THE BIOCHEMICAL ASSESSMENT OF IMPORTED FROZEN CHICKEN AND DETECTION THE EFFECT OF BIOGENIC AMINES ON THE MEAT QUALITY

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ABSTRACT

To assess the quality of imported frozen chicken sold in Sulaimani markets with respect to biogenic amines content and sensory properties, a total of 160 samples of frozen chicken meat belonged to four foreign trademarks were collected in two batches from different parts of Sulaimani city markets. The samples consisted of whole chickens (160 samples- 80 for each batch). HPLC- based detection of Biogenic amines revealed histamine within the acceptable limits in all marks, it ranged 0.00- 0.22 and 0.00- 0.03 mg/ Kg meat in batches 1 and 2 respectively for the four marks of carcasses. Putrescine, Cadaverine, Spermine and Spermidine were within the ranges reported in literatures for good chicken meat quality in all marks, except in DMIS. The biogenic amines indexes for all marks were within the acceptable limits except for DMIS, which significantly differed from the others. The sensory evaluation (organoleptic evaluation) of carcasses revealed highest scores gained by Gedik for all categories then by Sadia and Frinal, while DMIS had the lowest with the presence of significant differences among marks.

***Key Words**:* Biogenic Amines, Index Quality, Frozen Chicken, Sensory Evaluation.

Introduction

Estimation of Biogenic amines is important not only from the point of view of their toxicity, but also because they can be used as indicators of the degree of freshness or spoilage of food (Halasz *et al*., 1994; Bardocz, 1995 and Balamatsia *et al*, 2006). The biogenic amine occurrence is a consequence of the enzymatic decarboxylation of the precursor amino acids because of the microorganism activities. Polyamines: spermine and spermidine are natural amines produced by the body. The biogenic amines: putrescin, cadaverine, histamine, tyramine can be formed when storing the chicken meat due to microorganism action. Biogenic amines are low molecular-weight basic nitrogenous compounds that possess biological activity. They can be formed or degraded as a consequence of normal metabolic processes in animals, plants, and microorganisms (ten-Brink *et al*., 1990). Like other meat types, chicken meat is subject to deterioration in quality, due to lipid oxidation and microbial activity (Monahan, 1992), and spoiled foods are mentioned to be rich in biogenic amines and usually contain high levels of putrescine and cadaverine (Lovaas, 1991). Histamine possesses a powerful biological function, serving as a primary mediator of immediate symptoms noted in allergic responses (Taylor, 1986; Stratton *et al*., 1991). Polyamines, putrescine (PUT), spermidine (SPD), spermine (SPM) and cadaverine (CAD), are indispensable components of living cells as they are important in the regulation of nucleic acid function, cell division and protein synthesis and the stabilization of membranes (Maijala *et al*., 1993; Halasz *et al*., 1994). Putrescine and Cadaverine have been identified as potentiators that increase the toxicity of Histamine to human by depressing the activity of enzymes involving histamine detoxification (Ibe *et al*., 1991; Santos, 1996). Biogenic amines are potential precursors of carcinogenic N-nitroso compounds (Taylor, 1985; Stratton *et al*., 1991; Ruiz-Capillas & Jimenez-Colmenero 2004). The concentrations of some biogenic amines (tyramine, putrescine, and cadaverine) normally increase during the processing and storage of meat and meat products, whereas others (Spermidine and spermine) decrease or remain constant (ten- Brink *et al*., 1990; Halasz *et al*., 1994; Bardozc, 1995). Mietz and Karmas (1977) proposed a chemical quality index based on five types of biogenic amines, which are (Histamine, Putrescine, Cadaverine, Spermidine, Spermine). The main bacteria responsible for histidine decarboxylation are members of the family

Enterobacteriaceae as well as clostridium, *Bacillus* and *Lactobacillus* (Frank *et al.,* 1985; Taylor & Sumner, 1986; Ruiz-Capillas & Jimenez-Colmenero, 2004). *Pseudomonas* is one of the most important poultry spoilage bacteria; it possesses amino acid decarboxylase enzymes, particularly for the production of Putrescine (Rice *et al.,* 1986 and Halasz *et al*., 1994).

