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Biogenic Amines as Serious Residues in Some Meat Products Marketed in Qalyubia, Egypt.

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ABSTRACT

Biogenic amines are undesirable components resulting from the proteolysis and decarboxylation of FAAs by undesirable microorganisms contaminating the foods making them of great concern in food hygiene and public health. Practical applications were conducted to evaluate the levels of the certain four biogenic amines (histamine, tyramine, putrescine, and cadaverine) using HPLC; in different meat products by collecting ninety frozen samples of beef burgers, kofta, and sausage (30 of each) from various supermarkets in Qalyubia Governorate. The achieved results obtained that the average values of histamine, tyramine, putrescine and cadaverine, (mg/Kg) were 11.05 ± 0.62, 5.81 ± 0.45, 3.95 ± 0.29 and 2.43 ± 0.18 for beef burgers; 14.49 ± 0.87, 9.36 ± 0.73, 6.21 ± 0.37 and 3.91 ± 0.26 for kofta; and 19.71 ± 1.10, 12.97 ± 0.89, 7.88 ± 0.51 and 7.47 ± 0.50, respectively for sausage samples. According to the permissible limits recommended by EOS (2010), only 10% of beef burgers, 13.33% of kofta, and 20% of sausage samples were unaccepted based on their HIS and TYR levels, while only one (1.11%) sample of the examined sausage was unaccepted and exceeding the permissible limits of cadaverine.

1. INTRODUCTION

Meat products are important components of daily meals which have a role in filling the gap of protein deficiency and can be considered the best choice in solving human nutritional problems because of their taste, low-cost nutritional value. FAAs and positive decarboxylase microorganisms and suitable environmental conditions are considered the main factors of biogenic amines formation in the foods (Stadnik and Dolatowski, 2010). Thus, BAs become one of the concerns about food hygiene and are targeted by FDA and EFSA because of their toxicological effect on human health when intake in excessive amount inducing adverse reactions especially in sensitive people and in those with genetically impaired detoxification system, gastric intestinal diseases or due to taking of MAOIs and DAOIs that used as an antidepressant, antihistamines, antimalarial agents, psycho-pharmaceutics (Ruiz-Capillas and Herrero, 2019), also there are more than 1300 million smokers worldwide that consuming alcohol and smoking tobacco which proved to be part of the important factors causing potentiation to the toxicological consequences of biogenic amines poisoning because of their ability able to reduce MAO levels by 40% together with other components of cigarette (Broadley, 2010).

Histamine toxicological effect leads to allergic reactions that characterized by headaches and dizziness, excessive sweating, nasal discharge, skin rashes and itching, edema, urticaria, difficulty in breathing and inability to swallow, vomiting and diarrhea, bronchial spasm, tachycardia, and blood pressure disorders (Mor *et al*., 2005). On the other hand, tyramine causes the releasing of catecholamine from CNS and adrenal medulla which causes migraine, neurological manifestation, vomiting and nausea, respiratory manifestation, increased blood pressure and heartbeats that lead to hypertensive crisis, and brain disorders like schizophrenia, Parkinson's disease, and Reyes' syndrome which are more dangerous consequences (Ladero *et al*., 2010). Regarding cadaverine and putrescine, they are not considered toxic biogenic amines, but they are responsible for the formation of carcinogenic N-nitrosamines in the presence of nitrate and nitrite salts which are mainly used as curing agents in meat products processing. They also interfere with the detoxifying mechanism which is responsible for metabolizing and detoxifying biogenic amines in the human body enhancing the toxicological action of histamine and tyramine (De Mey *et al*., 2014).

Accurately, considerable research has been occurred recently for the detection of biogenic amines and reduce their occurrence in foods, but unfortunately, there is lower attention has been paid to evaluating the

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biogenic amines concentrations in meat products that are more consumed all over the world, even these studies that have been done were generally depended on the fermented meat products neglecting the other types of products. Therefore, this research has been carried out to determine the levels of four biogenic amines in some frozen meat products samples of beef burger, kofta, and sausage (30 of each) were collected from different supermarkets under different levels in Qalyubia governorate.

