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EFFECT OF FROZEN STORAGE (-20°C) ON SOME BIOGENIC AMINES FORMATION IN BEEF BURGER AND SALTED SARDINE

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ABSTRACT

Recent trends in food security are promoting an increasing search for trace compounds that can affect human health. Therefore, the objective of current study was determined some biogenic amines namely, histamine, tyramine and cadaverine in beef burger and salted sardine during storage at -20°C for two months. The obtained results revealed that, beef burger protein content was lower than that salted sardine. On the other hand, fibers, ash, fat and carbohydrates contents had approximately the same values in both products. The essential amino acids content of beef burger was higher than salted sardine especially, histidine, isoleucine, leucine, lysine and valine at zero time. Minerals content as Ca, Mg, Fe, Zn, Na, K and P of beef burger was higher than salted sardine samples at zero time.

Histamine recorded the highest value comparing with tyramine and cadaverine in beef burger whereas, histamine and cadaverine were higher than tyramine content in salted sardine sample. The histamine, tyramine and cadaverine contents of both commercial beef burger and salted sardine samples were higher than the prepared beef burger and salted sardine in laboratory during the storage period at -20°. After storage period at -20°for two months the total bacterial counts increased and minor contamination occurred. Meanwhile, *E. Coli* and *Salmonella* sp. not detected (-Ve) in all beef burger and salted sardine samples.

Generally, it could be clearly concluded that the optimum condition (Temperature, pH value, NaCl and Time) during storage period play a great role as limiting factors to obtain meat or fish products free or nearly free from biogenic amines.

Keywords: Salted Sardine, Beef burger, Biogenic amines, Optimum conditions

INTRODUCTION

Biogenic amines occur in a wide variety of foods such as fish and fish products, meat and meat products (Askar and Treptow, 1986 and Brink *et al*., 1990). Biogenic amines are low molecular weight organic bases with biological activity that are formed in foods by microbial decarboxylation of the corresponding amino acids or by transamination of aldehydes and kectones by amino acid transaminases (Maijala *et al*., 1993., Onal 2007., Brink *et at* ., 1990).

High amounts of certain amines may be found in food as a consequence of the use of poor quality raw materials, contamination and in appropriate conditions during food processing and storage (Braink *et al*., 1990 and Halasz *et al*., 1994).

Biogenic amines accumulation in foods requires the presence of microorganisms with amino acid decarboxylases and favorable conditions for their growth and decarboxylation activity(Zarei *et al.,* 2011 and Ayesh *et al*.,2012). Storage temperature is the most important factor contributing to BAs formation (Chong *et al*.,2011 and Fadhlaoui-Zid *et al*,2012). Other parameters (PH, water activity , NaCl concentration , additives i.e..) may influence the variation of microbiota composition and lead to the differences in BAs content (Munoz-Atienzae *et al*.,2011 and Hwang *et al*.,2012).

The most common amine found in fermented meats is tyramine , which is found at higher concentrations than other amines. The toxic level of biogenic amines is 100 mg per 100g of product (George *et al*., 2005)

Hassan, *et al* . (2014) reported that the histamine concentration was higher in sausage than burger , luncheon and nuggets . The highest concentration of tyramine and cadaverine were in susage, burger, nuggets and luncheon respectively. Simulation the biogenic amines (histamine-tyamine- cadaverine) increases as the storage time increase.

The relationship between the formation of BA in aquatic products and the growth of microbial flora during storage was investigated. The results showed that putrescine , cadaverine , histamine and tyramine were the predominate BA in the studied samples, but the concentrations of histamine and tyramine were mostly less than 50 and 100 mg/kg, respectively . Fressing can effectively prevent the formation of BA, but the levels of putrescine , cadaverine , histamine and tyramine significantly increasd ($p<0.05$)during storage (Huang and Youg , 2012). Otherwise , Matsheka (2013) reported that *bacillus* species isolated from all fermented food products were found to be weak histamine producers . The production of biogenic amines was not a widely distributed property among the lactic acid bacteria as previously documented for other fermented food product.

The aim of the present wark was to study the effect of frozen storage period at (-20°) for two months on the production of biogenic amines.

