



## Estimation of some biogenic amines on chicken meat products

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### ABSTRACT

Sixty random samples of raw thigh & breast, half cooked chicken shawerma & shiesh tawook, fully cooked chicken shawerma and fully cooked chicken shiesh tawook (10 of each) were collected from different markets in Gharbia governorates and analyzed for biogenic amines by HPLC. The obtained results indicated that the mean values of histamine, cadaverine and putrescine (mg/100g) were  $10.88 \pm 0.91$ ,  $12.65 \pm 1.10$  and  $4.54 \pm 0.32$  for raw thigh,  $4.54 \pm 0.32$ ,  $9.86 \pm 0.87$  and  $9.86 \pm 0.87$  for raw breast  $16.56 \pm 1.24$ ,  $19.81 \pm 1.52$ , and  $11.41 \pm 0.96$  for half cooked shawerma,  $12.29 \pm 0.97$ ,  $16.20 \pm 1.08$  and  $9.98 \pm 1.08$  for half cooked shiesh tawook,  $12.93 \pm 1.03$ ,  $16.69 \pm 1.33$  and  $8.01 \pm 0.63$  for fully cooked shawerma and  $11.68 \pm 0.97$ ,  $11.91 \pm 0.97$  and  $7.47 \pm 0.50$  respectively for fully cooked shiesh tawook. According to the permissible limits recommended by EOS (2005) 10%, 30%, 20%, 20% and 20% of the examined samples of raw thigh, half cooked shawerma, half cooked shiesh tawook, fully cooked shawerma and fully cooked shiesh tawook were unaccepted. All raw breast samples were accepted as a result of their histamine content. While, 20%, 40%, 30%, 30% and 20% of the examined samples raw thigh, half cooked shawerma, half cooked shiesh tawook, fully cooked shawerma and fully cooked shiesh tawook were unaccepted. Moreover, raw breast samples were accepted as result of their cadaverine content respectively. Accurately 10% of half cooked shawerma were unaccepted while all examined samples were accepted as a result of putrescine content.

**Key words:** Biogenic amines, chicken meat products, histamine, cadaverine, putrescine.

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### 1. INTRODUCTION

The worldwide poultry meat production and consumption have increased rapidly and consumption of poultry meat in many parts of the world will continue to grow. Competitive price, the absence of cultural and religious obstacles, and dietary and nutritional properties are the main factors explaining the poultry meat's attractiveness for consumers (Petracci and Cavani, 2012). The production and consumption of ready to eat chicken meat products have increased significantly throughout the world during the last decade, due to their desirable sensory characteristics, easiness and variety of preparation methods (Balamatsia et al., 2006). Biogenic amines in food are formed as a result of the growth of decarboxylase-positive microorganisms under conditions propitious for that enzyme activity (Halasz et al., 1994). Histamine poisoning is a chemical intoxication of short incubation period (30 minutes to 1 hour). It is often manifested by a wide variety of symptoms as urticaria, edema, localized inflammation and rash (Jean et al., 2001). Temperature dependent changes took place in the concentrations of putrescine and

cadaverine. The changes in the concentrations of these amines were related to the microbiological quality of broiler chicken cuts (Rokka et al., 2003). Putrescine is essential for the growth and function of normal cells. Due to- its electrostatic and covalent interactions with various macromolecules, it fulfils an array of important physiological roles. Its participation in human cell growth and proliferation has been of great interest for its role in tumor growth (Kozova et al., 2009; Krausova et al., 2006).

Therefore, the main goal of the present work was carried out to estimate the biogenic amines as quality index in chicken meat products.

### 2. MATERIAL AND METHOD

#### 2.1. Determination of biogenic amines by using HPLC:

Three biogenic amines including histamine, cadaverine and putrescine were determined in all examined samples according to the protocol

recommended by Krause et al. (1995) and Pinho et al. (2001).

