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THE EFFECT OF SOME TOXIC BIOGENIC AMINES ON THE QUALITY OF SOME MEAT PRODUCTS WITH A TRIAL TO REDUCE ITS LEVELS

(With 5 Tables and 4 Figures)

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تأثير بعض الأمينات الحيوية السامة على جودة بعض منتجات اللحوم ومحاولة تقليل مستواها

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تم تجميع عشرون عينة من منتجات اللحوم (١٠ عينات من كل من السجق الجاف واللانشون) من أماكن مختلفة بمدينة الأسكندرية لقياس مدى تواجد الأمينات الحيوية السامة وخاصة الهيستامين والتيرامين باستخدام جهاز الفصل الكروماتوجرافى (HPLC)، حيث أظهرت النتائج أن أعلى تركيز للهيستامين وجد فى عينات السجق الجاف تتراوح من ٢,٦٨ إلى ١٦,٤٧ مللجرام /١٠٠ جرام. وأقلها فى عينات اللانشون حيث تتراوح من لاشئ إلى ٣,٦٥ مللجرام /١٠٠ جرام. بينما تراوح تركيز التيرامين من لاشئ إلى ٣٢, ١٥ مللجرام /١٠٠ جرام فى عينات السجق الجاف ومن ١١,. إلى ٨٢,. فى عينات اللانشون. وقد أجريت تجربة لرفع جودة السجف المصنع معمليا من لحم مفروم مخلوط بالمواد اللازمة لتصنيع السجق ثم إضافة كل من القرنفل والقرفة كمواد طبيعية تعمل على خفض نسبة الأمينات الحيوية السامة على حدى بتركيزات مختلفة ٥,. , ١, ١,٥ % أثناء التصنيع ثم تخزين العينات بالثلاجة عند ٤° م لمدة شهر مع الفحص الدورى كل إسبوعين لقياس مستوى الأمينات الحيوية بها. وقد وجد أن أعلى نسبة إختزال للهيستامين هى ٦٠,٦٠ % , ٤٠,٢٥ % وجدت فى العينات المخزنة لمدة أربعة أسابيع بتركيز ١,٥ % من القرنفل والقرفة على التوالى. بينما أعلى نسبة إختزال للتيرامين هى ٣٧,٩٩ % , ٣٣,٢٥ % وجدت فى العينات المخزنة لمدة أربعة أسابيع بتركيز ١,٥ % من القرنفل والقرفة على التوالى. أى أنه كلما

زادت نسبة تركيز التوابل كلما كان تأثيرها أكبر فى خفض كمية الأمينات الحيوية السامة فى المنتج. وقد تمت مناقشة الأهمية الصحية للأمينات الحيوية وتأثيرها على الصحة العامة وكذلك الاقتراحات التى يجب وضعها فى الاعتبار لتحسين جودة اللحوم ومنتجاتها.

SUMMARY

Twenty samples of meat products (dry sausage and luncheon) ten samples of each were randomly collected from different localities of Alexandria city. Collected samples were subjected to biogenic amine examination. Histamine and tyramine were determined by HPLC. Histamine concentration was the higher in dry sausage (2.68 to 16.47 mg/100g) than luncheon not detected (ND) to 3.65 mg/100g. Tyramine was ranging between ND to 15.32 mg/100g in dry sausage and ranging between 0.11 to 0.82 mg/100g in luncheon. For improvement the quality of sausage manufactured by fresh minced meat that purchased from butcher and mixed with the ingredients were used in sausage mixture, the addition of Clove or Cinnamon at levels of 0.5, 1.0 and 1.5% during manufactured of fresh sausage is used to reduce the production of histamine and tyramine. All samples stored under refrigeration condition at 4°C and were periodically withdraw for analysis of biogenic amines at Zero, 2 and 4weeks, respectively. The highest reduction percentage of histamine (60.60% and 40.25%), were found in samples stored for 4 weeks with 1.5% clove and cinnamon, respectively. While the highest reduction percentage of tyramine (37.99% and 33.25%), were found in samples stored for 4 weeks with 1.5% clove and cinnamon, respectively. The public health significance of the biogenic amines as well as the suggested measures for improving the quality of produced products has been discussed.

