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BIOGENIC AMINES IN CHICKEN EDIBLE PARTS AND THEIR AFFECTION BY COOKING

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ABSTRACT

Biogenic amines were determined in chicken meat, liver and gizzard samples collected from a local market in Cairo. The proportion of samples containing biogenic amines, along with their maximum concentrations and averages, were recorded. The obtained data revealed that their was a great variation in biogenic amines content of the samples among the same kind and between the different kinds. However, relatively high levels of all the tested biogenic amines were found in liver samples compared to those detected in meat or gizzard samples. Regardless of sample kind, the highest concentrations were recorded for tyramine (TYR), followed by histamine (HIS), putrescine (PUT), cadaverine (CAD) and tryptamine (TRY), while β-phenylethylamine (PHE) had the lowest concentration. Both PUT and CAD were presented in the samples by similar proportions, yet the concentrations were different. The effect of two cooking methods on the biogenic amine contents was studied. Boiled samples had lower amounts of biogenic amines as compared with the raw samples. As well as, some biogenic amines were completely disappeared after boiling. On contrary, the amounts of biogenic amine detected in the stewed samples were more than the initial amounts presented in the fresh samples, due to loss of moisture during stewing.

INTRODUCTION

Biogenic amines are natural antinutritive factors important from a hygienic point of view, since they have been implicated as the causative agents in a number of food poisoning episodes, and they are able to initiate various pharmacological reactions (Shalaby, 1996). Histamine (HIS), putrescine (PUT), cadaverine (CAD), tyramine (TYR), tryptamine (TRY) and β-phenylethylamine (PHE) are considered to be the most important biogenic amines occurring in foods. HIS has been implicated as the causative agent in several outbreaks of food poisoning, while TYR and PHE, have been proposed as the initiators of hypertensive crisis. The toxicity of histamine appeared to be enhanced by the presence of other amines such as CAD, PUT and TRY (Shalaby, 1996).

Food substances that have been prepared by a fermentative process, or have been exposed to microbial contamination during ageing or storage, are likely to contain amines (Shalaby, 1996). In this concern, biogenic amines had been found in edible parts of chicken, i.e. meat, liver and gizzard during storage at 4°C (Vinice *et al*., 1995). Also, Schmitt and Schmidt-Lorenz (1992) detected HIS, TYR, CAD and PUT in refrigerated broilers, and CAD was the major biogenic amine detected. Moreover, biogenic amines had been appeared in chicken meat, liver and gizzard during storage at -20°C (Ayesh *et al*., 1997). Consequently, PUT and CAD were considered and used as a

good indicator for the onset of spoilage of poultry carcasses since the concentration of both amines could increase rapidly with advancing decomposition (Schmitt *et al.*, 1988 and Lebron, 1994). However, it is of important to point out that high levels of amines may be formed before the foods appear spoiled or being organoleptically unacceptable (Shalaby, 1996). The present work was undertaken to determine six biogenic amines, namely; TRY, HIS, CAD, PUT, TYR and PHE, content in chicken edible parts collected from a local market in Cairo. The biogenic amines content of the samples as affected by cooking was also considered.

MATERIALS AND METHODS

Source of samples

A total of sixty samples (two kgs each) of chicken edible parts (twenty samples each of meat, liver and gizzard) were collected randomly from a local market in Cairo, Egypt. About 500g of each sample were homogenized in a grinder (Moulinex, Paris, France); 50g of the homogenate were used for biogenic amines analysis. The rest of the samples was frozen and kept at -18 °C till cooking (the frozen period not exceeded 7 days).

Cooking of chicken edible parts

The rest samples of chicken edible parts containing high level of biogenic amines were used to demonstrate the effect of cooking on the biogenic amines content. The samples were divided into two parts; the first part was cooked by stewing till well done, while the second part was cooked by boiling. The cooked samples were left at room temperature to cool, homogenized and subjected to biogenic amines analysis.

