



## The impact of storage duration and conditions on the formation of biogenic amines and microbial content in poultry meat

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### Abstract

This research was conducted to estimate the safety of breast and thigh meat (Ross 308) stored in refrigeration and freezing for different periods (0, 3 and 6) and (0, 15 and 30) days, respectively (total samples 12). High pH found in the 6th day of refrigeration storage for thigh meat; 6.414, while low recorded in the 6th day for breast meat; 5.757. High pH was found in the freezing storage period 0 day for chicken breast meat; 6.168, and low pH was found in breast meat in the 30th day of freezing storage; 5.826. The 6th day of refrigeration storage gave the highest TPC for thigh meat;  $111.33 \times 10^6$  cfu/ gm. Also, the 15th day of freezing storage recorded significant increase in TPC for breast and thigh meat;  $244 \times 10^5$  cfu/ gm and  $274 \times 10^5$  cfu/ gm respectively. Significant differences were noted for histamine, cadaverine and spermidine during storage periods, high levels recorded in the 6th day of storage for breast meat; 0.395, 0.078 and 0.643 mg/ kg respectively. Significant differences were noted between the mean levels of biogenic amines for breast samples during all storage periods. High levels of histamine, putrescine, cadaverine, spermine and spermidine were recorded in the 15h day of storage; 2.654, 0.358, 1.589, 0.124 and 2.652 mg/ kg respectively. In thigh meat, significant differences were recorded for levels of biogenic amines during the freezing storage periods except putrescine. Histamine did not exceed the legal limit set by the US FDA; 50 mg/ kg in all samples.

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### Introduction

Animal proteins represent a significant group of dietary products which may be utilized instantly or in form of products after various manufacturing steps. Peoples are currently looking for healthy and good quality foodstuffs. Food quality is affected with different impacts and several complicated interactions within the biological characteristics in the living organism, containing at most the biological operations after death of the animal when muscles converted into meat, manufacturing and stockpiling (1, 2). World production and utilization of poultry protein increased day by day, and per person eating of chicken in different regions of the earth will go on to develop (3,4). According to the data, about 7.6 billion tons of poultry meat are produced

annually (5) and the average of annual chicken consumption is 14.2 kg per person (6). However, chickens considered a perishable food, and the period required for spoilage varies from four to ten or eleven days after slaughter, despite the fact that it is stored in refrigeration systems (7,8).

The Deterioration during storage of chilled chicken meat is caused by microorganism's activity and biochemical changes inside the produced food. To identify the initial deterioration, some chemical indicators were suggested like pH values of meat, total volatile basic nitrogen, content of biogenic amines, etc. The estimation of biogenic amines considered a significant measurement in fresh and produced meat, not only because they are toxic, but also because of their use as potential agents of freshness (9). Poultry that were not cool properly after slaughtering; before being

transferred to the refrigerator or fridge in order to be exposed to the appropriate refrigeration temperature, the numbers of microorganisms may increase in their muscles (10). In terms of food safety and assessment of potential toxic impacts of biogenic amines, it is remarkable to monitor and estimate what biogenic amines must be classified, several rapid and perfect procedures were used to estimate the level of mentioned compounds in various foodstuffs, such as ELISA to estimate histamine, GC, GC-MS, capillary electrophoreses and HPLC. HPLC is considered the most accepted method to estimate biogenic amines in foodstuffs (11). Several studies have been conducted on the evaluation of the biochemical and microbial content of refrigerated and frozen chicken meat, such as Odetunde *et al.* (12), Bhandari *et al.* (13), Ivanov *et al.* (9), and Mawlood and Khidhir (8) and many other studies. The aims of this study were to determine levels of biogenic amines in muscles of chicken, and to detect a correlation to microbial growth, value of pH during different storage periods and conditions.

## **Materials and methods**

### **Samples preparation and storage**

This study conducted in high education lab, Animal Sciences Department, College of Agricultural Engineering Sciences, Sulaimani University. The broiler chicken (Ross 308) brought from local farm and transport to slaughterhouse and then slaughtered, eviscerated, takes the breast and thigh, divided into two groups, one at refrigerator storage; 4 °C for 0, 3 and 6 days, and the other group at freezing storage; -18 °C for 0, 15 and 30 days, each sample types included 3 replicates for each storage condition (total samples 12).

### **pH Calculation**

The value of pH in chicken treatments was calculated as mentioned in the procedure of Naveena and Mendiratta (14). Ten gm of sample was mixed with fifty ml of distilled water, and filtered via filter paper (Whatman No.1). The concentration of H<sup>+</sup> were calculated via digital pH meter (WTW 2f40-11420 D. Produce of Germany).