2. MATERIAL AND METHODS

2.1. Sampling

Ninety samples of frozen meat products of beef burgers, kofta, and sausage (30 of each) were randomly collected from different supermarkets in Qalyubia Governorate. Samples were transferred as rapidly as possible to the laboratory for the measuring of four biogenic amines concentrations (histamine, tyramine, putrescine, and cadaverine) by HPLC according to the technique protocol recommended by Krause *et al*. (1995) and Pinho *et al*. (2001).

2.2. Samples extraction

In an alarm mixer, 25grams from each sample was mixed with 125 ml tri-chloro-acetic acid (5%) for three minutes, and then filtration was done using Whatman No. 1 filter paper. Then, 10 ml of the filtrated sample with 4-gram NaCl and 1 ml of NaOH (50 %) were moved to a suitable glass tube and were extracted three times (2 min of each time) with 5 ml of n-butanol: chloroform (1:1). The top clear supernatant was added to 15 ml n-heptane with 1.0 ml portions of 0.2 N-HCl in a separation funnel and made extraction three times and transferred the upper layer to a closed glass tube and put in a water bath at 95°C for evaporation till drying.

2.3. Formation of dansylamines

Accurately, from each extract or standard, 100 µl have been taken and transferred to 50 ml dried vial with adding 0.5 ml saturated $NaHCO_3$ and 1 ml of dansyl chloride then carefully closed the vial and shacked well using a vortex shaker to avoid spillage and spattering, after that the vials were incubated at 55°C/45 min. Further, adding 10 ml distilled water to the vial then closed well and vigorously shaken using a vortex shaker. Moreover, the dansylamines extraction was made using five ml diethyl-ether and then the top layers have been taken and carefully evaporated till dryness at 35°C. The dried contents were injected into HPLC after dissolving with adding 1.0 ml from Methanol. Finally, the achieved results were evaluated and calculated statistically using the T-Test based on Feldman *et al*. (2003) technique.

3. RESULTS

From the achieved results in the table (1), histamine concentrations were 53.33%, 63.33%, and 80% in beef burgers, kofta, and sausage examined samples which had the highest levels of histamine (average level was 19.71 ± 1.10 mg/Kg); followed by kofta samples with the mean value of 14.49 ± 0.87 mg/Kg; then beef burgers with the mean value of 11.05 ± 0.62 mg/Kg. It is also indicated that the 75.56% accepted meat product samples in histamine levels where 83.33% of beef burger, 76.67% of kofta, and 66.67% of sausage samples) were not exceeding the permissible limits of histamine concentration according to EOS (2010) which stipulated that the MRL mg/kg is 20 mg/Kg in meat product samples.

Table 1 Histamine levels and acceptability of the examined meat Products according to EOS (2010) (30 of each).

BAs	Products	+Ve samples		Min.	Max.	Mean ± S.E**	Acceptability *MRL= 20mg/kg			
							Accepted		unaccepted	
		No.	%				No.	%	No.	%
HIS	Beef burger	16	53.33	1.0	22.7	11.05 ± 0.62	25	83.33	5	16.67
	Kofta	19	63.33	1.3	26.9	14.49 ± 0.87	23	76.67	7	23.33
	Sausage	24	80	1.9	34.1	19.71 ± 1.10	20	66.67	10	33.33

Table 2 Tyramine levels and acceptability of the examined meat Products according to EOS (2010) (30 of each).

BAs	Products	+Ve samples		Min.	Max.	Mean ± S.E**	Acceptability *MRL= 20mg/kg			
							Accepted		unaccepted	
		No.	%				No.	%	No.	%
TYR	Beef burger	13	43.33	0.8	21.3	5.81 ± 0.45	27	90	3	10
	Kofta	17	56.67	1.1	23.6	9.36 ± 0.73	26	86.67	4	13.33
	Sausage	21	70	1.5	28.4	12.97 ± 0.89	24	80	6	20

Table 3 Putrescine levels and acceptability of the examined meat Products according to EOS (2010) (30 of each).

