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BIOGENIC AMINES IN CHICKEN CUT-UP MEAT PRODUCTS

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A B S T R A C T

Eighty random samples of frozen chicken (wings, thigh, shwerma and nuggets) represented by 20 of each were collected from different supermarkets during the first month of their production .Each sample was weighed about 100gm and stored at -18°C. The collected samples transferred in an insulated icebox to the laboratory and then subjected to the following examinations to be analyzed for biogenic amines by using High Performance Liquid Chromatography. The obtained results revealed that the average concentrations of histamine, tyramine, and cadaveine (mg / 100g) were 8.41 ± 0.33, 4.98 ± 0.17and 2.97 ± 0.06 for chicken wings, 10.75 ± 0.39, 5.45 ± 0.20, and 4.18 ± 0.09 for chicken thigh ,17.28 ± 0.52, 11.62 ± 0.31, and 9.35 ± 0.11 for chicken shawerma and 16.59± 0.46, 9.37 ± 0.24 and 8.82 ± 0.09 for chicken nuggets, respectively. In general, the levels of such amines were significantly higher ($p < 0.01$) in all examined sample. According to the permissible limits recommended by Egyptian Organization for Standardization and Quality Control, 5%, 5%, 25% and 20% of the examined samples of wings, thigh, shwerma and nuggets were unaccepted because of their histamine contents. While, all the examined samples of wings, thigh were accepted based on their tyramine contents but 15% and 10% of the examined shawerma and nuggets samples exceeded the safe permissible limits. Only 5% of the examined shawerma samples exceeded the safe permissible limits of cadavereine. In this respect, the acceptability of such examined samples for biogenic amines according to ***"EOS" was*** recorded.

Keywords: High Performance Liquid Chromatography, Histamine, Tyramine, Cadaverine

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1. INTRODUCTION

In foodstuffs, biogenic amines occur either as physiological constituents [21], as they are a natural part of cell structure, or because of enzymatic amino acid decarboxylation due to microbial enzymes [2]. In general, the most important biogenic amines in ready to eat chicken products are histamine, tyramine, tryptamine and putrescine, which formed by the enzymatic decarboxylation of histidine, tyrosine, trytophane and ornithine, respectively [13]. In some cases, biogenic amines may reach concentrations in foods, which are dangerous for consumers with enhanced sensitivity to biogenic amines determined by the inhibition of the action of aminooxidases, the enzymes involved in the detoxification of these substances [28]. Approximately 62% of respondents ranked chicken first in nutritional values, compared with 27% and 7% for beef and pork, respectively. In the case of in- home eating, 76% of respondents reported serving chicken at least once a week. The most important for buying chicken was nutrition, with economy, taste, versatility and convenience ranking equally in second place [5]. Aim of the present work was to determine histamine, tyramine, and cadaverine (biogenic amines) in chicken cuts, which could be indicative to its healthiness for human consumption.

2. Material and Methods

2.1. Collection of samples:

A grand eighty random samples of ready to eat chicken products represented by wings, thigh, shwerma and nuggets (20 of each) were collected from different markets in Kaluobyia, Gharbia and Menoufiya governorates. The samples were transferred to the laboratory under complete aseptic conditions without undue delay to be examined as follows:

1. Determination of APC
2. Estimation of biogenic amines:

The estimation of biogenic amines as histamine, tyramine, and cadaverine was recorded by using HPLC according to Mort, and Conte [17] as follows:

2.1. Amine extraction:

Accurately, 25 gm of the examined sample were homogenized with 125 ml of 5% trichloroacetic acid (TCA) for 3 minutes using a blender, and then filtered using filter paper Whatmann No. (1). Moreover, 10 ml of the extract were transferred into a suitable culture test tube with 4 gm NaCl and 1 ml of 50% NaOH, then shacked and extracted 3 times by 5 ml n – butanol chloroform (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 – heptane were added and extracted 3 times with one ml portions of 0.2 N HCl, then N HCl layer was collected in a glass stopper tube. Solution was evaporated just to dryness using water bath at 95^{o}C with air currents.

