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DEMONESTRATION OF BIOGENIC AMINES IN FAST FOODS

Faten S. Hassanien, Hassan, M.A., Amany M. Salem and El-Wakeel, M.E.
Food Control Department, Fac. Vet. Med., Benha University

A B S T R A C T

Ninety random samples of fast food (fried and grilled meat, chicken and fish) were used to analyze biogenic amines (histamine, tyramine and putrescine) by using HPLC. The obtained results revealed that the average concentrations of histamine, tyramine and putrescine for fried meat meals were 5.26±0.39, 12.65±0.81, 6.77±0.28 respectively, 8.94±0.31,16.29±0.97,7.95±0.46, respectively for grilled meat, 9.71±0.48, 7.98±0.57, 9.45±0.51, respectively for fried chicken, 11.25±0.59, 9.83±0.60, 13.08±0.89, respectively for grilled chicken, 14.05±0.82, 11.12±0.65, 8.18±0.39, respectively for fried fish and 18.43±1.04, 14.51±0.78, 10.37±0.64, respectively for grilled fish with interaction 3.12, 3.25 and 1.15 for histamine, tyramine and putrescine, respectively.

KEY WORDS: Biogenic amines, Fast food, Histamine, Putrescine, Tyramine

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1. I N T R O D U C T I O N

Biogenic amines are nitrogenous low molecular mass organic bases with aliphatic, aromatic or heterocyclic structures which will be formed and metabolized in the normal metabolism in human, animals, plants and microorganisms [29]. Biogenic amines are a natural part of cell structure, or as a consequence of enzymatic amino acid decarboxylation due to microbial enzymes [5] and - to a lesser extent-due to tissue activity [35]. In general, the most important biogenic amines in ready-to- eat meals are histamine, tyramine and putrescine which are formed by the enzymatic decarboxylation of histidine, tyrosine, and ornithine, respectively [14, 26]. In this respect, the mono aminoxidase inhibitors facilitate the absorption of both histamine and tyramine from the intestine and potentiate their action [22].

The excessive consumption of ready-to-eat foods containing biogenic amines can be of health concern because they are not equilibrate assumption in human [33] can initiate various pharmacological reactions as dilatation of peripheral blood vessels, hypotension, hypertension, headache, contraction of intestinal smooth muscles causing abdominal cramps, diarrhea and vomiting [30], intestinal ulcers, allergic responses [32], heart disease especially when coupled with additional factors as mono aminoxidase inhibitor drugs or alcohols, burning sensation in the mouth [8], nausea, respiratory distress, pyrexia and migraine [33]. Biogenic amines are also known as possible precursors of carcinogenic nitrosamines [34]. Considering all these hazards, the current study was applied to compare the biogenic amines levels with the Egyptian and international standards.

2. MATERIAL AND METHODS

A total of 90 random samples of ready-to-eat meals represented by fried meat, grilled meat, fried chicken meat, grilled chicken meat, fried fish and grilled fish (15 of each) were collected from different hotels and transferred directly to the laboratory. All collected samples were examined for determination of their contents of biogenic amines (histamine, tyramine and putrescine).

2.1. *Determination of biogenic amines*:
Cadaverine, histamine and putrescine were identified by Thin Layer Chromatography, as described by Shalaby [29] given as average of three replicates.

2.2. *Amine extraction (for meat samples)*:
25 g of meat sample were homogenized in a blender with 125 ml of 5% TCA for 3 minutes using a warning blender, then filtration: was achieved using filter paper Whatman (England). Accurately 10 ml of the extracts were 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 chloroform (1:1 v/v) stoppered and shacked vigorously for 2 minutes followed by centrifugation for 5 minutes 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 1.0 ml portions of 0.2 NHC1, then NHC1 layer was collected in a glass stopper tube. Solution was evaporated just to dryness using water bath at 95°C with aid of air currents.

2.3. *Separation of dansyl amines*:
The chromatographic separation was carried out to separate the three dansyl amines by one- dimensional TLC. On 2 cm from the bottom edge of the TLC plate and at intervals of 1 cm, the following samples were applied using a micro syringe: 10, 15, 20, 30 and 50 jal of dansyl. Amine standard and 10 µl of each of dansylated sample extracts. The plate was developed in chloroform/ benzene/triethyl amine (6:4:1) for 15 cm. The plate was taken out of the jar and allowed to dry. Then it was developed in the same direction in benzene/ acetone/triethyl amine (10:2:1) for 15cm. The plate was allowed to dry at room temperature until the excess of the solvent disappeared before interpretation.

2.4. *Interpretation of the chromatogram*:
The chromatogram after the second development was examined under long wave (360 run) of ultraviolet light to establish whether or not the dansyl amines of interest are present in the sample.