BAs	Products	+Ve samples		Min.	Max.	Mean ± S.E**	Acceptability *MRL= 20mg/kg			
							Accepted		unaccepted	
		No.	%				No.	%	No.	%
PUT	Beef burger	9	30	0.6	12.4	3.95 ± 0.29	30	100	0	0
	Kofta	12	40	1.0	20.9	6.21 ± 0.37	28	93.33	2	6.67
	Sausage	14	46.67	1.2	25.9	7.88 ± 0.51	26	86.67	4	13.33

Table 4 Cadaverine levels and acceptability of the examined meat Productsaccording EOS (2010) (30 of each).

BAs	Products	+Ve samples					Acceptability *MRL= 20mg/kg			
							Accepted		unaccepted	
		No.	%	Min.	Max.	Mean ± S.E**	No.	%	No.	%
CAD	Beef burger	8	26.67	0.5	7.2	2.43 ± 0.18	30	100	0	0
	Kofta	10	33.33	`1.0	9.5	3.91 ± 0.26	30	100	0	0
	Sausage	11	36.67	1.0	20.1	5.38 ± 0.40	29	96.67	1	3.33

From data recorded in table (2), it was obvious that the tyramine levels were 43.33%, 56.67%and 70%in beef burgers, kofta, and sausage, respectively. Furthermore, the examined sausage samples are containing the highest concentration of tyramine that the average level was 12.97 ± o.89 mg/Kg; followed by kofta samples (9.36±o.73 mg/Kg); then beef burger samples (5.81 ± o.45 mg/kg). Only14.4% of the examined meat product samples (10% of beef burger, 13.33% of kofta, and 20% of sausage samples) were unaccepted and exceeded the permissible limits of tyramine (20 mg/Kg) according to EOS, 2010.

Further results in table (3) showed the highest concentration recorded for putrescine (mg/Kg) was in the examined sausage (7.88 ± o.51 mg/Kg); followed by kofta (6.21 ± o.37mg/Kg), then beef burgers samples (3.95 ± o.29 mg/Kg). While putrescine prevalence was 30% in the examined beef burgers, 40% kofta, and 46.67% in sausage samples. The accepted samples were 93.3% of the examined meat product samples (100% of beef burgers, 93.33% of kofta, and 86.67% of sausage samples).

Moreover, the recorded results in table (4) revealed the prevalence of cadaverine levels that were 26.67%, 33.33%, and 36.67% in beef burgers, kofta, and sausage, respectively. The highest level of cadaverine was5.38 ± o.40 mg/Kg in sausage; followed by 3.91 ± o.26 mg/kg in kofta; then 2.43 ± o.18 mg/Kg in beef burgers. All samples of beef burger and kofta were accepted and it was only one sample (1.11%) of the sausage was unaccepted according to EOS (2010).

4. DISCUSSION

The most common biogenic amines that occurred in the examined meat product samples was histamine which formed from decarboxylation of histidine essential amino acid (Triki *et al*., 2018) that present in meat, followed by tyramine, putrescine, and cadaverine.

Accordingly, the collected frozen sausage samples were containing the highest levels of 4 biogenic amines (histamine, tyramine, putrescine, and cadaverine followed by kofta, then beef burgers. It may be related to the selection of bad quality raw materials or the use of starter cultures during the manufacturing (Roselino *et al*., 2020) of sausage which may affect biogenic amines contents in the final products.

Furthermore, the presence of other conditions that enhance the microbial load and decarboxylase activity during the processing such as the unfavorable temperatures during processing, the variation of the manufacturing process, pH, insufficient handling and hygienic conditions, additives, refrigeration, packaging, processing time, and the shelf life and storage conditions during the period stayed in stores which influenced the formation of biogenic amines (Algahtani *et al*., 2020).

According to obtained results of histamine recorded for the examined meat products, they relatively agree to some extent with those obtained by El-Shewey et al. (2003) and Hefny (2014) who recorded that the histamine levels in minced meat samples were 11.6 mg/Kg, and 12.8 mg/Kg, respectively. It was lower than Rekka (2002), Erkmen and Bozkurt (2004), Hassan (2004), El-Mossalami *et al.* (2011), Lu et al. (2010), EFSA (2011), Papavergou (2011), Abd El-Wahab (2016), Ekici and Omer (2018) and Morgan (2020) who recorded that the mean value of histamine was 73.6 ± 15.6 mg/Kg, while the lowest results of histamine levels were recorded Anastasio *et al.* (2010), Mokhtar *et al*. (2012), El-sheikh (2014) and Algahtani *et al*. (2020) who recorded that the mean value of histamine is 0.63±0.46 mg/Kg.