Key words: *Biogenic amines, histamine, tyramine, meat products, sausage, luncheon.*

INTRODUCTION

Biogenic amines are basic nitrogenous compounds with low molecular weight formed mainly by decarboxylation of amino acids or by amination and transamination of aldehydes and ketones (Halász *et al.*, 1994; Silla-Santos, 1996; Zaman *et al.,* 2009). This compounds are ubiquitous and play an important role in human and animal physiological function (Jansen *et al.,* 2003; Suzzi and Gardini, 2003; Önal, 2007). Low level of biogenic amines in food is considered no significance health risk for consumption Bodmer *et al.,* 1999.

Based on the mode of action, biogenic amines can be differentiated into vasoactive and psychoactive amines (Sellers *et al.,* 2006). Psychoactive amines influence neural transmitters in the central neural system, while vasoactive amines act either directly or indirectly on the vascular system. Histamine, putrescine and cadaverine are psychoactive amines, while tyramine, tryptamine and phenylethylamine are vasoactive amines. Histamine exerts its toxic by interacting with receptors (H_1, H_2 and H_3) on cellular membranes which are found in the cardiovascular system and in various secretory glands (Shalaby, 1997; Sellers *et al.,* 2006). Histamine causes dilatation of peripheral blood vessels, capillaries and arteries, resulting in hypotension, urticaria, flushing and headache. Histamine also induced contraction of intestinal smooth muscle, mediated by H_1 receptor, causes abdominal cramp, diarrhea, nausea and vomiting. Moreover, stimulation of sensory and motor neuron by histamine causes pain and itching (Russell and Maretic, 1986; Lehane and Olley, 2000; Gonzaga *et al.,* 2009). The action of vasoactive presser tyramine and phenylethylamine can cause hypertensive crisis in individuals on monoamine oxidase inhibitor (MAOI) drugs therapy. The physiological effects of tyramine include peripheral vasoconstriction, increase the cardiac output, increases respiration, elevated blood sugar, releases noradrenaline and causes migraine (Shalaby, 1997; Premont *et al.,* 2001; Sellers *et al.,* 2006). Putrescine and cadaverine can cause hypotension and potentiate toxicity of other amines, particularly histamine (Bjeldanes *et al.,* 1978; Shalaby, 1997; Emborg *et al.,* 2006).

The toxicological effect of biogenic amines can only occur when they are ingested in excessive amounts or when the natural mechanisms for their catabolism are inhibited or genetically deficient. Human intestinal tract normally detoxifies these compounds in food by enzyme monoamine oxidase (MAO) and diamine oxidase (DAO). Detoxification efficiency varies considerably among individual and was affected by several factors. Dietary intake of some MAO inhibitors is the main factor suppressed detoxification of biogenic amines (Brink *et al.*, 1990; Til *et al.*, 1997). Unfortunately, MAO inhibitors are still remaining an important class of drugs for a variety of psychiatric conditions, including depressive illnesses, anxiety and eating disorders (Walker *et al.*, 1996; Kalac, 2009). It has also been reported that drugs used for antidepressants were nonselective irreversible of MAO, while drugs used for anti Parkinsonism were selective reversible inhibitior (Sellers *et al.*, 2006). Therefore, those who are taking this kind if medicinal therapy should avoid consume food products with high potential concentration of biogenic amines. Putrescine and cadaverine hamper the detoxification of histamine and tyramine, tryptamine inhibit DAO and phenylethylamine inhibit HMT (histamine N-methyltransferase) (Eitenmiller *et al.*, 1980; Stratton *et al.*, 1991; Silla-Santos, 1996; Mohan *et al.*, 2009).