2.2. 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 stoppered and carefully mixed to prevent loss due to spattering. Carefully, one ml dansyl chloride solution was added and mixed thoroughly using Vortex mixer. The mixture was kept in a water bath at 70^{o}C for 10 min. then, the extraction of dansylated biogenic amines was carried out using 3 times of 5 ml portions of diethyl ether, stoppered, shacked carefully for 1 minute and the ether layers were collected in a culture tube using disposable pasture pipette. The combined ether extracts were carefully evaporated at 35^{o}C in dry film and dissolved in one ml methanol, then 10 micro liters injected in HPLC [22].

2.3. Interpretation of HPLC:

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. Furthermore, 10, 20, 30, 40 and 50 microlitre of dansyl amine standard as well as 10 micro liters of each dansylated sample extract was used. However, the chromatogram was examined under long wave of ultraviolet (254 nm) to establish weather or not the dansyl amines of interest are present in the examined sample.

Finally, the concentration of each biogenic amine in the samples was recorded as mg/100 gm according to the following formula:

Amine concentration (mg/100 gm) = CV / W

Where, C: concentration of amine standard (mg / gm), V: final dilution of sample extract (ml)

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

Finally, HPLC techniques were applied on the positive samples of each biogenic amine for confirmation and accurate estimation of its concentration as mg % (mg/100g) according to the method recommended byOrdonez et al. [20].

2.2. Statistical analysis:-

The obtained results were statistically analyzed according to Feldman et al. [7]. Analysis of variance (ANOVA) test was carried out to check the difference between the levels of each residue among the examined sample.

3. Results and Discussion

It is evident from the results recorded in table (1) that the histamine levels were varied from 1.3 to 20.1 with an average of 8.41 ± 0.33 mg % for Chicken wing, 2.6 to 20.5 with an average of 10.75 ± 0.39 mg % for thigh chicken meat , 4.2 to 31.8 with an average of 17.28 ± 0.52 mg % for chicken shawerma and varied from 3.8 to 28.4 with an average of 16.59 ± 0.46 mg % for chicken nuggets, for histamine level 5% , 5% ,

Table (1): Statistical analytical results of histamine levels (mg %) and unaccepted samples of the examined samples of chicken cut-up meat products (n=20).

Chicken samples	Histamine (mg/100gm)			Unacceptability According to Egyptian standards	
	Min.	Max.	Mean ± S.E.	No.	%
wings	1.3	20.1	8.41± 0.33 [c]	1	5
Thigh	2.6	20.5	10.75 ± 0.39 [b]	1	5
shawerma	4.2	31.8	17.28 ±0.52 [a]	5	25
nuggets	3.9	28.4	16.59 ±0.46[a]	4	20

Values within the same column with different letters were significant differences (P<0.01).

Table (2): Statistical analytical results of tyramine levels (mg %) and unaccepted samples of the examined samples of chicken cut-up meat products (n=20).

Chicken samples	Tyramine (mg/100gm)			Unacceptability According to Egyptaian standards	
	Min.	Max.	Mean ± S.E.	No.	%
wings	1.0	10.3	4.98± 0.17 [c]	0	0
Thigh	1.4	12.2	5.45 ± 0.20 [c]	0	0
shawerma	2.9	25.6	11.62 ±0.31 [a]	3	15
nuggets	1.8	21.9	9.37 ±0.24 [b]	2	10

Values within the same column with different letters were significant differences (P<0.01)

Table (3): Statistical analytical results of cadaverine levels (mg %) and unaccepted samples of the examined samples of chicken cut-up meat products (n=20).