### **Microbiological test**

The samples were prepared according the procedure mentioned in National Advisory Committee on Microbiological Criteria for Foods (15). Under aseptic conditions, 25 ± 0.1 g of the refrigerated and frozen sample were weighed separately, then made soft, placed in a fridge for 18 hours at 4°C, transferred to a sterile blender vessel, then 250 ml of sterile peptone water was added and mixed at high speed for two minutes. Here we got the stock solution 1:10. The froth was allowed to dissolve; ten ml of the stock solution was transferred via pipette into a 90 ml dilution blank to get a dilution of 1: 100. The assay was iterated to get sequent dilutions; 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup>, 10<sup>-6</sup>, and 10<sup>-7</sup>.

### **Total Plate Count (TPC)**

The prepared dilutions mentioned above were used; a 0.1 ml from 10<sup>-1</sup>, 10<sup>-2</sup>, 10<sup>-3</sup>, 10<sup>-4</sup>, 10<sup>-5</sup>, 10<sup>-6</sup>, and 10<sup>-7</sup>. Solutions were pipetted into each of two Petri dishes. We used extra dilution Petri dishes when high numbers of bacteria is predictable. The used plate count agar was left to harden, and then 1 series of repeated plates was put in an incubator; 35 ± 1°C for two days. The colonies in plates were counted via colony counter in a appropriate range; 30-300 colonies per plate. The mean number of counts was calculated from duplicate dishes, multiplied by the dilution factor, and stated the resulted value as the aerobic plate count (standard plate count)/ gram at the incubation centigrade temperature applied (16).

### **Biogenic amines analysis**

The biogenic amines were extracted from chicken meat according to the procedure of Gingerich *et al.* (17). A 30 ml of 8% Trichloroacetic acid (TCA) were mixed with twenty gm of treatment, ultra-purified water was used to get a volume of 100 ml mixture, and then centrifuged; 10 minute/ 6000 rpm. TCA was taken away after adding 50 ml of ether. A 0.45µm micropore filter was used to filter the produced supernatant. Per- column orthophthaldehyde (OPA) was derivatized after reaction of 20µl sample against 20µl OPA for 60 second. 20 µl of sample was injected on HPLC system under the appropriate separation conditions. The assay conditions were done as followed, the analysis was completed on Shimadzu HPLC system model 2010 (Koyota, Japan) under the next conditions: The used column: Shim-pack ISC-07 / 51504 Na (50 × 2.1 mm ID). The mobile phase: A: 0.6 sodium citrate 0.1 M boric acid, pH (10), B: acetonitrile, under gradient program as below. The rate of flow: 0.8 ml/ min. The temperature of column: 65 °C. Detection OPA, fluorometric detection excitation 340nm, emission 445nm. Biogenic amine (BA) concentration was obtained as concentration of BA (mg/kg)= Area of peak / (area of standard peak\*concentration of standarard\*dilution).

### **Statistical Analyses**

Data in current study were submitted to one-way analysis of variance (ANOVA) using XL Stat for Windows. The variations between the means were calculated via Duncan's multiple range tests. The significance concentration was chosen at (P< 0.05) and the final results were given as mean (18).

## **Results**

### **The Value of pH**

The table 1 displays the mean pH values for breast and thigh samples stored in refrigeration for 0, 3 and 6 days. High significant differences (P< 0.05) were observed in the 6<sup>th</sup> day

of refrigeration storage for thigh; 6.414, while low significant difference in the 6<sup>th</sup> day for breast meat; 5.757.

The table 2 data showed that high significant differences (P< 0.05) were found in the storage period 0 day for breast meat; 6.168, while the lowest pH was found in breast meat in the 30<sup>th</sup> day of freezing storage; 5.826, which is the best value.

Table 1: pH value of some chicken parts during storage in refrigeration

Sample	Storage period (day)	pH
Breast	0	6.047 <sup>bcd</sup>
	3	5.917 <sup>cd</sup>
	6	5.757 <sup>d</sup>
Thigh	0	6.240 <sup>ab</sup>
	3	6.084 <sup>bc</sup>
	6	6.414 <sup>a</sup>

All values are means of triplicate measurements. Means with different letters differentiated significantly (P<0.05).

Table 2: pH value of some chicken parts during storage in freezing

Sample	Storage period (day)	pH
Breast	0	6.168a
	15	5.900bc
	30	5.826c
	0	6.125ab
Thigh	15	5.970ab
	30	5.917abc

All values are means of triplicate measurements. Means with different letters differentiated significantly (P<0.05).