2.5. *Quantification of dansyl amines*:
The developed TLC plates were placed under a chromatogram scanner and the absorbance value for each separated spot is recorded at wave length of 254nm.

2.6. *Calculation*:
The concentration of each amine in the sample is made using the following formula: H g/g (mg/kg) =SYV/XW
S: amine standard equal to unknown.
Y: concentration of amine standard (µg/ml).
V: Final dilution of extract (ml).
X: sample extract given a spot intensity equal to S (µl).
W: weight of the sample represented by the final extract (in grams).

2.7. *Statistical analysis*:
The obtained results were statistically analyzed according to Feldman et al. [11].

3. RESULTS AND DISCUSSION

It is evident from the results recorded in table (1) that the histamine levels were varied from 1.8 to 14.7 with an average of 5.26±0.39 mg % for fried meat meal, 2.5 to 21.6 with an average of 9.71±0.48 mg % for fried chicken meat meal and 6.1 to 30.9 with an average of 14.05±0.82 mg % for fried fish meal. However, the histamine levels were varied from 2.4 to 19.8 with an average of 8.94±0.31 mg % for grilled meat meal, 3.3 to 27.2 with an average of 11.25±0.59 mg % for grilled chicken meal and 7.8 to 41.5 with an average of 18.43±1.04 mg % for grilled fish meal, with interaction between the examined products and cooking methods 3.12 significant differences (p>0.05).

Consequently, the examined fish meals had the highest level of histamine. Furthermore, the grilling process of cooking can increase the concentration of histamine contents of such examined meals rather than frying method of cooking. The present results agreed, quite well, with these reported by previous authors for meat meal [7, 15] and for fish meal [17, 20, 21].

The great fluctuations of biogenic amines content among products and in the same type of the product depend on many variables as the quail-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 [31, 33], with which amine-positive bacteria are mainly introduced to food playing a great role in the formation of biogenic amines [13].

The high level of histamine in the examined samples of fish meals may reflect the high counts of organisms lead to considerable accumulation of histamine in the fish flesh by histadine decrboxylation [2].

Table (1) declared that the tyramine levels were ranged from 4.9 to 29.2 with a mean value of 12.65±0.81mg % for fried meat meal, 2.7 to 18.5 with a mean value of 7.98±0.57 mg % for fried chicken meat meal and 4.1 to 24.8 with a mean value of 11.12±0.65 mg % for fried fish meal. Moreover, the mean values of the tyramine levels in the examined samples of grilled meat, chicken and fish meals were 16.29±0.97, 9.83±0.60 and 14.51±0.78 mg%, respectively. However, significant differences (P<0.05) appeared due to the interaction between the examined products and cooking methods.

Nearly similar results were obtained by former authors for meat meal, for chicken meal [3, 25, 31] and for fish meal [2].

It is observable that the examined meat meal samples had the highest concentrations of tyramine followed by fish and chicken meals. Hence, all grilled products of meat, chicken and fish meals contained tyramine more than those of fried meals. The higher concentration of tyramine in the examined samples of meat and fish meals may be due to the higher temperature which favored proteolytic and decarboxylase activities of micro-organisms resulting in increased tyramine concentrations in these food articles containing higher contents of tyrosine *[6, 27]*. Also, the addition of acidic materials to fish as lemon specially grilled fish lowers pH of the product resulting in the activation of acid producing bacteria to form tyramine *[18]*. The most important tyramine producers are *Enteroccus fecalis,* Pediococcus and Lactobacillus species as *L. brevis, L. buchneri, L. curvatus, L. divergens* and *L. carnis* [19].

Also results recorded in table (1) revealed also that the putrescine levels in the examined samples of fried ready-to-eat meals were varied from 1.5 to 16.2 with an average of 6.77±0.28 mg % for fried meat meal, 3.4 to 21.8 with an average 9.45±0.51 mg % for fried chicken meat meal and 2.2 to 19.7 with an average 8.18±0.39 mg % for fried fish meal. In the grilled ready-to-eat meals, the putrescine levels were varied from 1.9 to 20.1 with an average of 7.95±0.46 mg % for meat, 4.0 to 28.2 with an average of 13.08±0.89 mg % for chicken meat and 23.6 to 23.6 with an average of 10.37±0.64 mg % for fish. The present results were nearly similar with these reported by earlier studies in meat meal [5, 9, 26], chicken meat meal [1, 4, 23] and fish meal [2, 17]. The high concentration of putrescine in the examined samples of chicken and fish meals indicated inappropriate treatment, poor hygienic levels of manufacturing process, using raw materials of poor quality [13] and increased microbial contamination [16]. Moreover, putrescine may be formed as endogenous modulator of outer membrane permeability, possible as a part of adaptive response to acidic environment [28]. So, putrescine concentration may be considered as the limit for spoilage initiation [3] and objective indicator of acceptability of ready to eat chicken products [24].