Most sensory characteristics can only be measured meaningfully by humans. However, advances are being made in the development of instruments that can measure individual quality changes (Lawless & Heymann, 1999). Sensorial attributes of food products are perceived by consumers in a specific order: Odor, consistency and texture with flavor (Meilgaard *et al*., 1999 ; Schilling, 2007). As whereas the first consumer right is to have a product of good quality and not constituting any health hazard (Sahoo, *et al*., 1996). So our research aims to assess the quantities of these products and their effect on the sensorial quality of the chicken meat sold in Sulaimani markets, and then their affinity for human consumption.

Materials and methods

Sampling:

The ultimate inspection included (160) samples of frozen whole chicken carcasses belonged to (4) foreign trademarks which collected in two batches from different parts of Sulaimani city markets, (80 for batch 1 and 80 for batch 2), The duration of sampling batch (1) was from the first of February 2010 till end of April, while batch (2) was sampled from July 2010 till end of September. All samples were subjected for sensory analysis while samples for biogenic amines, were prepared as 3 random samples for each trademark with 3 replicates**.**

Biogenic amines analysis (Hernandez- Jover, *et al*., 1996 and Gingerich, 1999)

a- Preparing of Chicken extract: Thirty ml of 8% Trichloroacitic acid were added to 20 gm of chicken meat and deionized water added to make 100ml solution, centrifuged for 10 minute at 6000 rpm. Trichloroacetic acid was removed by using 50ml ether. The supernatant was filtered with 0.45µm micropore filter. Per- column orthopthaldehyde (OPA) was derivitized by reacting 20µl sample with 20µl OPA for 60 second. 9-20 µl injected on HPLC system under the optimum separation conditions.

b- Analytical conditions**:** The analysis was performed on Shimadzu HPLC system model 2010 (Koyota, Japan) under the following conditions, Column: Shim-pack ISC-07 / 51504 Na (50 × 2.1 mm ID). Mobile phase: A: O.6 sodium citrate 0.1 M boric acid, pH10, B: acetonitrile, under gradient program: Flow rate: 0.8 ml/min. Column temperature: 65 ºC. Detection OPA, florometric detection excitation 340nm, emission 445nm. Appendixes (1, 2, 3).,

c- Calculation: Concentration of sample was calculated: Concentration of sample = area of peak/ area of standard peak× conc.of standard × dilution .

Biogenic amines Index calculation: the formula of (Mietz and Karmas, 1977) was used:

$$\text{Quality Index} = \frac{\text{ppm histamine} + \text{ppm putrescine} + \text{ppm cadaverine}}{1 + \text{ppm spermidine} + \text{ppm spermine}}$$

Test	Retention time /min (standard)	Retention time/min (sample)	Concentration of standard µg/ml
Putrescine	3.76	3.76	5
Spermidine	4.5	4.54	5
Spermine	5.31	5.37	5
Cadaverine	5.99	5.90	5
Histamine	6.81	6.83	5

Sensory evaluation (Organoleptic evaluation)

An eight member semi trained descriptive sensory analysis panel evaluated the cooked samples using an established sensory lexicon (Cross *et al*., 1978 and Castillini, 2002). Each panelist evaluated 2 cubed subsections from each sample for all marks. Samples were served at temperature of 55ºC and presented to panelists.

Statistical analysis

The statistical analysis system (SAS, 2004) program was used to effect of study factors (Trademark and batch) in traits. Duncan multiple ranges used to significant compare between means ($p< 0.05$).

Results

Biogenic amines

Figure (1) showed the Chromatogram of high performance liquid chromatography (HPLC) for standards and frozen whole chicken carcass solutions of biogenic amines. While table (1) showed the biogenic amines mean values of the carcasses for two batches of four trademarks. The Putrescine mean values in batch (1) showed significant differences ($p<0.05$) among all marks except for DMIS and Frinal which were not significantly different in between. Batch (2) showed significant differences ($p<0.05$) among all marks. The Cadaverine mean values showed that there were significant ($p<0.05$) differences among all marks except for Gedik and Sadia which had no differences in between for both batches. The Histamine mean values only in Frinal was significantly ($p<0.05$) different with the other marks. The Spermine mean values in both batches showed significant ($p<0.05$) differences among all marks except for Gedik and Sadia which were not different in between. The Spermidine mean values in all marks were significantly ($p<0.05$) different at both batches. The biogenic amines dependent Quality index (QI) were been showed in figure (2). The mean values ranged 0.42- 1.32 mg/ kg meat for batch (1) and 0.42- 1.30 mg/ kg meat for batch (2). Batch (1 and 2) showed significant ($p<0.05$) differences among all marks except Gedik and Sadia which were significantly not different in batch (2). All marks showed no significant difference between the two batches for each mark.