Chicken samples	Cadaverine (mg/100gm)			Unacceptability According to Egyptaian standards	
	Min.	Max.	Mean ± S.E.	No.	%
wings	0.9	6.8	2.97± 0.06 [c]	Zero	Zero
thigh	1.0	9.5	4.18 ± 0.09 [b]	Zero	Zero
shawerma	2.4	20.3	9.35 ±0.11[a]	1	5
nuggets	2.1	17.7	8.82 ±0.09 [a]	Zero	Zero

Values within the same column with different letters were significant differences (P<0.01)

25% and 20% of the examined wings, thigh, shwerma and nuggets samples, respectively, exceeded such permissible limits. However, on comparing the obtained results with the permissible limits recommended by *"EOS" [6]*. The present results agree, quite well, with these reported by earlier studies for chicken meat [15, 18, 19]. On the other hand, the lowest histamine concentrations in the examined samples of chicken meat may be due to use large slices of them, which constitute a protective layer from the surface microorganisms to penetrate the meat and cause degradation of amino acids [8]. Actually, the presence of histamine in the examined samples of chicken meat is of great interest for two reasons: firstly, for their role as possible quality indicators and secondly, for their toxicological aspects in the sense that high levels of dietary histamine can be toxic for certain consumers [29]. Table (2) declared that

the tyramine levels were ranged from 1.0 to 10.3 with a mean value of 4.98 ± 0.17mg % for chicken wings, 1.4 to 12.2 with a mean value of 5.45 ± 0.20 mg % for chicken thigh ,2.9 to 25.6 with a mean value of 11.62 ± 0.31 mg % for chicken shawerma and 1.8 to 21.9 with a mean value of 9.37 ± 0.24 mg % for chicken nuggets , for tyramine level, none of the examined chicken wings and chicken thigh samples exceeded such permissible limits, while 15% and 10% of the examined chicken shawerma and chicken nuggets samples, respectively, exceeded such permissible limits recommended by "EOS" [6]. In the same time, nearly similar results were obtained by earlier authors for chicken meat [1, 23, 26]. Generally, tyramine is produced in any food item as result of decarboxylation of the amino acid tyrosine. Accordingly, the higher concentration of tyramine in the examined samples of meat may be due to the higher temperature which favored proteolytic and decarboxylase activities of microorganisms resulting in increased tyramine concentrations in these food articles containing higher contents of tyrosine [3, 25]. However, it should be mentioned that the capability to decarboxylate amino acids is strain dependant rather than species dependant [4], since some strains have a wide spectrum and able to decarboxylate many amino acids, whereas other strains have only strictly substrate specific decarboxylases leading to great variations between the rates of production of biogenic amine by different strains of the same species [16]. Only few histamine - positive bacteria possess the ability to decarboxylate tyrosine to form tyramine. The presence of other biogenic amines can potentiate the negative effect of tyramine on human health [14]. Tyramine acts mainly indirectly by releasing noradrenalin from the sympathetic nervous system which causes an increase of blood pressure by peripheral vasoconstriction and by increasing the cardiac output. Tyramine also dilates the pupils, dilates the peripheral tissue, causes lacrimation and salivation, increases respiration and increases the blood sugar [11]. Thus, high concentrations of tyramine derived from foods were accumulated in the blood leading to a hypertension crisis known as "cheese reaction" [9, 27]. The cheese reaction can lead to severe migraine headache, brain hemorrhage or heart failure [16]. Results recorded in table (3) revealed that the cadaverine levels in the examined samples of chicken cuts-up meat were varied from 0.9 to 6.8 with an average of 2.97 ± 0.06 mg % for chicken wings,1.0 to 9.5 with an average 4.18± 0.09 mg % for chicken thigh,2.4 to 20.3 with an average 9.35 ± 0.11 mg % for chicken shawerma and 2.1to 17.7 with an average 8.82 ± 0.09 mg % for chicken nuggets, for cadaverine level, none of the examined chicken wings , chicken thigh and chicken nuggets samples exceeded such permissible limits, while 5% of the examined chicken shawerma samples exceeded such permissible limits recommended by *"EOS"* [6]. In general, there were great fluctuations of biogenic amines content among types of products and in the same type of the product. These differences depend on many variables as the qualitative– quantitative composition of microflora, the chemico – physical variables, the hygienic procedure adopted during processing, the availability of precursors, the amount of meat used, types of ingredients added and the quality of the raw material [10, 26, 28], with which amine – positive bacteria are mainly introduced to food playing a great role in the formation of biogenic amines [12, 16].