MATERIALS AND METHODS

Materials

Fresh minced beef meat (4 kg) was purchased from butcher at Mashtul Al-Soq, Al-Sharqia Governorate, Egypt and transported immediately in an icebox to the laboratory.

A commercial beef burger sample (500 g) was purchased from retail markets in Mashtul Al-Soq, Al-Sharqia Governorate.

Fresh sardine *(sardinella aureate)* about 6 kg was purchased from Kafer El-Sheikh governorate, during August 2014. The fresh sardine sample was transported to the laboratory in an icebox.

One commercial salted sardine sample (500 g) was purchased from a retail markets at Mashtul Al-Soq, Al-Sharqia Governorate, Egypt as a control.

The NaCl salt was obtained from Al-Nasre Company, Cairo, Egypt.

Nutrient agar and malt extract agar were obtained from the Difco and Alkan Co. , Detroit I Michigan (U.S.A).

Methods

Preparation of raw materials

1-Preparation of beef burger

Beef burger contained lean beef meat, cow fat, rusk powdered, dried onion, starch, salt and ice-water with ratios of 78%, 11%, 4%, 0.9%, 1.25% and 4.45%, respectively (Ahmed et *al.,* 1999). Meat and fat were twice minced and mixed well, then the other ingredients were added and mixed well. The beef burger formulate using a patty marker (stainless steel model) to obtain round discs (10 cm diameter and 0.5 cm thickness). After preparation of each formulate, the beef burger discs (each disc 50 g) were packed polyethylene bags and stored immediately in a deep freezer at -20°C for up to two months. Samples were analyzed monthly.

2-Preparation of Salted Sardine

Sardine, *(Sardinella aureate)* was cleaned and treated with salt (NaCl 3.5%), adjusting the pH value to 4.5. the position of sardine fillets one on one after this the paked (each one kg) in bags were stored immediately in a deep freezer at - 20°C for up to two months. Samples were analyzed monthly.

Analytical Methods

Gross Chemical Composition

Moisture, protein, fat crude fibers and ash contents were determined according to the methods described by the A.O.A.C. (2012). Total carbohydrates content was calculated by differences.

Minerals

Minerals content was determined using atomc absorption spectrophotometer (Prekin Elmer Instrument, Model 2380), according to the method described by the A.O.A.C. (2012). Calcium was determined by EDTA titration according to the method described by the A.O.A.C (2012). Ammonium molybedate titration method was used for phosphorus determination as described by the A.O.A.C. (2012).

Peroxide value of beef burger fat was determined according to the method described by the A.O.A.C (2012). Acid value of beef burger fat was determined according to the method described by Gunstone *et al.* (1994).

Amino acids composition

Amino acids composition of all samples were analyzed using amino acid analyzer (Beckman 7300), according to the method of Lopez *et al.* (1991).

Extraction of biogenic amines

Ten grams of each sample were homogenized with 50ml of 6% trichloroacetic acid (TCA) for 3 min. The homogenates were filtered through whatmann No. 2 filter paper, then the filtrates were placed in volumetric flasks and TCA was added to bring a final volume of 100ml. The benzoyl derivatives of the standard biogenic amines and the fish extract were prepared according to Hwang *et al*., (1997). Briefly, one ml of 2M sodium hydroxide and 10ml of benzoyl chloride was added sequentially to 2ml of standard biogenic amines solutions or the fish extracts. The resulting solution was vortex mixed and allowed to stand at 30°C for 45 min. Benzoylation was stopped by adding 2ml of saturated NaCl solution and the mixed solution was extracted with 3ml of diethyl ether. After centrifugation, the uppel organic layer was transferred to a test tube and evaporated to dryness.

Determination of biogenic amines

The dried residue was dissolved in 500µl of methanol and 20µl aliquots were used for injection in to HPLC (Wald broon, Germemy model C1311A) equipped with UV detector model G1314A set at 254nm, auto sampler model G1329.

Physical properties

The pH values of beef burger and salted sardine were periodically measured using the pH meter (Jenway, model 3010, UK), according to the method of the A.O.A.C (2012).

Beef burger freshness (cooking loss, water holding capacity and plasticity) were determined according to the methods described by Solovier (1966) and Lin and Zayas (1987).