Sensory evaluation

The sensory evaluation mean values of the whole carcasses for two batches of four trademarks are shown in table (2). The colour scores revealed that there were no significant differences between Gedik and Sadia and between Frinal and DMIS while a significant ($p< 0.05$) difference appeared between the former and latter two trademarks. So, with this respect, all trademarks are within almost acceptable scores (Cross *et al*., 1978) in spite of that relative differences among them are noted. The flavour–aroma score in both batch revealed that DMIS significantly ($p< 0.05$) differed from all other three marks which have no differences in between. According to (Cross *et al*., 1978), DMIS and Frinal appeared to have low acceptable scores. The tenderness scores in batch (1) showed that DMIS significantly ($p< 0.05$) differed from all other three marks which have no differences in between. Batch (2) revealed no significant differences among all. According to (Cross *et al*., 1978), DMIS and Frinal had low scores. The Juiciness scores recorded that the highest score was of Gedik share for both batches while the lowest was of DMIS share for both batches. According to (Cross *et al*., 1978), DMIS and Frinal had low scores. The overall acceptability scores showed that both batches showed no significant differences among all marks. According to (Cross *et al*., 1978), DMIS and Frinal had low score

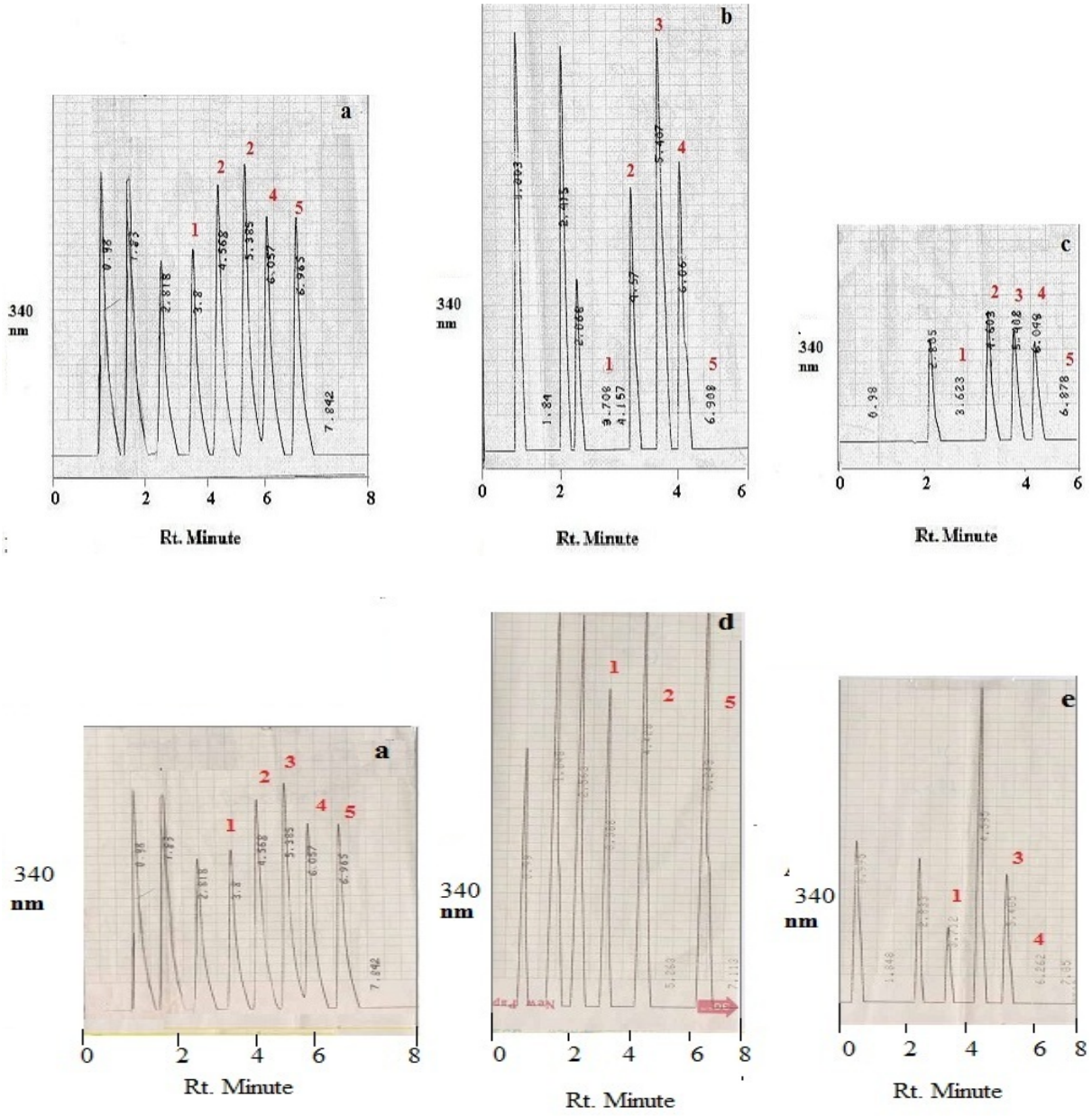