Biogenic amines formation in food products is either a result of endogenous amino acids decarboxylase activity in raw food material or the growth of decarboxylase positive microorganisms under conditions favorable for enzyme activity. Therefore, prerequisites for biogenic amines formation in foods including the availability of free amino acids, the presence of decarboxylase active microorganism and environmental condition enabling microorganism growth and their enzymatic activity (Brink *et al.*, 1990; Halász *et al.*, 1994; Silla-Santos, 1996; Shalaby, 1997). Biogenic amines formation was also possibly altered by the presence of proteolytic enzymes as they play an important role in the release of free amino acids from protein tissues which offer the substrate for decarboxylation activity.

Meat and meat products have repetitively been reported to contain biogenic amines (Maihala *et al.*, 1995; Eerola *et al.*, 1997; Hernández-Jover *et al.*, 1997; Kaniou *et al.*, 2001; Bover-Cid *et al.*, 2006; Galgano *et al.*, 2009). The most prevalent biogenic amines in meat and meat products are tyramine, cadaverine, putrescine and also histamine (Ruiz-Capillas and Jiménez-Colmenero, 2004; Stadnik and Dolatowski, 2010).

Some amines such as tyramine, putrescine and cadaverine can be formed during storage of meat (Hernández-Jover *et al.*, 1997; Galgano *et al.*, 2009). As tyramine concentrations in stored beef was found the highest on the meat surface, that it can be reduced effectively by washing (Kaniou *et al.*, 2001; Paulsen *et al.*, 2006).

Fermented meat products, constitute one of the foods in which considerable amounts of biogenic amines can be found as a consequence of the use poor quality raw materials, contamination and inappropriate conditions during processing and storage. Additionally, the microorganisms responsible for the fermentation process may contribute to biogenic amines accumulation (Bover-Cid *et al.*, 2006; Latorre-Moratalla *et al.*, 2010). The nonprotein nitrogen fraction which increases during fermentation includes the presence of free amino acids, precursors of biogenic amines. The major protease activity is derived from endogenous meat enzymes. Proteolysis is favoured by the denaturation of proteins as a consequence of acidity increase, dehydration and action of sodium chloride (Eerola *et al.*, 1997; Suzzi and Gardini, 2003).

Many authors (Maijala *et al.*, 1995; Eerola *et al.*, 1997; Komprda *et al.*, 2004) observed significant differences in biogenic amines content in fermented meat products depending on raw material hygienic quality. However, the same material can lead to very different amine levels in final products depending on the presence of decarboxylating microorganisms, either derived from environmental contamination or from starter cultures and the conditions supporting their growth and activity. Amines content and profiles may vary depending on various extrinsic and intrinsic factors during the manufacturing process, such as pH, redox potential, temperature, NaCl, the size of the sausage, hygienic conditions of manufacturing practices and effect of starter cultures (Gardini *et al.*, 2001; Komprda *et al.*, 2004; Latorre-Moratalla *et al.*, 2008).

Naturally occurring specific inhibitory substances in spices and additives cause inhibition of biogenic amines formation (Komprda *et al.*, 2004). Substances are clove and cinnamon. They delay biogenic amine production in Myeolchi-jeot (Mah *et al.*, 2009). The effect of spices have specific effects on bacteria that produce biogenic amines (Wendakoon and Sakaguchi, 1992). Ethanol extracts of allspice, cloves and cinnamon found to delay biogenic amine formation by *Enterobacter aerogenes* (Naila *et al.*, 2010). The inhibitory effect was improved with the addition of sodium chloride (Gardini *et al.*, 2001; Suzzi and Gardini, 2003).

Cinnamic aldehyde, a component of cinnamon and eugenol a compound of cloves were found to be the most effective inhibitors of biogenic amine formation by specific bacteria, *E. aerogenes* (Zaika, 1988; Thoroski *et al.*, 1989; Wendakoon and Sakaguchi, 1995).
This study was planned to fulfill the following points:

1- Quantitative determination for histamine and tyramine in Luncheon and dry sausage sold in Alexandria markets using high performance liquid chromatography (HPLC).
2- Investigate the effect of clove and cinnamon added during preparation of sausage on the level of histamine and tyramine content during storage.

