

## **Incidence of Biogenic Amines and Its Relation to Chemical Preservatives Effects in Canned Beef and Tuna**

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### **Abstract**

Presence of biogenic amines can lead to several problems for susceptible consumers. A total of forty six random samples of canned beef and canned tuna (23 samples of each divided as 20 samples in date of production collected as 10 samples after one month of production, other 10 samples before two months of expired date and 3 samples expired) were collected from different markets at El Menofea Governorate for detection of biogenic amines and preservatives in these samples. The obtained results indicated that the average of histamine, putrescine and tyramine in canned beef samples shortly after production were  $1.17 \pm 0.31$ ,  $9.31 \pm 2.18$  and  $3.05 \pm 0.92$  mg/100g respectively, the averages in samples shortly before expiry date are  $2.62 \pm 0.43$ ,  $12.46 \pm 2.72$  and  $4.71 \pm 1.15$  mg/100g for histamine, putrescine and tyramine, respectively. In expired canned beef samples the averages were  $3.93 \pm 0.53$ ,  $16.41 \pm 2.75$  and  $5.47 \pm 1.33$  mg/100g for histamine, putrescine and tyramine, respectively. In canned tuna samples, the numbers of unaccepted sampled due to increase the level of histamine in both examined samples shortly after production and samples before expiring is 10 were 50% of examined samples in the two groups in date of production. While, all examined samples of expired tuna samples were unaccepted due to increased histamine. Miss handling of meat and bad storage conditions for food minimize the date of expiring, so care must be taken to keep food in the most suitable conditions to avoid the spoilage or chemical changes in its components.

**Keywords:** Biogenic amines, Canned Beef, Tuna and preservatives.

### **Introduction**

Biogenic amines in foods originated from two sources. They represent the natural component of cell structures of plants, animals and microorganisms, or may arise during the process of food production and storage as the result of metabolic action of microorganisms. The BAs levels were indicators of spoilage both in red and white meat. Particularly a determination of the cadaverine concentration could be used to monitor spoilage in both red and white meat and also the tyramine concentration is useful indicator to control red meat during storage (**Vinci and Antonelli,2002**).

Biogenic amines (BAs) can be found in all foods containing proteins or free amino acids and are found in a wide range of food products including fish products, meat

products, dairy products.....etc. In non-fermented foods the presence of BAs is mostly undesired and can be used as indicator for microbial spoilage (**Wikipedia.Org**).

Biogenic amines are natural antinutritional factors and are important from a hygienic point of view as they have been implicated as the causative agents in a number of food poisoning episodes, and they are able to initiate various pharmacological reactions. Histamine, putrescine, cadaverine, tyramine, tryptamine,  $\beta$ -phenylethylamine, spermine, and spermidine are considered to be the most important biogenic amines occurring in foods. Analysis of biogenic amines is important because of their toxicity and their usage as indicators of the degree of freshness or spoilage of food (**Armağan,2006**).

Biogenic amines are normally formed in food as a consequence of the metabolic process during storage, spoilage or ripening. After degradation of proteins either by self autolysis or by bacterial proteolysis, free amino- acids are formed which act as precursors of biogenic amine with the aid of decarboxilase enzymes (**Koehler and Eitenmiller, 1978 and Davidek and Davidek, 1995**). Therefore, the production of biogenic amines requires the availability of amino acids, the presence of microorganisms capable of decarboxylating amino acids, and favourable conditions for their growth and for the development of their decarboxylase activity (**Moret and Conte, 1996 and Ordonez et al., 1997**).

High levels of biogenic amines in foods are of public health significance because of their potential toxic effects. Toxic levels of biogenic amines cause reddening of the skin, stomach trouble and migraine. Histamine and other food amines as putrescine and cadaverine have vaso-active properties and, in some cases, they can reach concentrations in foods, which are dangerous for the most sensitive consumers (**Maijala et al., 1993**).

**Fiigen et al. (2001)** recorded the great fluctuations of amine content are reported in the same type of meat product. These differences depends on many variables including the quali-quantitative composition of microbial microflora, the chemico-physical variables and the hygienic procedure, respectively. Hence, the major biogenic amines produced by *Enterobacteriaceae* are putrescine, cadaverine, tyramine and histamine, both in culture medium and meat products.