### 2.2. Extraction of samples:

Twenty-five grams of each sample was blended with 125 ml of 5% Tri chloro acetic acid (TCA) for 3 min using a warning blender then filtration was achieved using filter paper (Whatman No1.) Then, 10 ml of the filtrate were transferred into a suitable glass tube with 4 g NaCl and 1 ml of 50 % NaOH. The filtrate was extracted three times (2 min each) by using 5 ml n-butanol: chloroform (1:1 v/v) and the upper clear layer was transferred to 100 ml separating funnel by using disposable Pasteur pipette. To combine the organic extracts (upper layer), 15 ml of n-heptane was added in separating funnel and extracted three times with 1.0 ml portions of 0.2 N HCl, the HCl layer was collected in a glass Stoppered tube. Solution was evaporated just to dryness using water bath at 95°C with aid of a gentle current of air.

### 2.3. Formation of dansylamines:

One hundred µl of each stock standard solution (or sample extract) were transferred to 50 ml vial and dried under vacuum. About 0.5 ml of saturated NaHCO<sub>3</sub> solution was added to the residue of the sample extract (or the standard). Vial was stoppered and carefully mixed to prevent loss due to spattering. Carefully, 1.0 ml dansyl chloride solution was added and mixed thoroughly using vortex mixer. The reaction mixture was incubated at 55°C for 45 min. About 10 ml of distilled water were added to the reaction mixture. Then vial was stoppered and shaken vigorously using vortex mixer. The extraction of dansylated biogenic amines was carried out using 5 ml of diethyl ether for 3 times again vial was stoppered, shaken for 11 min and the ether layers were collected in a culture tube using disposable Pasteur pipette. The combined ether extracts were carefully evaporated at 35°C in dry bath with aid of air current. The obtained dry material was dissolved in 1ml methanol and 10 µl was injected in HPLC.

### 2.4. Statistical analysis

The obtained results were statistically evaluated by application of Analysis of Variance (T-TEST) test according to Feldman et al. (2003).

## 3. RESULTS

It is evident from the results recorded in table (1) that the mean values of the histamine levels in the examined samples were  $10.88 \pm 0.91^+$  mg% for raw thigh chicken meat,  $8.17 \pm 0.53$  mg% for raw breast chicken meat,  $16.56 \pm 1.24$  mg % for half cooked chicken shawerma,  $12.29 \pm 0.97$  mg % for half cooked chicken shiesh tawook,  $12.93 \pm 1.03$  mg % for fully cooked chicken shawerma and  $11.68 \pm 0.97$  mg % for fully cooked chicken shiesh tawook.

Table (2) indicated that all examined samples of raw chicken breast were accepted based on their contents of histamine and come in accordance with EOS (2005) which stipulated that the maximum permissible limit of histamine in chicken meat products should not exceed 20 mg %. In contrast, 10%, 30%, 20%, 20% and 20% examined samples of raw thigh meat, half cooked shawerma, half cooked sheish tawook, fully cooked shawerma and fully cooked sheish tawook respectively exceeded such permissible limit.

Table (3) declared that the mean values of cadaverine levels were  $12.65 \pm 1.10$  mg% for raw thigh,  $9.86 \pm 0.87$  mg% for raw breast,  $19.81 \pm 1.52^+$  mg% for half cooked shawerma,  $16.20 \pm 1.08$  mg% for half cooked sheish tawook,  $16.69 \pm 1.33$  mg% for fully cooked shawerma and  $11.91 \pm 0.97$  mg% for fully cooked sheish tawook. Table (4) demonstrated that the acceptability of cadaverine levels in the examined samples of raw and heat treated chicken meat products. All raw breast chicken meat was accepted while 20%, 40%, 30%, 30% and 20% of examined samples of raw thigh chicken meat, half cooked chicken shawerma, half cooked shiesh tawook, fully cooked chicken shawerma and fully cooked sheish tawook were unaccepted, respectively, based on their contents of cadaverine according to EOS (2005).

Table (5) revealed that the mean values of putrescine in the examined samples were  $4.54 \pm 0.32$  mg% for raw thigh chicken meat,  $4.15 \pm 0.25$  mg% for raw breast chicken meat,  $11.41 \pm 0.96$  mg% for half cooked shawerma,  $9.98 \pm 1.08$  mg% for half cooked sheish tawook,  $8.01 \pm 0.63$  mg % for fully cooked shawerma and  $7.47 \pm 0.50$  mg% for fully cooked sheish tawook. Table (6) demonstrated that the acceptability of putrescine levels in the examined samples of raw and heat treated chicken meat products. 10% of the examined samples of half cooked shawerma were unfit for human consumption, while all examined samples were accepted according to Egyptian Organization for Standardization "EOS" (2005).