Figure (1): Chromatogram of high performance liquid chromatography (HPLC)for standards and frozen whole chicken carcass solutions of biogenic amines by using mobile phase: A- 0.6 Sodium citrate, 0.1 M boric acid, pH10; B- Acetonitrile.
a-Standard solutions ; b-DMIS mark. ; c-Gedik mark.; d- Sadia mark.; e-Frinal mark.
1-Putrescine(3.8).;2-Spermidine(4.568).;3-Spermine(5.385).;4 Cadaverine(6.057).; 5-Histamine(6.965).

Table (1) : Biogenic amnines mean values of imported frozen whole chicken carcasses for two batches of four trademarks (mg/ kg meat).

Traits	Batch	Trade mark			
		DMIS	Gedik	Sadia	Frinal
Putrescine	1	9.68±0.065 b A	11.58±0.060 a B	7.80 ±0.60 c A	9.25±0.310 b A
	2	9.30 ± 0.09 b A	12.22 ± 0.09 a A	7.71 ± 0.15 d A	8.45 ± 0.07 c A
Cadaverine	1	5.51 ± 0.25 a A	0.00 ± 0.00 c A	0.00 ± 0.0 c A	2.54 ± 0.45 b A
	2	5.27 ± 0.27 a A	0.00 ± 0.00 c A	0.00 ± 0.00 c A	3.05 ± 0.15 b A
Histamine	1	0.02 ± 0.00 a A	0.00 ± 00 a A	0.00 ± 0.00 a A	0.22 ± 0.22 a A
	2	0.03 ± 0.01 b A	0.00 ± 0.00 b A	0.00 ± 0.00 b A	0.44 ± 0.03 a A
Spermine	1	5.24 ± 0.30 c A	8.83 ±0.06 ab A	10.40 ± 0.81 a A	7.66 ± 0.33 b A
	2	5.24 ± 0.40 c A	9.13 ± 0.18 a A	10.00 ± 0.79 c A	7.20 ± 0.25 b A
Spermidine	1	5.24 ± 0.30 d A	11.40 ± 0.12 a A	7.04 ± 0.13 c A	8.67 ± 0.55 b A
	2	5.01 ± 0.20 d A	11.80 ± 0.80 a A	7.05 ± 0.24 c A	8.84 ± 0.06 b A

-Means having different lower-case at the same row (Trademarks) and upper-case at the same column (Batch) are significantly different at ($p < 0.05$).