Table l Statistical analytical results of Histamine/Tyramine and putrescine levels (mg%)in the examined samples of ready-to-eat meals(n=15)with the correlation coeffecient for each biogenic amines:-

Product	Cooking method	Frying			Grilling		
		Min.	Max.	Mean ±S.E	Min.	Max.	Mean ±S.E
Histamine							
	Meat meal	1.8	14.7	5.26±0.39	2.4	19.8	8.94±0.31*
	Chicken meal	2.5	21.6	9.71±0.48	3.3	27.2	11. 25±0.59
	Fish meal	6.1	30.9	14.05±0.82	7.8	41.5	18.43±1.04
Tyramine							
	Meat meal	4.9	29.2	12.65±0.81	6.3	37.6	16.29±0.97*
	Chicken meal	2.7	18.5	7.98±0.57	3.5	25.4	9.83±0.60
	Fish meal	4.1	24.8	11.12±0.65	4.6	32.0	14.51±0.78
Putrescine							
	Meat meal	1.5	16.2	6.77±0.28	6.3	1.9	7.95±0.46**
	Chicken meal	3.4	21.8	9.45±0.51	3.5	4.0	13.08±0.89
	Fish meal	2.2	19.7	8.18±0.39	4.6	23.6	10.37±0.64

*Signficant difference ($p<0.05$) and **Non significant difference

Results achieved in table (2) indicated that all examined samples of fried and grilled meat meals were accepted based on their contents of histamine and come in accordance with EOS [10] which stipulated that the maximum permissible limit of histamine in foods should not exceed 20 mg%. In contrast, 6.67% & 1.33% of fried and grilled chicken meals and 13.33% & 26.67% of fried and grilled fish meals were exceeded such safe standard limit, respectively. In regard to FDA [12], 6.67%, 26.67% and 40% of the examined samples of fried meat, chicken and fish meals were unfit for human consumption where they exceeded the safe permissible limit of histamine (10 mg%). While, 20%, 46.67% and 66.67% of grilled meat, chicken and fish meals were unaccepted according to their levels of histamine, respectively. In regard to tyramine, table (2), indicated that 5(33.33%), 2 (13.33%) and 4(26.67%) of the examined samples of grilled meat, chicken and fish were unaccepted based on their contents of tyramine according to EOS [10] stated that the maximum permissible limit of tyramine in ready-to-eat meals must not exceed 20 mg%, respectively. Moreover, 3 samples of fried meat (20%) and one sample of fried fish (6.67%) exceeded this permissible limit and unfit for human consumption. In contrast, all the examined samples of fried chicken were accepted and agree with the recommended limit of EOS [10] also, FDA [12] stipulated that the maximum permissible limit of tyramine in ready-to-eat meals should not above 10 mg% to be safe for human consumption. Accordingly, 40% & 73.33% of fried and grilled meat meal, 13.33% & 46.67% fried and grilled chicken meal and 33.33% & 53.33% of fried and grilled fish meal samples exceeded such standard limit of FDA [12], respectively as shown in table (2). In other words, the consumption of these food articles may constitute, at times, public health hazard.

EOS [10] recommended 20 mg% as a maximum permissible level of putrescine in ready-to-eat meals. Subsequently, 6.67%, 20% and 13.33% of the examined samples of grilled meat, chicken and fish meals were unaccepted where they exceeded such limit, respectively. For fried ready-to-eat meals, only 13.33% of the examined samples of chicken meat meal were unaccepted. While, all examined samples of fried meat and fish meals come in accordance with the standard limit stipulated by EOS [10] as described in table (2) and results recorded in table (2) pointed out that 13.33% & 20% of fried and grilled meat meal, 33.33% & 46.67% of fried and grilled chicken meat meal and 20% & 40% of fried and grilled fish meal exceeded the maximum permissible limit of putrescine (10 mg%) recommended by FDA [12].