MATERIALS and METHODS

Collection of samples: 10 samples (500g each) from each of luncheon and dry sausage were collected from different localities of Alexandria City. Fresh minced meat (8kg) was purchased from the butcher and transported immediately in an ice box to the laboratory for manufactured of fresh sausage. Samples of meat products were prepared by mincing and 50 g was taken for extraction of biogenic amines.
Determination of biogenic amine content

Extraction of biogenic amines: biogenic amines were extracted by using the method previously described by (Lang *et al.*, 2002; Ónal, 2007). Ground samples (50g) were extracted with 5% trichloroacetic acid (TCA) by 3 x 75 ml using a warring blender. Each blended mixture was centrifuged and the clear extracts were combined. The volume was adjusted to 250 ml with trichloroacetic acid TCA (5%) solution. The equivalent of 2 g of samples as the TCA extract (10 ml was made alkaline by adding 1ml 50% sodium hydroxide) and then extracted with n-butanol/chloroform mixture (1:1 v/v) 3 x 5 ml. The combined organic phase after addition of an equal amount of n-heptane (15) was extracted with several portions of 0.02 n HCl (1ml each) and the aqueous extract was dried by using current of air and water bath at 30°C.

Derivative formation: (Paleologos *et al.*, 2004)

The dansyle derivatives of the biogenic amines were formed by adding saturated sodium bicarbonate solution (0.5ml) to the residue (dry film), stopper and carefully mixed using vortex mixture, then carefully adding 1ml dansyle chloride solution (500 mg in 100 ml acetone) and thoroughly mixed. After standing for more than 10 hours at room temperature, the dansyle-amines were extracted by adding 15 ml HPLC grade water and then the mixture was extracted with several portion (5ml each) of diethyl ether (HPLC grade). The combined ether extracts were evaporated to dryness by the aid of current of air and water bath at 35°C. The residue was dissolved in 1ml acetonitrile.

Preparation of standard solution:

Histamine: Aliquot 41.40 mg of histamine dihydrochloride (sigma chemical Co., N.7505) was dissolved in 50 ml water HPLC grade (stock solution 0.5mg/ml).

Tyramine: Aliqout 31.39 mg of tyramine (4-hydroxyphenylethylamine) hydrochloride (N, T. 2879) was dissolved in 50 ml water HPLC grade. (stock solution 0.5mg/ml). 200 µl of each stock standard solution was transferred to glass tube (using micropipette), then evaporated using current of air. The residue was subjected to dansylation as described above. The residue was dissolved in 5ml acetonitrile. (1ml = 20µg or 10µl= 0.2µg each amine as derivative).

Detection: (Takagi and Shikata, 2004). Shimadzue HPLC was used for the quantitative estimation of biogenic amines. The conditions used as follow:

Mobile solvent: Solvent A: acetonitrile: 0.02 N acetic acid (1:9 v/v).

Solvent B: 0.02 N acetic acid: acetonitrile: methanole (2:9:9 v/v/v).

Program: gradient program 60% solvent B in solvent A to 100% solvent B using linear program over 30 min period and 1ml constant flow rate.

Detector: UV-Vis at 254 nm.

Column: reversed phase C18 Shim Pack CLC. ODS. 0.15 m x 6.0.

Injection: 10 µl of standard solution (as derivative) or sample was injected into HPLC apparatus.

Effect of addition of natural ingredients during preparation of sausage on the decrease of histamine and tyramine content during storage at 4°C:

The following ingredients were used in the sausage mixture according to Ali, 1985: Fresh minced meat (8kg containing 20% fat), 3% sodium chloride, 0.5% black pepper, 0.25% cubeb, 0.25% red pepper, 1% parsley, 0.25% ascorbic and 10% ice flakes. The solid spices were prepared using an electric mill. Minced meat was mixed with other ingredients except ice. Then the ice was added the mixture was minced and divided into seven batches. The first was left as control. Three batches were mixed with 0.5%. 1.0% and 1.5% cinnamon, respectively. The last three batches were mixed with 0,5%, 1.0% and 1.5% cloves, respectively. All batches were subjected to stuffing into cleaned natural mutton casing and stored under refrigeration condition at 4°C for one month. Samples were periodically withdrawn for analysis for biogenic amines at zero, 2 and 4 weeks, respectively.