**Seyed et al. (2009)** mentoned that high biogenic amine levels found in the food indicated poor handling and/or processing of these products. Therefore, education on hygienic handling and manufacturing of raw materials and proper process control is recommended for local health authorities and producers.

**Munoz, et al. (2008)** recorded that the biogenic amines may lead to dilatation of blood vessels, capillaries and arteries, causing headache, gastrointestinal distress and

odema. Also tyramine causes the increase of nor adrenaline concentration in blood as an indirect effect, acting like vasoconstrictor causing hypertension and migraine. Also **Stratton *et al.* (1991)** said that the histamine isn't the only amine responsible for the illness, other biogenic amines, such as putrescine and cadaverine have been shown to potentiate histamine toxicity when present in spoiled fish by inhibiting both intestinal histamine metabolizing enzyme.

**Bentley, *et al.* (1995) and Roig-Sagues, *et al.* (1997)** recorded that the production of histamine and putrescine has been related to the presence of histidine and lysine decarboxylase enzymes which can be synthesized by auto-enzymes or bacteria as *E.coli*, *Enterobacter*, *Lactobacilli*, *Pseudomonas*, *Streptococci*, *Micrococci* and aerobic spore- former species.

Presence of sodium chloride activates tyrosine decarboxylase activity and inhibits histidine decarboxylase activity (**Silla-Santos, 1996**). The ability of *Lactobacillus buchneri* to form histamine is partly inhibited when sodium chloride concentration was 3.5 % and at the level of 5.0 % histamine formation is stopped.

**Paleologos, *et al.* (2003)** reported that the advantages of micellar point extraction combined with a surfactant-assisted separation in HPLC system are presented as a method for the effective separation and determination of nine biogenic amines in fish substrates. Benzoyl derivatives of the amines are extracted inside the micelles of a non-ionic surfactant, Triton X-114, and separated with gradient elution micellar liquid chromatography. Quantification was performed by measuring the UV absorbance of the benzene ring at 254 nm. Detection limits of the nine biogenic amines were in the vicinity of 0.01 mg l<sup>-1</sup> which are 10 times lower than those of the conventional method (HPLC–UV) and 100 times lower than those of micellar electrokinetic capillary chromatography. The correlation coefficients of determinations were 0.9911–0.9996. The method was applied for the determination of putrescine, cadaverine, agmatine, tyramine, tryptamine, phenylethylamine, spermine, spermidine and histamine in fish samples. Recovery of the proposed method ranged from 95 to 103.5%.

Therefore, the present work was planned out for detection of biogenic amines as histamine, Putrescine and tyramine in the examined canned beef and tuna samples as well as detection of preservatives as sodium chloride and nitrate in in these samples and study the effect of preservatives on biogenic amines formation and its percentage.

## Materials and Methods

### Collection of Samples

A total of forty six random samples of canned beef and tuna ( 23 samples of each divided as 20 samples in date of production from them 10 samples shortly after

production by 1 month, another 10 samples before expiring by 2 months and 3 samples expired) were collected from different markets at El Menofia Governorate for detection of biogenic amines and preservatives in these samples, and transferred to the laboratory to be examined chemically.

### **1. Determination of biogenic amines by using HPLC**

Three biogenic amines including histamine, putrescine and tyramine were determined in all examined samples according to the protocol recommended by **Krause *et al.* (1995)** and **Pinho *et al.* (2001)** including

#### **1. Reagents preparation:**

#### **2. Extraction of samples:**

#### **3. Formation of dansylamines:** to be injected in HPLC.

#### **Apparatus HPLC conditions:**

High performance liquid chromatography (HPLC) used for dansylamines determination was an Agilent 1100 HPLC system, Agilen Technologies, Waldbronn, Germany, equipped with quaternary pump model G 1311A, UV detector (Model G 1314A) set at 254nm wavelength, auto sampler (model G1329A VP-ODS) and Shim pack (150× 4.6 mm) column (Shimadzu, Kyoto, Japan) was used for biogenic amines separation. Data were integrated and recorded using Chemstation Software program.