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مجلة بنها للعلوم الطبية البيطرية

الأحماض الأمنية في منتجات لحوم الدواجن المجزأة

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الملخص العربي

تعد لحوم الدواجن مصدرا هاما من مصادر البروتين الحيوانى نظرا لاحتوائهاعلى نسبة عاليه من البروتين الحيوانى سهلة الهضم لذلك تم التطور فى تصنيع وإعداد لحوم الدواجن فى صورة مختلفة من لحوم الدجاج المجمدة مثل الاجنحة والدبوس ولحوم الدجاج الجاهزة للأكل مثل الشاورما والناجتس. ولما كانت هذه القطعيات تتعرض أثناء تجهيزها ونقلها وتداولها فى الأسواق للتلوث والغش الذي قد يؤدى إلى فسادها قبل إستهلاكها مما تشكل خطرا على صحة المستهلك بما قد تسببه له من أمراض مختلفة. لذلك أجريت هذه الدراسة على عدد80 عينه بواقع 20 عينه لكل منتج وتم قياس نسبة الهستامين وقياس نسبة التيرامين وقياس نسبة الكادفرين .وقد دلت نتائج الدراسة على أن متوسطات تركيز الهستامين في عينات أجنحة الدجاج المجمد هي8.41 ±0.33 مجم %، في قطعيات دبوس الدجاج هي10.75± 0.39 مجم %، في قطعيات الشاورما هي 17.28± 0.52 مجم % وفي قطعيات الناجتس وقد وجد ان قطعيات الشاورما تحتوي على أعلى نسبه من تركيز الهستامين وهي 16.59± 0.46 مجم %. وقد أوضحت النتائج أن5% من عينات أجنحة الدجاج والدبابيس المجمدة و25% و20% من عينات قطعيات الشاورما وقطعيات الناجتس على التوالى قد تجاوزت الحدود القياسية المصرية الخاصة بالهستامين (20مجم %). وقد تراوح متوسط تركيز التيرامين 4.98±0.17 مجم % في عينات أجنحة الدجاج، 5.45± 0.20 مجم % في عينات الدبابيس المجمدة، 11.62 ± 0.31 مجم % في عينات قطعيات الشاورما و9.37±0.24 مجم % في عينات قطعيات الناجتس. وتبين أن15%و10% من عينات قطعيات الشاورما وقطعيات الناجتس قد تجاوزت الحدود القصوى للتيرامين تبعا للمواصفات القياسية المصرية، على التوالى. بينما كانت جميع عينات أجنحة الدجاج وعينات الدبابيس المجمدة مقبولة ولم تتخطى الحدود الأمنة. وعلى الجانب الاخر، كان متوسط تركيز الكادفرين 2.97± 0.06 مجم % في عينات أجنحة الدجاج، 4.18± 0.09 مجم % في عينات الدبابيس المجمدة، 9.35±0.11 مجم % في عينات قطعيات الشاورما و8.82±0.09 مجم % في عينات قطعيات الناجتس. وقد أوضحت النتائج أن 5% من عينات قطعيات الشاورما قد تجاوزت الحد الاقصى لكادفرين تبعا للمواصفات القياسية المصرية. بينما كانت جميع عينات أجنحة الدجاج، عينات الدبابيس المجمدة وقطعيات الناجتس مقبولة ولم تتخطى الحدود الأمنة للمواصفات القياسية المصرية. وقد أثبتت نتائج التحليل الاحصائى أن الاختلافات بين العينات الاربع كانت عالية المعنوية كنتيجة للأحتوائها على الأمينات الحيوية سواء الهستامين، التيرامين أو الكادفرين.

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