#### Total Plate Count (TPC)

The table 3 shows the total plate count for breast and thigh meat stored in refrigeration, microbial count increased significantly (P< 0.05) during storage periods. No significant differences (P< 0.05) were recorded between all storage periods except the 6<sup>th</sup> day for thigh meat which gave the highest bacterial count;  $111.33 \times 10^6$  cfu/ gm.

The table 4 shows significant increase (P< 0.05) in TPC with increasing freezing storage period. The 30<sup>th</sup> day of storage recorded significant increase (P< 0.05) in microbial

counts for breast and thigh meat;  $244.00 \times 10^5$ ,  $274.00 \times 10^5$  cfu/ gm respectively. No significant differences (P< 0.05) were recorded for the same samples in the storage period 0 and 15 days respectively.

Table 3. Total plate count of some chicken parts during storage in refrigeration

Sample	Storage period (day)	Total Plate Count (cfu/ g meat)
Breast	0	$44.66 \times 10^3$ <sup>b</sup>
	3	$63.66 \times 10^5$ <sup>b</sup>
	6	$302.00 \times 10^6$ <sup>ab</sup>
Thigh	0	$40.00 \times 10^3$ <sup>b</sup>
	3	$92.33 \times 10^5$ <sup>b</sup>
	6	$111.33 \times 10^6$ <sup>a</sup>

All values are means of triplicate measurements. Means with different letters differentiated significantly (P<0.05).

Table 4: Total plate count of some chicken parts during storage in freezing

Sample	Storage period (day)	Total Plate Count (cfu/ g meat)
Breast	0	$13.87 \times 10^{3c}$
	15	$235.67 \times 10^{4bc}$
	30	$244.00 \times 10^{5ab}$
Thigh	0	$6.57 \times 10^{3c}$
	15	$371.33 \times 10^{4bc}$
	30	$274.00 \times 10^{5a}$

All values are means of triplicate measurements. Means with different letters differentiated significantly (P<0.05).

#### Biogenic amines levels

The table 5 shows the mean levels of biogenic amines in refrigerated chicken samples. Significant differences (P< 0.05) were noted for histamine, cadaverine and spermidine during storage periods for breast and thigh samples. The mentioned biogenic amines recorded the highest levels in the 6<sup>th</sup> day of storage in breast meat; 0.395, 0.078 and 0.643 mg/ kg, respectively. In contrast, no significant differences (P< 0.05) were recorded for the biogenic amines putrescine and spermine in breast and thigh meat during all storage periods.

Table 5: Biogenic amines levels (mg/ kg meat) of some chicken parts during storage in refrigeration

Sample	Storage period (day)	Histamine	Putrescine	Cadaverine	Spermine	Spermidine
Breast	0	0.103 <sup>e</sup>	0.000 <sup>a</sup>	0.000 <sup>b</sup>	0.000 <sup>a</sup>	0.222 <sup>e</sup>
	3	0.256 <sup>c</sup>	0.001 <sup>a</sup>	0.001 <sup>b</sup>	0.001 <sup>a</sup>	0.587 <sup>c</sup>
	6	0.395 <sup>a</sup>	0.001 <sup>a</sup>	0.078 <sup>a</sup>	0.001 <sup>a</sup>	0.643 <sup>a</sup>
Thigh	0	0.001 <sup>f</sup>	0.001 <sup>a</sup>	0.000 <sup>b</sup>	0.001 <sup>a</sup>	0.005 <sup>f</sup>
	3	0.207 <sup>d</sup>	0.005 <sup>a</sup>	0.001 <sup>b</sup>	0.005 <sup>a</sup>	0.559 <sup>d</sup>
	6	0.369 <sup>b</sup>	0.005 <sup>a</sup>	0.078 <sup>a</sup>	0.005 <sup>a</sup>	0.612 <sup>b</sup>

All values are means of triplicate measurements. Means with different letters differentiated significantly (P<0.05).

The table 6 shows the mean levels of the biogenic amines of the breast and thigh samples stored in freezing for the previously mentioned storage periods. Significant differences ( $P < 0.05$ ) were noted between the mean levels of biogenic amines in breast samples during all storage periods. High concentrations of histamine, putrescine, cadaverine, spermine and spermidine were recorded in the 30<sup>th</sup> day of storage; 2.654, 0.358, 1.589, 0.124 and 2.652 mg/ kg in

breast muscle respectively. In thigh meat, significant differences ( $P < 0.05$ ) were recorded between levels of most biogenic amines during the freezing storage periods.