Biogenic amines analysis

Biogenic amines content of the samples were determined by TLC – densitometry method according to Shalaby (1999) as follows:-

Extraction of Amines

Homogenated sample (50 g) was successively extracted three times with 5% trichloroacetic acid (TCA) (3 x 75 ml) using a Waring Blender. Each blended mixture was centrifuged and the clear extracts were combined. The volume was adjusted to 250 ml with 5% TCA. An aliquot (5 ml) of the TCA extract, equivalent to 1 g of sample, was introduced into a screw-capped tube and washed three times with an equal amount of diethylether to remove the acid, and the ether remaining with the aqueous extract was removed by heating in water bath. Two drops of concentrated hydrochloric acid were added to the washed extract and the solution was evaporated just to dryness using a current of air and hot water bath.

Derivative formation

The dansylated derivatives of the amines were formed by dissolving the residue with 1 ml of saturated sodium bicarbonate solution, and 1 ml of dansyl chloride reagent (5 g l^{-1} acetone) was added. The sealed tube was

immediately mixed for 30 sec. using a Vortex mixer and the reaction mixture was then left for 1 hr at 40° C. The dansylamines were extracted by adding water (about 10 ml) and extracting the mixture with several portions of diethylether. The combined ether extracts were evaporated to dryness and the residue was dissolved in 2 ml acetonitrile for TLC.

Preparation of the standard solution

A mixed standard solution, as dansyl derivatives, was prepared using 100 μl of each amine stock standard solution (0.5 mg ml^{-1}). Using a current of air and a steam bath, the prepared solution was evaporated to dryness. The dansyl derivatives were prepared as described above. The residue was dissolved in 10 ml acetonitrile (intermediate standard). Afterwards, 1 ml was diluted to 5 ml using acetonitrile.

Separation of dansylamines

One-dimensional TLC technique was used to separate the eight dansylamines under investigation. The standard amines and the dansylated food extracts, indicated below, were applied 2 cm from the base of the TLC plate and at 1 cm intervals using a microsyringe:

- 10, 20, 30, 40 and 50 μl of dansylamine standards.
- 10 μl of each dansylated food extract.

The plate was developed in chloroform: benzene: triethylamine (6:4:1, v/v/v) for 15 cm. The plate was removed from the jar and allowed to dry. Then, it was developed in the same direction in benzene: acetone : triethylamine (10:2:1, v/v/v) for 15 cm. The plate was allowed to dry at room temperature, and then dried with a hair dryer to remove excess solvent before interpretation.

Interpretation of the chromatogram

The chromatogram after the second development was examined under long-wave (360 nm) ultraviolet light to establish whether or not the dansylamines of interest are present in the sample.

Quantification of dansylamines

The developed TLC plate was placed under a Shimadzu CS-9000 chromatogram scanner and the absorbance value for each separated spot is recorded at wavelength of 254 nm.

Recovery assay

For the recovery assay, a known amount of each biogenic amine was added as a mixture to a sample of chicken edible parts (meat, liver and gizzard), detected to be free from biogenic amine, to a level of 10 mg kg^{-1} (giving a final solution, to be spotted, with a concentration of 5 μg ml^{-1}). Three replicates were conducted on each contaminated sample. The recoveries were calculated by comparing the densitometer peak areas of each sample to the areas of the same standard solution.

RESULTS AND DISCUSSION

Recovery of the method used

Three replicates 50-g samples of chicken meat, liver and gizzard free of biogenic amines were spiked with sufficient quantities of standard solution to give 10 mg kg^{-1} of each amine in the sample, after extraction, washing and derivatization, the percent recoveries were calculated and the results are given in Table (1). It could be noticed that recoveries were high for all samples subjected to analysis. The highest recovery was reported for CAD and the lowest recovery was for PHE in all samples analyzed. The CAD recoveries were 99.0, 100.0 and 97.0% for chicken meat, liver and gizzard, respectively. The corresponding recoveries recorded for PHE were 73.0, 70.0 and 74.0%. The overall recovery ranged from 98.7 to 72.3% as calculated for CAD and PHE, respectively. It is of interest to point out that statistical analysis (Snedecor & Cochran, 1979) of the obtained data indicates that there was no significant differences ($p > 0.05$) between the recoveries of all samples analyzed, which reflect the suitability of this method to determine biogenic amines in chicken edible parts.