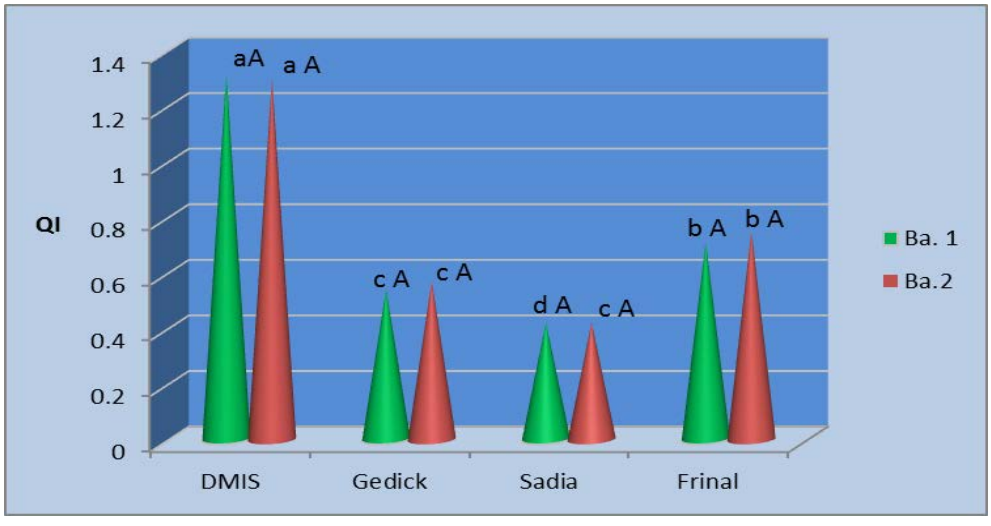

Figure (2): Quality index of imported frozen whole chicken carcasses of two batches for four trademarks.
Means having different lower-case at the same row (Trademarks) and upper-case at the same column (Batch) are significantly different at ($p < 0.05$). Ba. Batch

Discussion

Although both of DMIS and Gedik marks had high Putrescine content, this is due to, Putrescine and Cadaverine are formed during storage of meat (Galgano *et al*., 2009), and the high Cadaverine content in DMIS mark also could be due to the low pH value where pH is a key factor influencing the amino acid decarboxylase activity (Maijala *et al*., 1995). And it was recorded that the Spermine content decreases in time, possibly due to its use as nitrogen source for microorganisms and also due to enzymatic action of polyaminooxidases (Baston *et al*., 2010). The current results revealed lower bioamines values in comparison to more similar inspections achieved by researchers in different countries. They indirectly indicate no putrefaction because of microbial activity if they are standardized by other results. In a study by (Tamim and Doerr (2003) showed that freshly killed poultry carcasses contained 2.0 μg/g meat of Putrescence, 39.2 μg/g meat of Cadavarin, 110.5 μg/g meat of Histamine, 36.8 μg/g meat of Spermidine, and 54.5 μg/g meat of Spermine. They further indicated that the level of all amines increased with an increase of putrefaction except for Spermine and Spermidine. Silva &Gloria (2002) recorded the presence of biogenic amines in chicken breasts through 4, 10 .15 days of storage as 0, 0, 10.4 mg/kg meat for Putrescine and 0 ,0 , 0.3 mg/kg meat respectively for Cadaverine and 0,0 ,10.3 mg/ kg meat respectively for Histamine; 0.3, 6.0,8.7 mg/kg meat respectively for Spermidine 17.2 , 12.5 , 11.2 mg/kg meat respectively for Spermine. Baston *et al*. (2010) recorded the amounts of biogenic amines in chicken breasts through seven days of storage; Putrescine was not detected in the fifth day, when Histamine was 2 mg/kg meat, then both were raised in the seventh day to 3.01 and 4 mg/kg meat respectively, while the content of Cadaverine, Spermidine, Spermine were 4, 20, 4.5 mg / kg meat respectively at day seven. However, in both researches fresh meat was used and stored under chilling condition but not freezing.

It is well-known that meat freshness is a quality indicator that decreases in time because of meat spoilage. Biogenic amines are indicators for microbial activity, so that the loss of freshness indicates that meat has started spoiling. So, the (BAI) is a confirmative index for the freshness depending on the values of biogenic amines. As shown in figure (1), the frozen whole chicken carcasses revealed the highest biogenic Amines index (BAI) for DMIS mark which reflects the high levels of total biogenic amines in it. The current results seemed higher than that of (Baston *et al*. (2010) who applicated the freshness index proposed by (Mietz and Karmas, 1977), they recorded it as 0.2; this may be due to the fact that they used fresh chicken meat. However, results here appeared higher than those of (Balamstae *et al*., 2008) who recorded the quality index as 0.27. It should be noted that the histamine content is a health hazard were that 5–10 mg of histamine/ Kg can be considered potentially hazardous for some sensitive people, 10 mg - 100 mg may induce a medium toxicity and a dose of 1000 mg histamine/ Kg meat is highly toxic (Shalaby, 1996; Karovičová & Kohajdová, 2005). Also, the levels of Spermine and Spermidine in all inspected marks were not in threat levels, because high Spermine contents, usually between 20 and 60 mg/kg, are usual in meat and meat products of warm-blooded animals and Spermidine levels in meat rarely exceed 10 mg/kg meat. According to (Mietz and Karmas, 1977) who invented the biogenic amines index, whenever the quality index was low, whenever the meat quality is high, so with this respect, the biogenic amines dependent quality indexes for carcasses of all marks were below the suspicious limits except for DMIS mark.