The results of the current study show a positive relationship between the pH values of the refrigerated and frozen chicken treatments, and the microbial growth and formation of biogenic amines during storage periods as shown in table 7.

Table 6: Biogenic amines levels (mg/ kg meat) of some chicken parts during storage in freezing

Sample	Storage period (day)	Histamine	Putrescine	Cadaverine	Spermine	Spermidine
Breast	0	0.108 <sup>e</sup>	0.000 <sup>b</sup>	0.000 <sup>e</sup>	0.000 <sup>b</sup>	0.465 <sup>d</sup>
	15	0.646 <sup>c</sup>	0.002 <sup>b</sup>	0.114 <sup>c</sup>	0.001 <sup>b</sup>	1.006 <sup>b</sup>
	30	2.654 <sup>a</sup>	0.358 <sup>a</sup>	1.589 <sup>a</sup>	0.124 <sup>a</sup>	2.652 <sup>a</sup>
Thigh	0	0.107 <sup>e</sup>	0.001 <sup>b</sup>	0.000 <sup>e</sup>	0.001 <sup>b</sup>	0.245 <sup>e</sup>
	15	0.449 <sup>d</sup>	0.015 <sup>b</sup>	0.078 <sup>d</sup>	0.005 <sup>b</sup>	0.485 <sup>c</sup>
	30	0.988 <sup>b</sup>	0.015 <sup>b</sup>	0.139 <sup>b</sup>	0.010 <sup>a</sup>	1.015 <sup>b</sup>

All values are means of triplicate measurements. Means with different letters differentiated significantly ( $P < 0.05$ ).

Table 7: Correlation between TPC and biogenic amines in stored chicken parts

Storage	Sample	Traits	Histamine	Putrisine	Cadverine	Spermine	Spermidine
Refrigeration	Breast	TPC	0.433	0.337	0.580	0.337	0.218
	Thigh		0.811	0.029	0.951	0.029	0.573
Freezing	Breast		0.625	0.614	0.625	0.623	0.625
	Thigh		0.640	0.679	0.578	0.341	0.661

## Discussion

The difference in pH values can be caused by the variance in metabolism after animal death. Internal factors, such as age of animal, muscle kind and muscle position, glycogen content, and external factors like state of the poultry before slaughter, slaughter conditions, post-slaughter treatment in addition to temperature; all these impacts may influence the level of post-mortem glycolysis, and thereafter the final pH. The raise of pH level correlates with production of amine compounds, which considered the main products of microbial deterioration and are contingent by packaging category (1). The pH has a major function in controlling the growth of microorganisms, e.g. at pH 5.4-5.8, the growth of Enterobacteria and Psychotropes inhibited (19).

The reduction in pH level in chicken muscles may be due to the destroyed of glycogen with the production of lactic acid and a increasing in pH may be associated with fractional proteolysis, leading to produce more free alkaline groups depending on the state of such variable, in addition, higher pH levels of the muscles of the breast may be associated with an increase in the concentration of lactic acid as a result of anaerobic metabolism (19).

These results agreed with Baston *et al.* (20) they noticed a logarithmic increase in the microbial counts in chicken breast and thigh samples during refrigerated storage; 4 °C. High microbial count of thigh meat may be resulted from skin which could be polluted through handling (9).

In general, meat contains different species of microorganisms, which may increase if they get some suitable conditions for growth, such as temperature, humidity, and oxygen. Storage temperature -18 °C affects and inhibits the growth of microorganisms (21). Microorganisms isolated from frozen meat may be come from the environment, or the persons through handling and freezing (10). High TPC in the studied samples presented here indicates that product contamination may be due to poor sanitation during processing and handling (13). The standard concentrations for the Iraqi quality regulations IQS 2270/4 and 3725/4 have set that the TPC for frozen poultry between 105- 107 cfu/ gm meat (22). The results of this research in first and second periods for refrigeration and freezing storage not exceed the Iraqi standard, but in last period in two types of storage periods recorded high count and near to spoilage limits count.

The obtained results agreed with Baston *et al.* (20), a significant increased occurred in histamine level during refrigeration storage periods 1, 3, 5 and 7 days for breast and thigh meat respectively. This increase resulted from microbial activity; the main producer of biogenic amines, cells biochemical and enzymatic activity (23). Therefore, because of slight level of histamine content in studied chicken meat, it will be safe if the refrigeration conditions are good.