Table (1). Recovery (%) of biogenic amines added to chicken edible parts.

Amines	Chicken meat		Chicken liver		Chicken gizzard		Overall	
Tested	Average	SE	Average	SE	Average	SE	Average	SE
TRY	84.0	7.77	86.0	7.21	85.0	7.51	85.0	3.76
PUT	96.0	11.40	98.0	11.14	92.0	7.57	95.3	5.16
CAD	99.0	9.71	100.0	11.53	97.0	9.54	98.7	5.17
HIS	98.0	10.26	95.0	8.39	96.0	8.89	96.3	4.63
TYR	87.0	6.11	91.0	5.86	90.0	4.62	89.3	3.40
PHE	73.0	8.51	70.0	8.62	74.0	9.29	72.3	4.45

SE: Standard error.

Biogenic amine contents of chicken edible parts

Biogenic amines in chicken edible parts were studied and the proportion of sample containing biogenic amines along with maximum amounts and average concentration of the positive samples were given in Table (2). There was a great variation in biogenic amines content of the samples among a kind and between the different kinds. However, relatively high levels of all tested biogenic amines were found in liver samples compared to those presented in meat or gizzard samples. Regardless of sample kind, the highest concentrations were recorded for TYR, followed by HIS, then PUT, CAD and TRY, while PHE had the lowest concentration. The maximum concentration presented in liver samples were 170.3, 158.1, 142.6, 103.3, 92.1 and 8.0 ppm for TYR, HIS, PUT, CAD, TRY and PHE, respectively. The corresponding values in meat samples were 120.7, 113.5, 93.2, 80.7, 73.0 and 7.2 ppm. The proportion of positive samples were also high in liver samples compared to both meat and gizzard samples. It could be noticed that 90, 85, 80, 80, 75 and 55% of the tested liver samples were

contained TYR, HIS, PUT, CAD, TRY and PHE, respectively. The corresponding positive proportion of the meat samples were 75, 70, 65, 65, 50 and 45%. Moreover, it could be observed that both PUT and CAD were presented in the samples, regardless the kind of chicken parts, by similar proportions, yet the concentrations were different.

The obtained results are supported by the previous findings of many researchers. Ayesh *et al.* (1997) showed that biogenic amines could be formed in the edible parts of chicken (meat, liver and gizzard) during frozen storage. Liver samples had the highest biogenic amine concentration, while the gizzard possessed the lowest content. Vinci *et al.* (1995) and Schmitt and Schmidt (1992) stated that PUT, CAD, HIS and TYR were formed in chicken meat during refrigerated storage. Ayesh (1992) nominated liver as the target edible part of chicken in concern to its HIS content. Yamanaka (1989) evaluated chicken meat during storage at 0° C for their biogenic amine contents and concluded that some biogenic amines, i.e. putrescine and cadaverine, were useful as an index for freshness and decomposition of chicken meat. Tarijan and Janossy (1978) observed that chicken liver showed a high TYR content.

Biogenic amines in chicken edible parts may be formed as a result of contamination during preparation, storage and handling. It was reported that increases of biogenic amines in chicken samples indicating that these samples had been stored at excessive temperature prior to sale (Slemr and Beyermann, 1985). In this concern, Lebron (1994) indicated that cadaverine has excellent potential as a microbiological quality indicator of poultry. The variable concentrations of biogenic amines noticed in the tested samples (Table, 2) could be due to the variability and the difference on decarboxylase activities of the contaminated bacteria from one side and both biosynthesis and metabolism of such biogenic amines from the other side (Shalaby and Ragab, 1997). Nevertheless, the observed differences in biogenic amines concentration between the kinds of samples might be due to the degree and kinds of bacterial contamination which differ in their decarboxylase activities (Shalaby, 1996).