Table2 Unacceptability of the examined samples of ready-to-eat meals based on histamine, tyramine and putrescine contents according to EOS [10] and FDA[12] standards (n=15)

Product	Cooking Method	Unacceptable fried samples				Unacceptable grilled samples			
		EOS 10 mg%		FDA 20 mg%		EOS 10 mg%		FDA 20 mg%	
		No.	%	No.	%	No.	%	No.	%
Histamine									
	Meat meal	-	-	1	6.67	-	-	3	20.00
	Chicken meal	1	6.67	4	26.67	2	13.33	7	46.67
	Fish meal	2	13.33	6	40.00	4	26.67	10	66.67
Tyramine									
	Meat meal	3	20.22	6	40.00	5	33.33	11	73.33
	Chicken meal	-	-	2	13.33	2	13.33	7	46.67
	Fish meal	1	6.67	5	33.33	4	26.67	8	53.33
Putrescine									
	Meat meal	-	-	2	13.33	1	6.67	3	20.00
	Chicken meal	2	13.33	5	33.33	3	20.00	7	46.67
	Fish meal	-	-	3	20.00	2	13.33	6	40.00

*EOS=Egyptian Organization for standardization and quality Control. **FDA=Food and Drug Administration

Finally, the high concentrations of biogenic amines in the examined samples of ready-to-eat meals indicated poor handling and / or processing of these food items. Therefore, the profile of biogenic amines in foods could be used an important index of quality assurance of fresh and processed meals and may be used as chemical indicators of their spoilage. However, the complexity of food matrix, the presence of potential interferences and the occurrence of several biogenic amines simultaneously are typical problems encountered in the analysis of food for biogenic amines.

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مجلة بنها للعلوم الطبية البيطرية

تحديد الأمينات الحيوية في الوجبات السريعة

فاتن سيد حسنين، محمد احمد حسن، امانى محمد سالم، متولى الوكيل
قسم المراقبة الصحية على الاغذية– كلية الطب البيطرى–جامعة بنها

الملخص العربى

تحظي الأغذية الجاهزة للكل بإقبال كبير من قبل المستهلكين في جمهورية مصر العربية وذلك لطعمها الشهي وسهولة تداولها بما يتناسب مع ظروف ومتطلبات الحياة الحديثة. وتتميز الأغذية ذات الأصل الحيواني باحتوائها علي نسبة مرتفعة من البروتين عالي القيمة الغذائية الغني بالأحماض الأمينية الضرورية والدهون والفيتامينات والمعادن والتي بدورها تؤثر علي صحة وحيوية المستهلك. وتعتبر أيضا الأغذية الجاهزة للأكل من أكثر أنواع الأغذية المعرضة للتلوث مما يساعد علي تحويل الأحماض الأمينية الموجودة بها إلي مواد أخري تسمي بالأمينات الحيوية والتي تؤثر علي صحة المستهلك بشكل خطير حينما تتجاوز الحدود المسموح بها في هذه المنتجات وقد أجريت هذه الدراسة علي عدد تسعين عينة (90) من كل من وجبات اللحوم المقلية واللحوم المشوية والدجاج المقلي والدجاج المشوي والسمك المقلي والسمك المشوي (15 عينة من كل منتج) والتي تم جمعها بصورة عشوائية من الفنادق المختلفة بجمهورية مصر العربية. وقد تم قياس الأمينات الحيوية (الهستامين والثيرامين والبيوترسين) في تلك الأغذية الجاهزة للأكل ومقارنتها بالحدود القصوي المسموح بها في المواصفات القياسية المصرية والعالمية. وكانت النتائج أن متوسط تركيز الهيستامين، التيرامين والبيوتريسين للحوم المقلية كانت 5.26 ±0.39، 12.65±0.81، 6.77±0.28 علي التوالي وكانت 8.94±0.31، 16.29±0.97، 7.95±0.46 علي التوالي للحوم المشوية، بينما كانت 9.71±0.48، 7.98±0.57، 9.45±0.5 علي التوالي للدجاج المقلي وكانت 11.25±0.59، 9.83±0.60، 13.08±0.89 علي التوالي للدجاج المشوي، أما بالنسبة للأسماك المقلية كانت 14.05±0.82، 11.12±0.65، 8.18±0.39 علي التوالي بينما كانت 18.43±1.04، 14.51±0.78، 10.37±0.64 علي التوالي للأسماك المشوية. وقد أثبتت نتائج التحليل الإحصائي أن الاختلافات بين عينات اللحوم، الدجاج والأسماك كانت عالية المعنوية كنتيجة للإحتوائها علي الأمينات الحيوية سواء الهستامين، التيرامين أو البيوترسين. كما تبين وجود اختلافات معنوية بين هذه العينات كنتيجة لطرق الطهي سواء القلي أو الشي. هذا وقد تم دراسة كيفية تكوين الأمينات الحيوية في تلك المنتجات محل الدراسة، ومدي خطورتها علي الصحة العامة، كما تم وضع بعض التوصيات لتجنب وجودها في هذه المنتجات حفاظا علي صحة المستهلك.

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