-5): 225-240.

# الكشف عن بعض الأمينات الحيوية السامة في بعض منتجات اللحوم الجاهزة للأكل.

نسرين زكريا حلمي عليوة
معهد بحوث صحة الحيوان - المعمل الفرعي بطنطا

## الملخص العربي

للحوم أهمية كبيرة في غذاء الإنسان لما تحتويه من قيمة عالية من الأحماض الآمينية والدهون والفيتامينات والمعادن والتي بدورها تؤثر على صحة وحيوية الإنسان . ولما كانت اللحوم المفرومة تمثل المادة الخام لصناعة منتجات اللحوم فلذا فانها أكثر مصادر التلوث بالميكروبات التي تساعد على تحويل الأحماض الأمينية الموجودة في اللحوم إلى مواد أخرى تسمى بالأمينات الحيوية والتي قد تؤثر على صحة الإنسان بشكل خطير حينما تتجاوز الحدود المسموح بها لتواجدها في هذه المنتجات . ولذلك أجريت هذه الدراسة على عدد ٦٠ عينة من منتجات الشاورمة والكفتة والكباب من مطاعم مختلفة بمحافظة الغربية.
وأوضحت النتائج أن متوسط تركيز الهستامين كان (٩١ ,١٢ ± ٩ ,١ مجم/ ١٠٠ جم)، ( ٨٦ ,٨ ± ٣ ,١ مجم/ ١٠٠ جم ) و ( ٢٤ ,٥ ± ٦ , مجم/ ١٠٠ جم ) في عينات الشاورمة ،الكفتة والكباب على التوالي . بينما تمثل التيرامين بنسبة عالية في عينات الكفتة و بأقل تركيز في عينات الكباب . وعلى الجانب الآخر كانت عينات الشاورمة هي الأعلى مستوى للبيوتريسين يليها عينات الكفتة ثم الكباب. هذا وبمقارنة النتائج للحدود المسموح بها (٢٠مجم /١٠٠جم) بالمواصفات القياسية المصرية لهذه الأمينات الحيوية (١٩٩٦ ) وجد أن ٢٥% ، ١٥% و٣٥ % من عينات الشاورمة قد فاقت الحدود المسموح بها للهستامين ،التيرامين والبيوتريسين.بينما ١٠%،٣٠% و٢٥% من عينات الكفتة كانت غير مسموح بها على التوالي . وقد احتوت فقط نسبة ٥% و٢٠%من عينات الكباب على تركيز من كل من التيرامين والبيوتريسين أعلى من الحدود المسموح بها.
وقد خلصت هذه الدراسة الى أن الشاورمة تمثل أكثر منتجات اللحوم احتواءاً على تلك الأمينات الحيوية السامة والتي تتكون نتيجة تحويل الأحماض الأمينية الموجودة بها بفعل الميكروبات الملوثة لذلك المنتج أو لاستخدام درجات حرارة أثناء الطهي ودرجة حموضة تساعد على تنشيط عملية التحويل.
ولذا فقد أوصت الدراسة إلى استخدام نوعية جيدة من اللحوم وكذلك التوابل والإضافات التي تستخدم فى تصنيع منتجات اللحوم والعمل على منع أو تقليل التلوث الميكروبي لها .هذا وقد نوقشت الأهمية الصحية لهذه الأمينات الحيوية وتأثيرها على صحة المستهلك وكيفية الحد من تكوينها وآثارها الضارة ..