Table (2). Biogenic amines content of raw collected chicken edible parts.

Amines	Chicken meat			Chicken liver			Chicken gizzard		
	P.S.	M.C.	A.	P.S.	M.C.	A.	P.S.	M.C.	A.
TRY	50	73.0	42.6	75	92.1	60.2	40	75.0	32.3
PUT	65	93.2	53.1	80	142.6	59.2	60	43.4	16.6
CAD	65	80.7	29.8	80	103.3	86.6	60	38.5	13.5
HIS	70	113.5	30.6	85	158.1	104.3	65	105.0	87.4
TYR	75	120.7	54.8	90	170.3	107.8	70	119.2	48.7
PHE	45	7.2	3.4	55	8.0	3.5	35	5.1	3.0

P. S.: Positive samples (%).
M. C.: Maximum concentration detected (ppm).
A.: Average concentration (ppm) of the positive samples.

Effect of cooking on biogenic amines content of chicken edible parts

The behavior of biogenic amines during cooking the chicken meat, liver and gizzard samples was studied to throw light on the effect of heat on the biogenic amine contents. The biogenic amines content was determined in fresh samples and after cooking by boiling and stewing. Table (3) shows that boiling resulted in low amounts of biogenic amine compared to the raw samples. Some of the biogenic amines present in the raw samples disappeared completely from the samples after boiling. The obtained data indicated that biogenic amines may be released into the water during boiling.

Table (3). Effect of cooking (boiling and stewing) of chicken edible parts on their biogenic amine contents.

Samples	Biogenic amines content (ppm)*					
	TRY	PUT	CAD	HIS	TYR	PHE
Meat						
Before cooking	83.0	93.2	80.7	113.2	120.7	7.2
after boiling	nd	nd	nd	13.4	12.8	nd
after stewing	90.2	97.3	90.6	135.6	146.4	8.4
Liver						
Before cooking	92.1	142.6	103.3	158.1	170.3	8.0
after boiling	nd	12.7	nd	14.2	16.4	nd
after stewing	117.8	162.8	129.5	195.5	193.0	8.4
Gizzard						
Before cooking	75.0	43.4	38.5	105.0	119.2	5.1
after boiling	nd	nd	nd	8.6	9.3	nd
after stewing	95.6	56.9	44.6	136.3	167.3	6.2

*** Calculated on wet weight.**
nd : not detected.

The extractability of biogenic amines into the cooking water was enhanced by boiling. Similar trend was reported for biogenic amines content of legume sprout during cooking (Shalaby, 2000). Also, mashing process (in which 1 kg malt is boiled with 10 L of water) during beer production led to transform of all biogenic amines presented in the malt to the boiling water (Izquierdo-Pulido *et al.*, 1994; Shalaby and Ragab, 1997). In this concern, Shalaby (1990) stated that biogenic amines of sardine were migrated to the separated drip as a result of pre-cooking during canning process with partially destroying of biogenic amines. Also, Ayesh (1988) found higher concentration of biogenic amines in stick water (separated water) during fish meal production.

It seems that cooking the chicken edible parts by stewing had no effect on the biogenic amines content of meat, liver and gizzard (Table, 3), where the amounts of biogenic amines detected in cooked samples were more than the initial amounts presented in the fresh samples due to evaporation of water from samples during stewing. Moreover, some biogenic amines may be formed during heat treatment as result of thermal amino acid decarboxylation. It was reported that during boiling the wort (during beer production), formation

or decrease of biogenic amines can occur (Izquierdo-Pulido *et al.*, 1994; Shalaby and Ragab, 1997) due to thermal amino acid decarboxylation (Narziβ *et al.*, 1984a&b, Wackerbauer and Toussaint, 1984) or transformation into other compounds (Smith 1981), respectively. Moreover, Toyama *et al.* (1982) attributed the decrease of histamine observed during fish meal preparation to chemical changes induced by heat treatment in the presence of carbohydrates and/or proteins rather than physical loss. Nout *et al.* (1993) found that home cooking of soy bean Tempe by stewing had little effect on the concentration of either putrescine and tyramine. Moreover, it was found that processed cheeses (which produced by high temperature treatment of its ingredients including ripened cheese which contain biogenic amines) had appreciable amounts of biogenic amines (El-Sayed, 1996).