Colour is very complex and is a major component of appearance in poultry meat or products (Lyon and Lyon, 2001). The present results for whole chicken carcasses showed that DMIS mark had the lowest colour score which tended to be dark colour (Cross *et al*., 1978 and Castellini *et al*., 2002). This could be due to the effect of several thawing and freezing processes that lead to drip loss, which contains juice, exudation of juices from the muscle tissue (extracellular space) due to large ice crystal formation in case of slow freezing which leads to tissue injuries (Barbut & Mittal,1990) and then loss of protein component in muscles as the general precipitation of protein increases the light diffusion on the surface and is responsible for the light colour appearance of the cooked meat (Terra *et al*., 2009) or due to environmental conditions, such as feed and housing, which may affect meat colour as well (Du & Ahn, 2002).

With respect to that DMIS has the lower flavour score, it is known that flavour and aroma are influenced by age, whether young or mature at slaughtering, diet and diet ingredients, and

meat flavour is minorly influenced by bird strain, environmental conditions (litter, ventilation, etc.), scalding temperatures, chilling, product packaging, and storage (Mead, 2004 and Northcutt, 2009). Practically, it is not possible to evaluate these parameters to know the precise reason for decreasing score, so we suggest that information about these parameters should be considered with importing chicken.

During frozen storage, lipid oxidation occurs, which affect mainly the meat flavour (Raharja *et al*., 1992). The score of juiciness depends on moisture content in the meat (lyon *et al*., 1990)) and on the fat content with water holding capacity (Mckee, 2007). So, the high fat content means the lowest water content, then whenever the moisture content was less, the juiciness scores decreased. DMIS recorded lowest texture scores; this could be due to the amount of collagen in meat, that whenever the bird advanced in age, the collagen content will increase (Liu *et al*., 1996; Coró *et al*., 2003 and Lyon *et al*., 2004). Age and genetic strain are two further inherent factors that affect meat texture (Lyon *et al*., 2004). The overall acceptability for whole carcasses samples refers to that both DMIS and Frinal marks receive low scores, this could be due to the decline in scores of flavour-aroma, tenderness (texture) and colour where overall acceptability is a reflection of changes in scores of sensory attributes (Singh *et al*., 2011). According to Mead (2004), it is quite difficult to compare results obtained in different countries because sensory tests depend strongly on the experience of consumers or other panel members and their eating habits. So that comparing of the current results with those of other countries is not significantly important. Therefor Gedik mark reaped the highest sensory evaluation among all inspected marks, Sadia has come in the second order while DMIS gained the lowest scores due to the reasons mentioned above.

Conclusion

The findings related to the sensory evaluation shows that all inspected marks for whole chicken carcasses, were with good quality except for DMIS mark. According to the biogenic amines indices which refer to the quality of the products before freezing, the finding referred to the satisfied handling procedures and storing conditions for the vendors and sellers in the region. This study recommend that biogenic amines should be included in the quality regulation standards as freshness indicators, and more studies are needed to be done for researching the quantity of these amines in imported chicken through handling and storing stages.

Table (2): Mean values of Sensory evaluation of imported whole frozen chicken carcasses for two batches of four trademarks.

Traits	Batch	Trade mark			
		DMIS	Gedik	Sadia	Frinal
Colour	1	2.50 ± 0.29 b A	4.00 ± 0.00 a A	3.75 ± 0.25 a A	2.75 ± 0.25 b A
	2	2.75 ± 0.25 b A	4.00 ± 0.00 a A	3.50 ± 0.29 a A	2.75 ± 0.25 b A
Flavour -aroma	1	2.50 ± 0.29 b A	3.50 ± 0.29 a A	3.50 ± 0.28 a A	2.75 ± 0.25 ab A
	2	2.50 ± 0.29 b A	3.50 ± 0.29 a A	3.25 ± 0.25 ab A	2.75 ± 0.25 ab A
Tenderness	1	2.50 ± 0.29 b A	3.75 ± 0.25 a A	3.50 ± 0.29 a A	3.00 ± 0.00 ab A
	2	3.00 ± 0.00 a A	3.50 ± 0.29 a A	3.25 ± 0.25 a A	2.75 ± 0.25 a A
Juiciness	1	2.75 ± 0.25 a A	3.50 ± 0.29 a A	3.25 ± 0.25 a A	3.00 ± 0.00 a A
	2	2.75 ± 0.25 a A	3.25 ± 0.25 a A	3.25 ± 0.25 a A	2.75 ± 0.25 a A
Over all acceptability	1	2.50 ± 0.29 b A	4.00 ± 0.00 a A	3.25 ± 0.25 b A	2.50 ± 0.29 b A
	2	2.50 ± 0.29 c A	3.50 ± 0.29 ab A	3.75 ± 0.25 a A	2.75 ± 0.25 bc A