Regarding the documented results of tyramine for the examined meat products, they relatively agree to some extent with those recorded by Mokhtar *et al*. (2012) where the tyramine levels in sausage samples were ranged from 13.25 mg/Kg to 14.9 mg/Kg. Accordingly, the obtained levels of tyramine in the examined meat product samples were lower than Rekka (2002), Gençcelep *et al*. (2008), Anastasio *et al*. (2010), El-Mossalami *et al*. (2011), Lu *et al*. (2010), EFSA (2011), Papavergou (2011), Elsisy *et al.* (2016), and Ekici and Omer (2018) who recorded that the tyramine mean values were ranged from 53.50±17.65 to 273.91±33.8 mg/Kg; the lowest results of tyramine levels were recorded El-sheikh (2014) and Algahtani *et al*. (2020) who haven't detected tyramine in the examined sausage samples.

Concerning the results recorded in the examined meat product samples, it was nearly like that detected by Ekici and Omer (2018) which reported that putrescine was ranged from 1.008±1.65 to 24.602±5.71 mg/Kg in sausage. Accurately, lower results were recorded by Erkmen and Bozkurt (2004), Gençcelep *et al.* (2008), Anastasio *et al.* (2010), Lu *et al*. (2010), EFSA (2011), Papavergou (2011), and El-sheikh (2014) who recorded that the mean values of putrescine were 15.7± 14.73mg/Kg in sausage samples, but our putrescine levels were higher than recorded by Mokhtar *et al*.(2012) and Algahtani *et al.* (2020) which was 1.18± 0.36 mg/Kg in sausage samples.

Accurately, the achieved results of cadaverine recorded for the examined meat products were lower than Gençcelep *et al*. (2008), Anastasio *et al*. (2010), Lu *et al.* (2010), EFSA (2011), Papavero (2011), El-sheikh (2014), and Ekici and Omer (2018) reported the mean value of cadaverine was ranged from 23.8±4.98 to 681.2±113.6 mg/Kg. However, the lowest level of cadaverine was recorded by Mokhtar*et* *al.* (2012) and Algahtani *et al.* (2020) were 2.1 mg/Kg in sausage samples.

Hence, biogenic amines concentrations can be considered as chemical indicators for meat spoiling as high contents BAs significantly indicate low-quality raw materials, miss handling, and poor hygienic conditions during processing Ekici and Omer (2018a), and consuming food with high amounts of histamine, putrescine, cadaverine and/or tyramine can have toxic effects and become one of the most important food safety issues as in case of histamine poisoning which causes skin rashes, vomiting and fever, increased blood pressure and cardiac rates, migraine, diarrhea and vomiting, skin rashes and inflammation and may lead to death in severe toxic (Suzzi and Torriani, 2015) especially when associated with putrescine and cadaverine. Moreover, tyramine, cadaverine, and putrescine when exposed to high temperatures, formed the secondary amines which combined with nitrites, consequently forming nitrosamine that believed to be one of the main carcinogenic reasons Tofalo *et al.* (2016). Thus, consumers' safety needs to manufacture histamine free-food and therefore prevent histamine food poisoning Naila *et al*. (2015)

5. CONCLUSION

Accordingly, the sausage samples were containing the highest levels of biogenic amines which indicated low-quality raw materials, poor hygienic conditions during manufacturing. The consumption of such products may cause foodborne toxicity caused by histamine and/or tyramine particularly when they are associated with cadaverine and putrescine that enhance the toxic effect of tyramine and histamine causing serious threatening health hazards for consumers. Additionally, consumption of meat products containing putrescine and cadaverine is such a great problem as these biogenic amines are precursors for nitrosamines formation in combination with nitrate and nitrite salts which are already used during meat production as curing substances.

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