It is of important to mention here that 3 mg of phenylethylamine causes migraine headaches in susceptible individuals (Sandler *et al.*, 1974), while 6 mg total tyramine intake was reported to be a dangerous for patients receiving monoamine oxidase inhibitors (Blackwell and Mabbitt, 1965). Chicken liver was shown to cause hypertensive attacks similar to those of tyramine toxicity (Shalaby, 1996). On the other hand, the possible carcinogenicity of biogenic amines should be considered parallel to their toxicity effects. The biogenic amines have been reported to be a possible mutagenic precursor, since some of them may be nitrosated or act as precursors for other compounds capable of forming nitrosamines which are carcinogenic to various species of animals and pose a potential health hazard to humans (Shalaby, 1996). In this concern, Tannenbaum (1980) stated that humans are exposed to N-nitrosamines through in vivo nitrosation of ingested amines, where amines can form N-nitrosamines when exposed to nitrite under conditions similar to those found in the stomach (Lijinsky, 1980). A reaction product of tyramine and nitrite, 3-diazotyramine, induced oral cavity cancer in rats. This mutagenic compound may be formed in the stomach, where incubation of tyramine and nitrite at 37 °C and a pH of 1-2 for 60 minutes led to significant amounts of 3-diazotyramine (Joosten, 1988).

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الأمينات الحيوية فى الأجزاء المأكولة من الدجاج وتأثرها بطرق الطهى

على رجب شلبى ، وفاء حسين إمام ، نادية إبراهيم إبراهيم ، هناء عبد المنعم البنا
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تم فى هذا البحث تسجيل المحتوى الأمينى لبعض عينات أجزاء الدجاج (اللحوم والكبد والقوانص) المعروضة للبيع فى أحد الأسواق المحلية بالقاهرة ، حيث تم تسجيل نسبة العينات المحتوية على الأمينات الحيوية وأعلى تركيز تم كشفه مع حساب متوسط التركيز للعينات المحتوية على هذه الأمينات . وقد وجد إختلافات كبيرة فى محتوى الأمينات الحيوية ليس فقط بين أنواع العينات المختلفة ولكن أيضا بين العينات من نفس النوع . كما وجد أن عينات الكبد تحتوى على أعلى تركيزات من الأمينات الحيوية المختبرة مقارنة بعينات اللحوم والقوانص . وبغض النظر عن نوع العينة المختبرة فإن أعلى تركيزات سجلت كانت للتيرامين تلاه الهيستامين ثم الكادافرين والبيوتراسين والتريبتامين أما البيتا فينيل إيثايل أمين فسجل أقل التركيزات . وقد لوحظ أن كل من الكادافرين والبيوتراسين قد وجدا فى العينات بنسب متشابهة أما التركيزات فكانت مختلفة .

وقد تناول البحث دراسة مدى تأثر الأمينات الحيوية المتواجدة فى العينات بالطهى بطريقتين مختلفتين هما السلق والتشويح (التسوية فى سوائل العينة نفسها) حيث دلت النتائج المتحصل عليها على أن سلق العينات يؤدى إلى تقليل محتواها من الأمينات الحيوية بالاضافة إلى أن بعض هذه الأمينات يختفى تماما نتيجة عملية السلق . وعلى العكس من ذلك فقد وجد أن تركيز الأمينات الحيوية

يزداد نتيجة عملية التشويح وقد يرجع ذلك إلى فقد العينات لنسبة من الرطوبة أثناء عملية التشويح مما يؤدى إلى تركيز الأمينات فى العينة .