### Gradient solvent program for separation of biogenic amines:

Time /min.	Flow rate ml/min.	Solvent A%	Solvent B%	Solvent C%
0	1	60	20	20
10	1	20	40	40
15	1	15	35	50
20	1	60	20	20
25	1	60	20	20

A=0.02N acetic acid

B=Methanol

C= Acetonitrile

### 2. Determination of Sodium chloride % (AOAC, 2000):

### 3. Determination of sodium nitrites:

#### 3.1. Sample preparation:

The samples were treated according to the procedures recommended by **Cassens (1997)** and **Rashid (2003)**.

#### 3.2. Procedure: According to the method recommended by **Ferreira et al., (1999)**.

The reagent blank was prepared in the same way but in the absence of nitrite, and the absorbencies were measured at 427 nm. The absorbance readings obtained are directly related to the intensity of the color, which in turn is directly related to the concentration of nitrite present. The absorbencies of the standard solutions provide a direct measure of the amount of nitrite present in the known solutions. By comparing the absorbance of the meat solution to the absorbance of the known concentrations, the amount of nitrite in the meat solution may be estimated.

## Results and Discussion

The production of biogenic amines requires the availability of amino acids, the presence of microorganisms capable of decarboxylating amino acids, and favourable conditions for their growth and for the development of their decarboxylase activity (**Ordenez, et al., 1997**).

**Seyed et al. (2009)** mentioned that the presence of biogenic amines can cause several problems for susceptible consumers, such as nausea, respiratory disorders,

hot flushes, sweating, headache, bright red rash, oral burning, hypo or hypertension, whose intensity is depend on quantitative and qualitative differences. Fish is the most commonly implicated food item associated with histamine intoxication, besides histamine, tyramine has been implicated in adverse reactions involving headache and hypertensive crisis in patients taking MAOI (monoamine oxidase inhibitors).

**Egyptian Organization for Standardization and Quality Control (EOS, 2005)** had laid down permissible limit for histamine in canned tuna which is 10mg/100g.

The results recorded in table (1) showed that the concentration of histamine in the examined canned beef samples ranged from 0.3 to 2.2 mg/100g, with an average of  $1.17 \pm 0.31$  mg/100g in shortly after production samples. While it ranged from 0.8 to 4.5 mg/100g, with an average of  $2.62 \pm 0.43$  mg/100g in shortly before expiry date. In the expired samples, the results ranged from 1.5 to 6.7 with an average of  $3.93 \pm 0.53$ .

The histamine level can be minimized by appropriate food safety and quality management systems (**FAO/WHO, 2012**).

The obtained results showed that the concentration of putrescine in the examined canned beef samples ranged from 1.0 to 20.9 mg/100g, with an average of  $9.31 \pm 2.18$  in samples shortly after production date , ranged from 3.5 to 23.6 mg/100g, with an average of  $12.46 \pm 2.72$  mg/100g in samples shortly before expiry date. In the expired samples the results ranged from 4.8 to 29.0 with an average of  $16.41 \pm 2.75$  mg/100g. These results less than that recorded by **Min et al., (2007)** who recorded the changes of BAs contents in beef loin during 15 days of storage. As storage time increased, putrescine, and tyramine concentration increased significantly ( $p < 0.05$ ), especially, putrescine level increased from 0.97 to 202.54  $\mu$ g/g during this period.

It is also evident from the results recorded in table (1) that the tyramine concentration in samples shortly after production ranged from 0.7 to 6.4 mg/100g, with an average of  $3.05 \pm 0.92$  mg/100g, ranged from 2.0 to 8.1 mg/100g, with an average of  $4.71 \pm 1.15$  mg/100g in samples shortly before expiry date. In the expired samples the results ranged from 2.2 to 10.4 with an average of  $5.47 \pm 1.33$  mg/100g. These results lower than that recorded by **Min et al.,(2007)** that tyramine ranged from  $3.1 \pm 0.33$  to  $17.4 \pm 2.43$  in beef lion samples during 15 days of storage period.

The results recorded in table (1) revealed also that the average of the concentration of sodium chloride is  $2.49 \pm 0.32$ ,  $2.08 \pm 0.26$  and  $1.63 \pm 0.14$  in shortly after production, shortly before expiring and expired samples respectively. But for nitrite the average of the concentration is  $31.14 \pm 2.06$ ,  $39.55 \pm 3.80$  and  $31.94 \pm 1.86$  in shortly after production, shortly before expiring and expired samples respectively.