Microbiological examination

Total viable bacterial count and *E. coli* were enumerated according to the methods of (Difco Manual, 1977). *Salmonell Sp* was isolated according to (Ellis *et al.,* 1976).

RESULTS AND DISCUSSION

Chemical composition of tested materials

The results in Table (1) show the chemical composition of beef burger and salted sardine. Protein and fat contents were higher in salted sardine (19.32%) than beef burger (3.92%), while total carbohydrates (1.12%) were lower in salted sardine than beef burger (3.10%). These results are nearly with those of Zygmunt *et al.,* (2009).

Table (1) Chemical composition of beef burger and salted sardine prepared under optimum condition at zero time.

Samples / Constituents %	Beef burger	Salted Sardine
Moisture	70.25	70.22
Protein	15.20	19.32
Crude fibers	3.62	3.03
Ash	2.22	1.38
Fat	3.92	5.92
* Carbohydrate	3.10	1.12

*** Total carbohydrate was calculated by differences.**

The data of Table (2) show that the minerals contents of beef burger and salted sardine prepared under optimum conditions at zero time. Salted sardine had the highest values of Ca (875 mg/kg) and Mg (444 mg/kg) comparing with beef burger that had 55.4 and 13.06 mg/kg for Ca and Mg, respectively. The phosphorus content of beef burger recorded the highest level (9960 mg/kg) and presented the minor elements. After that, the data of all tested samples nearly recorded the same values (Table 3). These result are in agreement with those reported by Ibrahim (2015).

Table (2) Minerals content of beef burger and salted sardine salted prepared under optimum conditions at Zero times (on dry weight basis).

Minerals content (mg/kg)	Beef burger	Salted sareline
Ca	55.4	875
Mn	15.4	0.16
Mg	13.06	444
Fe	15.4	14.49
Zn	15.6	12.49
Cu	3.38	2.36
Cr	2.19	2.78
F	1.98	2.31
Mo	1.84	2.08
Se	1.23	2.04
Na	2604	2786
K	2134	2188
P	9960	1007

Amino acid composition

Fish is an important dietary source of high quality animal proteins and amino acids and play an important role in human nutrition. So, amino acids composition of beef burger and salted sardine processed under optimum conditions were analyzed and identified by amino acids analyzer and the results are presented in Table (3). The higher percentages of glutamic acid, aspartic acid, isoleucine, leucine, alanine and arginine while the lower percentages of methionine, cysteine, lysine, phenylalanine, histidine, proline, glycin, thrionine and serine. These results similar with those obtained by El-Adawy *et al.*, (2001).

Table (3) Amino acids composition of beef burger and salted sardine prepared under optimum conditions at Zero times (on dry weight basis) (gm/100g sample).

Amino acid composition	Beef burger	Salted sardine
Essential amino acids		
Arginine	5.77	5.92
Histidine	4.92	3.43
Isoleucine	4.72	5.42
Leucine	8.62	1.69
Lysine	6.22	3.78
Methionine	1.92	1.48
Phenylalanine	3.91	2.64
Threonine	3.33	4.11
Tyrosine	2.36	2.45
Valine	5.19	4.40
Trptophan	N.D.	N.D.
Cysteine	1.92	1.82
Nonessential amino acids		
Clutamic acid	22.12	15.32
Glycine	4.90	4.37
Proline	4.66	2.46
Alanine	3.62	5.44
Aspartic acid	9.32	6.55
Serine	3.11	5.52

N.D.: Not detected.

Biogenic amines content

1-In prepared beef burger and salted Sardine samples

The contents of biogenic amines formed in beef burger and salted sardine under optimum conditions (Temp. 20-37°C, pH value 4.0-5.5 and NaCl 3.5-5.01%) during storage period (0,1.2 months) were extracted and identified by HPLC and the results are presented in Table (4).

From the data illustrated in Table (4), it could be clearly noticed that histamine, tyramine and codaverine contents gradually increased with increasing the storage period.