Table (1): results of histamine levels (mg %) in the examined samples of chicken meat products (n=10).

Chicken meat	Positive samples		Min	Max	Mean $\pm$ S.E.*
	No.	%			
Raw thigh	8	80	1.7	22.4	10.88 $\pm$ 0.91 <sup>+</sup>
Raw breast	8	80	1.3	15.5	8.17 $\pm$ 0.53
Half cooked shawerma	10	100	2.7	32.5	16.56 $\pm$ 1.24 <sup>+</sup>
Half cooked shiesh tawook	10	100	2.1	26.9	12.29 $\pm$ 0.97
Fully cooked shawerma	10	100	2.5	28.2	12.93 $\pm$ 1.03 <sup>NS</sup>
Fully cooked shiesh tawook	10	100	1.8	23.2	11.68 $\pm$ 0.97

S.E.\* = standard error of mean. +Application of t-test indicated significant differences ( $P < 0.05$ ) between (thigh & breast) and half cooked (shawerma & shiesh tawook). NS= Non-significant differences ( $P < 0.05$ ) between full cooked (shawerma & shiesh tawook)

Table (2): Acceptability of the examined samples of raw and heat treated chicken meat products based on their levels of histamine (n=10).

Chicken Products	MRL (mg %)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Raw chicken meat:					
Thigh	20	9	90	1	10
Breast		10	100	0	0
Half cooked product:					
Shawerma	20	7	70	3	30
Shiesh Tawook		8	80	2	20
Full cooked product:					
Shawerma	20	8	80	2	20
Shiesh Tawook		8	80	2	20

\*Maximum Residual Limit stipulated by Egyptian Organization for Standardization "EOS" (2005).

Table (3): results of cadaverine levels (mg %) in the examined samples of chicken meat products (n=10).

Chicken meat	Positive samples		Min	Max	Mean $\pm$ S.E
	No.	%			
Raw thigh	10	100	1.9	23.7	12.65 $\pm$ 1.10 <sup>+</sup>
Raw breast	10	100	1.4	18.2	9.86 $\pm$ 0.87
Half cooked Shawerma	10	100	4.0	39.6	19.81 $\pm$ 1.52 <sup>+</sup>
Half cooked Shiesh Tawook	10	100	2.8	31.5	16.20 $\pm$ 1.08
Fully cooked Shawerma	10	100	2.9	33.1	16.69 $\pm$ 1.33 <sup>+</sup>
Fully cooked Shiesh Tawook	10	100	2.2	27.9	11.91 $\pm$ 0.97

+Application of t-test indicated significant differences ( $P < 0.05$ ) between products

Table (4): Acceptability of the examined samples of raw and heat treated chicken meat products based on their levels of cadaverine

Chicken Products	MRL (mg %)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Raw chicken meat:					
Thigh	20	8	80	2	20
Breast		10	100	0	0
Half cooked product:					
Shawerma	20	6	60	4	40
Shiesh Tawook		7	70	3	30
Full cooked product:					
Shawerma	20	7	70	3	30
Shiesh Tawook		8	80	2	20

\*Maximum Residual Limit stipulated by Egyptian Organization for Standardization "EOS" (2005).

Table (5): results of putrescine levels (mg %) in the examined samples of chicken meat products (n=10).

Chicken meat	Positive samples		Min	Max	Mean $\pm$ S.E
	No.	%			
Raw thigh	7	70	1.0	9.6	4.54 $\pm$ 0.32 <sup>NS</sup>
Raw breast	6	60	1.0	7.3	4.15 $\pm$ 0.25
Half cooked Shawerma	9	90	1.6	20.2	11.41 $\pm$ 0.96 <sup>+</sup>
Half cooked Shiesh Tawook	7	70	1.4	16.7	9.98 $\pm$ 1.08
Fully cooked Shawerma	8	80	1.4	15.8	8.01 $\pm$ 0.63 <sup>NS</sup>
Fully cooked Shiesh Tawook	7	70	1.1	13.5	7.47 $\pm$ 0.50