The results recorded in table (2) illustrated that the concentration of histamine in the examined canned tuna samples ranged from 7.1 to 44.8 mg/100g, with an average of  $19.73 \pm 2.25$  mg/100g in samples shortly after production . Also ranged from 8.5 to 93.7 mg/100g, with an average of  $51.94 \pm 6.28$  mg/100g in samples shortly before expiring. In the expired samples the results ranged from 26.9 to 97.4 with an average of  $58.74 \pm 6.90$ . The number of unaccepted samples was 10 represented as 50% in shortly after production and shortly before expiring date. All expired tuna samples un accepted as showed in table (2). The obtained results were more than to the results recorded by **Shaltout *et al.*, (2015)** who examined one hundred random samples of local and imported canned Tuna and Mackerel fish (25 of each), the mean value of histamine level was  $22.86 \pm 0.72$  mg/kg. But lower than that recorded by **Lonberg *et al.* (1980)**, they estimated histamine in 16 tuna fish products (49 batches: 245 cans) form 6 countries. The content was more than 100 mg/kg for 4% of cans and 80% of samples had 50 mg/g or less. Cans from Malaysia, Thailand and Taiwan had most histamine, which can be attributed to poor refrigeration before canning, contents above 50 mg/kg inevitably lead to scombroid poisoning with symptoms as skin reddening and severe headache.

It is evident from the results of putrescine in canned tuna recorded in table (2) that the concentration ranged from 3.3 to 25.9 mg/100g, with an average of  $10.14 \pm 1.87$  mg/100g in samples shortly after production. The results ranged from 10.1 to 42.5 mg/100g, with an average of  $27.51 \pm 4.13$  mg/100g in samples shortly before expiring. In expired samples the results ranged from 11.6 to 50.2 mg/100g with an average of  $31.43 \pm 4.07$  mg/100g. Putrescine is an organic chemical compound with bad odor that produced with cadaverine from breakage of amino acids in living or dead organisms. Putrescine is the cause of bad odor due to putrefaction and decay of amines. Putrescine is very toxic; it can change to spermdine by decarboxylation of S-adenozylmethionin (**Abouzar Fathi *et al.*, 2014**). The mean value of putrescine was lower than that recorded by **Shaltout *et al.*, (2015)** who recorded the mean value of putrescine in canned tuna as  $2.237 \pm 0.119$ .

Furthermore, the concentration of tyramine in the shortly after production samples ranged from 1.0 to 5.3 mg/100g, with an average of  $2.67 \pm 0.92$  mg/100g in, ranged from 2.7 to 14.0 mg/100g, with an average of  $6.93 \pm 1.45$  in samples shortly before expiring. In expired samples, the results ranged from 2.9 to 16.3 with an average of  $9.36 \pm 1.12$  mg/100g (Table 2). **Lapa-Guimarães and Pickova (2004)** determined histamine and tyramine in fish by thin-layer chromatography (TLC) and found that the detected limits was 10 mg for these amines.

The results recorded in table (2) showed that the averages of the concentration of sodium chloride were  $1.58 \pm 0.39$ ,  $1.27 \pm 0.22$  and  $1.06 \pm 0.08$  in shortly after production, shortly before expiring and expired canned tuna samples respectively.

The averages of nitrite as preservative in canned tuna are  $27.35 \pm 2.06$ ,  $34.82 \pm 2.57$  and  $22.87 \pm 2.57$  in shortly after production, shortly before expiring and expired canned tuna samples, respectively. **Gabriel et al. (2006)** recorded that the highest specific formation rate of histamine and putrescine were observed at the NaCl concentration 3.0 % and 0.5 % respectively. The sodium nitrite activates tyrosine decarboxylase activity (**Maijala, 1993**).