The results in Table (4) are in agreement with those of Stadnik and Dolatowki 2010, who reported that, histamine content in poultry sausage was the highest concentration after one day (2.72mg/100g) and the concentration of histamine increased by increasing the storage time in all samples. Also, at the same results with Henry Chin and Koehler (1986) they, demonstrated that NaCl concentration ranging from 3.5% to 5.5% could inhibit histamine production. This influence can be attributed to reduced cell yields obtaimed in the presence of high Nacl concentration and to a progressive disturbed of the membrane located microbial decarboxylase enzymes.

Amino acid decarboxylase was highly active in an acidic environment, being the optimum pH between 4.0 and 5.5. Enterobacteriaceae isolated from sausages are generally considered as microorganisms with a high decarboxylase activity, particularly in relation to production of histamine, cadaverine and tyramine.

Table (4) The effects of biogenic amines content formation in beef burger and salted sardine during storage period.

Biogenic amines	Storage period	Beef burger	Salted sardine
Histamine	(0)	2.5	2.5
	(1)	2.6	3.5
	(2)	3.5	4.5
Tyramine	(0)	0.85	0.66
	(1)	1.53	1.22
	(2)	1.80	1.45
Cadaverine	(0)	1.82	1.92
	(1)	2.36	2.30
	(2)	3.00	3.66

*** Time (0-1-2 month)** *** Temperature°C (20-37°C)**
*** pH (4.0-5.5) Freezing (-18°C)** *** NaCl (3.5 – 5.0%)**

2-In commercial beef burger and salted sardine samples

Biogenic amines formation in beef burger and salted sardine is a result from endogenous amino acids decarboxylase activity in raw food materials or the growth of decarboxylase microorganisms under conditions favorable for enzyme activity. Therefore, three types of biogenic amines namely, histamine, tryamine and cardaverine were isolated and identified by HPLC in commercial beef burger and salted sardine during storage period and the results obtained in Table (5). Increasing the time of storage period increased the biogenic amines. These results, are in agreement with those reported by accumulation George *et al*., (2005) they reported that the storage time and temperature after manufacturing can also increase the amount of biogenic amines present increase of tyramine content from 15 to 90 mg per kg during ripening, and then another increase from 90 to 200 mg per kg during storage.

Table (5) Biogenic amines contents (mg/100g) in commercial beef burger and salted sardine from local markets during the storage period (zero month, 1 month and 2 month):

Biogenic amines	Storage period	Beef burger	Salted Sardine
Histamine	(0)	30mg/kg	42mg/kg
	(1)	34mg/kg	44mg/kg
	(2)	36mg/kg	48mg/kg
Tyramine	(0)	45mg/kg	54mg/kg
	(1)	48mg/kg	58mg/kg
	(2)	50mg/kg	62mg/kg
Cadaverine	(0)	49mg/kg	60mg/kg
	(1)	50mg/kg	62mg/kg
	(2)	52mg/kg	64mg/kg

*** Time (0-1-2 month)** *** Temperature°C (20-37°C)**
*** pH (4.0-5.5) Freezing (-18°C)** *** NaCl (3.5 – 5.0%)**

Stadnik Dolatowski (2010) reported that the amine formation by bacteria is decisively influenced by temperature. Temperature between 20°C and 37°C is optimal for the growth of the most bacteria containing decarboxylases, decreased temperature stops their growth.

Biogenic amines accumulation decreases markedly with the increase of NaCl concentration, while

proteolytic NaCl activity is higher for intermediate concentration of salt Karovicová and Kohajdova (2005).

Higher concentrations of salt (3.5 to 5.5%) could inhibit the histamine production of some histamine-forming bacteria Gardin and Suzzi (2003).

Huang and Yang (2012) showed that during storage at -20°C for 12 days, no significant change in the concentrations of BA was observed for the four tested species (blue scad, golden thread, belt fish and octopus), except for cad and put with slight decreases in blue scad from the sixth day and in octopus from the initial time. The levels of cad and put in octopus remained unchanged from the third day of storage.

Cooking properties of beef burger prepared under optimum conditions

Cooking loss, water holding capacity, plasticity, peroxide number and acid value were measured in beef burger prepared under obtamin conditions and the results illustrated in Table (6).

Cooking loss of meat freshly prepared beef burger was 35.10%. the obtained data are in agreement with those reported by Ahmed *et al.,* (1999).