NS= Non-significant differences ( $P < 0.05$ ) between raw (thigh & breast) and between fully cooked (shawerma & shiesh tawook)  
 + Significant differences ( $P < 0.05$ ) between half cooked (shawerma & shiesh tawook)

Table (6): Acceptability of the examined samples of raw and heat treated chicken meat products based on their levels of putrescine

Chicken Products	MRL (mg %)*	Accepted samples		Unaccepted samples	
		No.	%	No.	%
Raw chicken meat:					
Thigh	20	10	100	0	0
Breast		10	100	0	0
Half cooked product:					
Shawerma		9	90	1	10
Shiesh Tawook	20	10	100	0	0
Full cooked product:					
Shawerma		10	100	0	0
Shiesh Tawook	20	10	100	0	0

\* Maximum Residual Limit stipulated by Egyptian Organization for Standardization "EOS" (2005).

#### 4. DISSUCSION

Proteolysis, either autolytic or bacterial, may play a significant role in the release of free amino acid from tissue proteins which offer a substrate for decarboxylases reactions forming the biogenic amines (Balamatsia et al., 2007; Shalaby, 1996).

The results of histamine agreed with those reported by Abd-Zahir (2013) (8.41 $\pm$ 0.33mg%) for chicken wing, (10.75 $\pm$ 0.39 mg%) for thigh and (17.28 $\pm$ 0.52 mg%) for chicken shawerma. Nearly similar results were obtained by El-Wakeel (2012) (11.25 $\pm$ 0.59 mg%) for grilled chicken and Ntzimani et al. (2008) (11.9 mg/100gm) in smoked turkey breast fillets. While, higher than Afifi and Amin (2010) (9.61 $\pm$ 1.44) for ready to eat chicken shawerma and also higher than Nassar and Emam (2002) in all heat treated chicken products

The high level of histamine in the examined half cooked and fully cooked shawerma & sheish tawook samples may be attributed to unfavorable temperature of processing, presence of additives as pepper, tomatoes and other spices, which played an important role in growth and multiplication of such histamine forming microorganisms (Fonberg – Broczek and Sawilska – Rautenstrauch, 1995).

These results of cadaverine were higher than those of Abdel-Zahir-Hager (2013) (4.18 $\pm$ 0.11 mg%) for thigh, ( 9.35 $\pm$ 0.11 mg%) for shawerma and Nassar and Emam (2002) (3.14 $\pm$ 0.47, 3.71 $\pm$ 0.57, 4.06 $\pm$ 0.85mg%) for chicken frankfurter and luncheon.

Results of putrescine were lower than those of El-Wakeel (2012) (13.08 $\pm$ 0.89 mg%) for grilled chicken, higher than those of Afifi and Amin (2010) (5.69 $\pm$ 0.83 mg%) for ready to eat shawerma and Eliassen (Eliassen et al., 2002) *et al.* (2002) (in grilled breast 2 mg/kg), and Patsias et al. (2006) (in fried breast 0.7 mg/kg).

Cadaverine and putrescine could be indicators for onset of spoilage of poultry, especially since they were detected in the pre-spoilage stages from colony count 10<sup>5</sup> cfu/cm<sup>2</sup> (Schmitt and Schmidtlorenz, 1992).

The high concentration of putrescine in the examined samples of half cooked and fully cooked chicken shawerma & shiesh tawook indicated inappropriate treatment, poor hygienic levels of manufacturing process, using raw materials of poor quality (Kalac, 2006) and increased microbial contamination (Krausova et al., 2006; Ruiz-Capillas et al., 2004).

One direction in cancer therapy research is to limit the intake of dietary putrescine (Cipolla et al., 2007).

From the public health point of view, putrescine persists an important interest because it can react with nitrates to form nitrosamine which have carcinogenic effects on human being (Oliveira et al., 1995).

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 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 (Silva and Glória, 2002; Suzzi and Gardini, 2003).

In conclusion, the formation of biogenic amines depends on addition of acidic materials to shawarma and sheish tawook as onion juice lowers pH of the product, so activating the acidic bacteria to form biogenic amines.

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