Some studies have reported minimum toxic levels for some Biogenic Amines as mentioned by **Wöhrl et al. (2004)** reported that 75 mg of pure liquid oral histamine - a dose common in normal meals - can provoke immediate as well as delayed symptoms in 50% of healthy females with no history of food intolerance. A concentration of over 125 mg/ kg of tyramine is considered to be toxic in normal individuals, almost 100 times the concentration considered potentially toxic when ingested in combination with mono amine oxidases inhibitors (MAOIs) (**McCabe-Sellers 19860**). Threshold values of 100 mg kg for tyramine and 30 mg kg for phenylethylamine have been suggested by (**Brink, et al., 1990**).

Acceptability of the examined samples of canned tuna based on their levels of biogenic amines and preservatives is shown in table (3). Accurately, 40%, 60% and 100% canned tuna shortly after production, before and after expiring were unfit for human consumption where they exceeded the safe permissible limit of histamine (10 mg/100g) stipulated by **EOS (2005)**.

Table (4) indicated the presence of an inverse relationship between the concentrations of histamine and putrescine in the examined samples either canned tuna or beef from one side and their levels of sodium chloride and nitrites from the other side. In other words, both sodium chloride and nitrites had a significant effect in reducing histamine and putrescine in such products. In contrast, these preservatives had minor influence on the concentration of tyramine either in canned tuna or canned beef.

The current results could be attributed to the action of these chemical preservatives on certain spoilage bacteria which responsible for production of such harmful biogenic amines (**Fiigen et al., 2001 and Gabriel et al., 2006**).

In general, the biogenic amines are biologically active compounds synthesized from amino acids. Food borne biogenic amines are most commonly synthesized by spoilage microorganisms and are usually considered to be potential toxins. A sub group of the biogenic amines are the mammalian polyamines: putrescine, spermidine and spermine. Biogenic amines should not always be considered as potential toxicants, but can also be considered to be non-hormonal growth promotants (**Codex Alimentarius, 2001**).

Vasoactive amines that include histamine may be present in food in quantities capable of producing clinically apparent pharmacologic effects. The prototype of these amines is histamine, the mediator of immediate allergic reactions triggered by foods and other allergens. Endogenous histamine is released from basophiles and mast cells. Endogenous histamine can also be released by intrinsic histamine-releasing food components. In addition to the release of endogenous histamine during allergic reactions, certain foods contain histamine produced by decarboxylation of histidine by enzymes produced by bacterial contamination of foods (**Munoz *et al.*, 2008**).

The obtained results allowed concluding that most of canned products exposed for consumption were contaminated with various chemical residues such as biogenic amines residues (histamine, putrescine and tyramine). The high level of histamine in some investigated samples is probably related to bacterial decarboxylase activity due to quality of raw material, mishandling during their shelf- life. The level of these amines can be minimized by appropriate food safety and quality management systems as application of Hazard Analysis and Critical Control Point (HACCP) system in all points of fish and beef products manufacturing to ensure a maximum safety to consumers. Also, setting maximum limits for these biogenic amines rather than histamine in the Egyptian standards is very important to avoid their risks.

**Table (1): Statistical analytical results of biogenic amines and chemical preservatives in the examined samples of canned beef.**

Canned Beef Parameters	Shortly after production (A) (n=10)			Shortly before expiring (B) (n=10)			Shortly after expiring (C) (n=3)		
	Min	Max	Mean ± S.E	Min	Max	Mean ± S.E	Min	Max	Mean ± S.E
<b><u>Biogenic amines:</u></b>									
<b>Histamine (mg %)</b>	<b>0.3</b>	<b>2.2</b>	<b>1.17± 0.31</b>	<b>0.8</b>	<b>4.9</b>	<b>2.62± 0.43</b>	<b>1.5</b>	<b>6.7</b>	<b>3.93± 0.53<sup>++</sup></b>
<b>Putrescine (mg %)</b>	<b>1.0</b>	<b>20.9</b>	<b>9.31± 2.18</b>	<b>3.5</b>	<b>23.6</b>	<b>12.46± 2.72</b>	<b>4.8</b>	<b>29.0</b>	<b>16.41± 2.75<sup>++</sup></b>
<b>Tyramine (mg %)</b>	<b>0.7</b>	<b>6.4</b>	<b>3.05± 0.92</b>	<b>2.0</b>	<b>8.1</b>	<b>4.71± 1.15</b>	<b>2.2</b>	<b>10.4</b>	<b>5.47± 1.33<sup>++</sup></b>
<b><u>Preservatives:</u></b>									
<b>Na Cl (%)</b>	<b>2.1</b>	<b>2.9</b>	<b>2.49± 0.32<sup>+</sup></b>	<b>1.6</b>	<b>2.3</b>	<b>2.08± 0.26</b>	<b>1.4</b>	<b>1.9</b>	<b>1.63± 0.14<sup>+</sup></b>
<b>Nitrites (ppm)</b>	<b>18.7</b>	<b>49.8</b>	<b>31.14± 2.06</b>	<b>25.2</b>	<b>51.9</b>	<b>39.55± 3.80</b>	<b>22.7</b>	<b>42.2</b>	<b>31.94± 1.86<sup>+</sup></b>