- Means having different lower -case at the same row (Trademarks) and upper -case at the same column (Batch) are significantly at ($p < 0.05$).

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پوختە

ئامانجي ئەم لێكولينەوە هەلسانگدني جۆرايەتي گوشتي مريشكي بەستووي هاوردە كە لە بازارەكاني شاري سلێمانيدا لە ريگەي ئەمينە بايوجينەكان و هەلسانگدني هەستي. لە كۆي 160 نموونەمريشكي بەستوو كە لە چۆار ماركەي بازرگاني لە خۆگرتبوو، بە دوو تاووەوە (باچ) ، وەهەر تاوێك 80 نموونەبوو . وەبەرێگەي HPLCبشكينيني ئەمينە بايوجينيناكاني بۆكرا، كە لە ئەنجامدا دەركەوت كە رێژەي هستامين لە سنووري رێگاپێدرا بوولە هەموو نموونەكاندا. كە بە رێژەي 0.00– 0.22 و 0.00–0.03 ملغم/كغم گۆشت لە باچەكاني يەكەم و دووەمدا يەك بە دوا يەك. رێژەي پيوتراسين كادافارين ، سپرمين وە سپيرميدين بەشيويكي رێكەوتبوو لە گەل رێژەي باسكرابوو لە لايني تۆيژەرێكي ديكە، كە هەمووي بە رێژێكي گونجاوبوو وە رێپيدراو بوو لە هەموو ماركەكان تەنها لە ماركەي DMIS. نيشاندەري ئەمينەبايۆوجيينييەكان (BAI) بۆ هەموو ماركەكان لە سنووردا رێگەپێدراووكاندابوو تەنها بۆ ماركەي DMIS كە جياوازي متمانەداري هەبوو لەگەل ماركەكاني ديكەدا . هەلسانگدني هەستي پيشاندي دا كە ماركەي Gedik بەرزترين خالەكاني بەدەست هێنا، دواي ئەوەس ماركەي Sadia وە Frinal لە كاتێكدا ماركەيDMIS كەمترين خالي بەدەست هێنا لەگەل هەبووني جياوازي متمانەدار لە نێوانياندا.

التقييم الكيموحيوي للدجاج المستورد وتاثير كمية الامينات الحيوية على نوعية لحم الدجاج

الخلاصة

لتقييم نوعية الدجاج المجمد المستورد والموجود في اسواق مدينة السليمانية اعتمادا على محتواها من الامينات البايوجينية و التقييم الحسي لها. من مجموع 160 عينة من الدجاج المجمد تضمنت اربعة علامات تجاريةو بواقع وجبتين (80 عينة لكل وجبة) من اسواق مختلفة في مدينة السليمانية. الامينات البايوجينية جرى فحصها باستخدام طريقة HPLC؛والتي بينت ان الهستامين كان ضمن الحدود المقبولة في كل العينات اذ كان مدياه في الذبائح 0.00–0.22 و 0.00–0.03 ملغم/ كغم لحم في الوجبتين الاولى والثانية على التوالي، كانت قيم الامينات البايوجينية الاخرى، البوتراسين والكادافرين و السبيرمين والسبيرميدين، في كل العلامات ضمن المعدلات المؤشره على نوعية اللحوم الجيده ماعدا علامة DMIS. وكان مؤشر الامينات البايوجينية (BAI) ضمن الحدود المقبولة في كل العينات لجميع العلامات باستثناء DMIS والذي اختلف معنويا عن باقي العلامات. اما التقييم الحسي فقد اظهر حصولGedik على اعلى النقاط يليها Sadia ثم Frinal بينما حصلت DMIS على اقلها مع وجود فروقات معنوية فيما بينها.