The water holding capacity WHC and plasticity, of meat freshly prepared beef burger were 2.11 cm^2 and 2.12 cm^2, respectively. While, perioxide number and acid value recorded 4.91 ml/kg and 0.33 ml/kg, respectively.

Table (6) Some Cooking properties of Beef burger (on dry weight basis)

Properties	Cooking loss %	Water holding capacity $(cm)^2$	Plasticity $(Cm)^2$	Peroxide number mg/kg	Acid value ml/kg
Beef burger	35.10	2.11	2.12	4.91	0.33

Microbiological examination of beef burger and salted sardine during storage

Table (7) illustrated that the total bacterial counts of both the beef burger and salted sardine reduced during storage period may be due to increase in support the bacterial growth or during the wing operation, which exposure the sampels to bacterial growth from the environment.

E.Coli and *Salmonella* spp. counts of all samples beef burger and salted sardine, not detected till the end of storage period. These results are in accordance with the results.

Table (7) Microbiological examination of beef burger and salted sardine prepared under optimum conditions

Samples	Microbiological analysis		
	Total bacterial count	*Escherichia. Coli*	*Salmonella*
Beef burger	3.1×10^4 cell/g	(-)	(-)
Salted sardine	3.1×10^4 cell/g	(-)	(-)

(-): Negative.

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" تأثير التخزين المجمد (-٢٠°م) على بعض الأمينات الحيوية المتكونة في البيف بورجر والسردين المملح"

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المركز الإقليمي للأغذية والأعلاف – مركز البحوث الزراعية – الجيزة

منذ عهد قريب تم الاتجاه نحو إنتاج الغذاء الآمن والترويج له وزيادة البحث عن المركبات الصغيرة التي تؤثر على صحة الإنسان. وعلى ذلك تم في هذا ابحث تقدير التركيب الكيماوي للأحماض الأمينية والعناصر المعدنية التي يجهز منها البيف بورجر والسردين المملح تحت الظروف المثلى مثل (درجة الحرارة ومستوى الـ pH وكلوريد الصوديوم والوقت). ومقارنة ذلك بالعينات التجارية. وإلى غير ذلك تم تقدير بعض أسماء الأمينات الحيوية مثل الهستامين والتيرامين والكيدوفيرين وذلك أثناء مرحلة التخزين بعد شهرين.

والنتائج التي حصلنا عليها تعتمد على ظهور نسبة البروتين وقلته في البيف بورجر عن السردين المملح. وعلى الجانب الآخر الألياف والدهن والكربوهيدرات تكاد تكون متقاربة في كلا من البيف بورجر والسردين المملح، وأن نسبة الأحماض الأمينية الأساسية في البيف بورجر مرتفعة عن نسبتها في السردين المملح وخاصة الهستامين والأيزوليوسين والليوسين والفالين عند الـ Zero time, وأن نسبة العناصر المعدنية مثل الكالسيوم والماغنسيوم والحديد والزنك والصوديوم والبوتاسيوم والفوسفور في البيف بورجر عند الـ Zero time أعلى من نسبتها في عينات السردين المملح.

الهستامين التي حصلنا عليه ومقارنته مع التيرامين والكيدوفيرين في البيف بورجر حيث ان الهستامين والكيدوفيرين نسبته أعلى عن نسبة التيرامين في عينات السردين المملح.

الهستامين والتيرامين والكيدوفيرين في البيف بورجر والسردين المملح التي تم شراؤهم من الأسواق (التجارية) نسبتهم أعلى من البيف بورجر والسردين المملح التي تم تجهيزها معملياً تحت ظروف التخزين المجمد(-٢٠°م).

وبعد مرحلة التخزين تحت الظروف المثلي بعد شهرين نجد أن العدد الكلي للبكتريا يزداد عند أقل تلوث قد يحدث. وأن الأيكولاى والسالمونيلا أعطت نتائج سلبية في عينات البيف بورجر والسردين المملح. عامة أن الظروف المثلي مثل درجة الحرارة ومستوى الـ pH وكلوريد الصوديوم والوقت يلعبوا دوراً كبيراً أثناء فترة التخزين في العوامل المحددة التي تعتمد عليها اللحوم والأسماك ومنتجاتهم الحرة أو الأمينات الحيوية القريبة منها.