<sup>++</sup> High significant differences (P<0.01) by ANOVA test.

<sup>+</sup> Significant differences (P<0.05).

**Table (2): Statistical analytical results of biogenic amines and chemical preservatives in the examined samples of canned tuna.**

Canned Tuna Parameters	Shortly after production (A) (n=10)			Shortly before expiring (B) (n=10)			Shortly after expiring (C) (n=3)		
	Min	Max	Mean ± S.E	Min	Max	Mean ± S.E	Min	Max	Mean ± S.E
<b><u>Biogenic amines:</u></b>									
<b>Histamine (mg %)</b>	7.1	44.8	19.73± 2.25	8.5	93.7	51.94± 6.28	26.9	97.4	58.74± 6.90 <sup>++</sup>
<b>Putrescine (mg %)</b>	3.3	25.9	10.14± 1.87	10.1	42.5	27.51± 4.13	11.6	50.2	31.43± 4.07 <sup>++</sup>
<b>Tyramine (mg %)</b>	1.0	5.3	2.67± 0.92	2.7	14.0	6.93± 1.45	2.9	16.3	9.36± 1.12 <sup>++</sup>
<b><u>Preservatives:</u></b>									
<b>Na Cl (%)</b>	1.2	1.8	1.58± 0.39 <sup>+</sup>	1.1	1.5	1.27± 0.22	0.9	1.3	1.06± 0.08 <sup>+</sup>
<b>Nitrites (ppm)</b>	16.3	42.4	27.35± 2.06	17.5	53.1	34.82± 2.57	13.5	37.9	22.87± 2.57 <sup>+</sup>

<sup>++</sup> High significant differences (P<0.01) by ANOVA test.

<sup>+</sup> Significant differences (P<0.05).

**Table (3): Acceptability of the examined samples of canned tuna based on their levels of biogenic amines and preservatives.**

Parameters	MRL (mg %)*	Unaccepted samples (A) (n=10)		Unaccepted samples (B) (n=10)		Unaccepted samples (C) (n=3)	
		No.	%	No.	%	No.	%
Histamine	10	4	40	6	60	3	100
Putrescine	-----	0	0	0	0	0	0
Tyramine	-----	0	0	0	0	0	0
Na Cl	-----	0	0	0	0	0	0
Nitrites	-----	0	0	0	0	0	0

\*Egyptian Organization for Standardization "EOS" (2005), 804/2005 for canned tuna.  
 The permissible limit of histamine is 10mg/100 g.

**Table (4): Correlation coefficient (r) between chemical preservatives Vs biogenic amine levels in the examined samples of canned beef and tuna.**

Parameters	Canned tuna		Canned beef	
	A	B	A	B
<b>NaCl %:</b>				
<b>1.Histamine</b>	-0.39*	-0.61**	-0.35*	-0.52*
<b>2. Putrescine</b>	-0.28	-0.44*	-0.40*	-0.57**
<b>3.Tyramine</b>	-0.21	-0.26	-0.14	-0.23
<b>Nitrite content:</b>				
<b>1.Histamine</b>	-0.55**	-0.71**	-0.42*	-0.49*
<b>2. Putrescine</b>	-0.36*	-0.51*	-0.25	-0.38*
<b>3.Tyramine</b>	-0.27	-0.33*	-0.11	-0.29

\* Significant inverse correlation

\*\* High significant inverse correlation

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