After slaughter, several changes occur in animal tissues, including the muscles that begin to decompose. The spoilage

bacteria produce enzymes able to remove carboxyl group from free amino acids, which result in formation of biogenic amines. Each amino acid produced a certain biogenic amine (24). The biogenic amines, particularly histamine, in this study did not exceed the legal limit set by the US FDA; 50 mg/kg, because histamine is the most toxic biogenic amine (11). In contrast, the concentrations of putrescine, cadaverine, spermine and spermidine were detected but in little amount which presented in table 6, because freezing temperature may affect on bacterial activity to produce these compounds (9).

The results showed that most of biogenic amine concentrations are higher in breast meat in comparison to thigh meat; as shown in tables 5 and 6, same results recorded by Silva and Gloria (25), they observed putrescine, cadaverine, histamine, and tyramine, were higher in breast meat as compared to thigh meat, after the 15th day of refrigerated storage. Positive relationship between the pH values, Microbial count and amine mention by Delgado-Pando *et al.* (26).

The pH of muscles considers an internal impact that can affect organism growth. In a living animal, the pH is being neutral, but after death there is a conversion from neutral to acid. Most bacteria can live in the range of pH 5.4-7.0, but they prefer a more neutral pH (27). For the biogenic amines, there was an increase in their concentration by increasing the bacterial numbers during storage periods (28).

## Conclusion

We can conclude that there was positive relationship between the pH values of the refrigerated and frozen chicken treatments, and the microbial growth and formation of biogenic amines during storage periods, and histamine concentrations didn't exceed the acceptable limits; 50 mg/kg in all treatments.

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## Conflict of interest

The authors announced that there are no conflicts of interest concerning the publication of this research paper.

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## تأثير مدة الخزن وشروطه على تكوين الأمينات الحيوية والمحتوى الميكروبي في لحوم الدواجن

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### الخلاصة

أجريت الدراسة الحالية لتقييم جودة لحم الصدر والفخذ لدجاج روز ٣٠٨ المخزن بالتبريد والتجميد لمدد زمنية مختلفة صفر و ٣ و ٦ أيام و ١٥ و ٣٠ يوماً على التوالي (عدد العينات الكلي ١٢). سجلت أعلى قيمة للرقم الحامضي عند مدة الخزن ٦ أيام للحم الفخذ بلغت ٦,٤١٤، بينما أدنى قيمة سجلت عند المدة ٦ أيام للحم الصدر ٥,٧٥٧. أما اللحم المحفوظ بالتجميد فقد سجلت أعلى قيمة للاس الهيدروجيني عند المدة صفر يوم للحم الصدر بلغت ٦,١٦٨، بينما أدنى قيمة وجد في لحم الصدر عند اليوم ٣٠ للخزن ٥,٨٢٦. سجل ارتفاع في أعداد الميكروبات بتقدم مدد الخزن بالتبريد، أعطت المدة ٦ أيام للحم الفخذ أعلى أعداد بكتيرية ١١١,٣٣ × ١٠<sup>٦</sup> وحدة تكوين المستعمرات/ غرام. بتقدم الخزن بالتجميد حصلت زيادة في أعداد البكتيريا وسجلت في اليوم ١٥ لخزن لحم الصدر والفخذ ٢٤٤ × ١٠<sup>٥</sup>؛ ٢٧٤ × ١٠<sup>٥</sup> وحدة تكوين المستعمرات/ غرام على التوالي. سجلت فروق معنوية لكل من الهستامين، الكادفرين والسبيرميدين خلال خزن لحم الصدر والفخذ بالتبريد، وسجلت أعلى مستويات عند اليوم السادس لخزن لحم الصدر بلغت ٠,٣٩٥، ٠,٠٧٨ و ٠,٦٤٣ ملي غرام/ كيلو غرام على التوالي. سجلت فروق معنوية بين متوسطات تركيزات الأمينات الحيوية لعينات لحم الصدر خلال مدد الخزن بالتجميد، ولوحظ أن أعلى تركيزات لكل من الهستامين، البيوتريسين، الكادفرين، السبيرمين والسبيرميدين سجلت في اليوم 15 للخزن ٢,٦٥٤، ٠,٣٥٨، ١,٥٨٩، ٠,١٢٤ و ٢,٦٥٢ ملغم/ كغم على التوالي. سجلت فروق معنوية لمستويات الامينات الحيوية في لحم الفخذ خلال الخزن بالتجميد باستثناء البيوتريسين. لم يتجاوز الهستامين الحد المسموح به الذي حدته إدارة الغذاء والدواء الأمريكية البالغ ٥٠ ملغم/ كغم.