Statistical analysis: The obtained data was analyzed statistically according to Perrie and Waston (1999).

RESULTS

The obtained data are recorded in Tables, 1, 2, 3 , 4 , 5 & Fig.,1.

Table 1: Histamine and tyramine content (mg/100g) in some meat products.

Type of meat product	Histamine (mg/100g)		Tyramine (mg/100g)	
	Range	M ± SD	Range	M ± SD
Dry sausage	2.68 - 16.47	9.93 ± 5.01	ND- 15.32	5.15 ± 6.49
Luncheon	ND- 3.65	1.39 ±1.54	0.11 - 0.82	0.32 ± 0.25

Table 2: Influence of clove and cinnamon on histamine content (mg/100g) in fresh sausage stored at 4°C for 4 weeks.

Storage Time / Week	Control	Cloves						Cinnamon					
		0.5%		1.0%		1.5%		0.5%		1.0%		1.5%	
		M ± SD	R %	M ± SD	R %	M ± SD	R %	M ± SD	R %	M ± SD	R %	M ± SD	R %
0	1.44± 0.05	2.05 ± 0.09	-	1.40 ± 0.09	2.78	1.15 ± 0.06	20.14	1.31± 0.11	9.03	1.20 ± 0.07	16.67	1.33 ± 0.01	7.64
2	18.47 ± 0.30	15.44 ± 0.75	16.40	11.39 ± 0.39	38.33	8.67 ± 0.42	53.06	18.42 ± 1.32	0.27	12.95 ± 1.34	29.89	11.89 ± 0.30	35.63
4	28.20 ± 1.00	20.20 ± 0.68	28.37	15.57 ± 0.06	44.79	11.11 ± 0.31	60.60	27.06± 2.18	4.04	20.24± 0.83	28.23	16.85 ± 0.11	40.25
Mean	16.04 ± 11.74	12.55 ± 8.16	21.76	9.45± 6.31	41.08	6.98 ± 4.50	56.48	15.60 ± 11.42	2.74	11.46 ± 8.36	28.55	10.02 ± 6.86	37.53

M ± SD = Mean of Triplicate Samples ± Standard Deviation

Table 3: Influence of clove and cinnamon on tyramine content (mg/100g) in fresh sausage stored at 4°C for 4 weeks.

Storage Time / Week	Control	Cloves						Cinnamon					
		0.5%		1.0%		1.5%		0.5%		1.0%		1.5%	
		M ± SD	R %	M ± SD	R %	M ± SD	R %	M ± SD	R %	M ± SD	R %	M ± SD	R %
0	0.65 ± 0.07	0.80 ± 0.02	-	0.58 ± 0.08	10.77	0.39 ± 0.03	40.00	0.61± 0.02	6.15	1.06 ± 0.05	-	0.52 ± 0.05	20.00
2	12.22 ± 0.33	11.04 ± 0.76	9.66	6.70 ± 0.29	45.17	3.82 ± 0.68	68.74	9.62 ± 0.92	21.28	9.20 ± 0.81	24.71	7.09 ± 0.49	41.98
4	16.90 ± 1.47	13.29 ± 0.64	21.36	10.48 ± 0.06	37.99	4.26 ± 0.03	74.79	15.02± 0.96	11.12	14.31± 0.49	15.33	11.28 ± 0.81	33.25
Mean	9.92 ± 7.28	8.38 ± 5.79	15.52	5.92 ± 4.33	40.32	2.82 ± 1.86	71.57	8.42 ± 6.33	15.12	8.19 ± 5.81	17.44	6.30 ± 4.72	36.49

M ± SD = Mean of Triplicate Samples ± Standard Deviation

Table 4: Pair wise comparison of histamine content in sausage samples as a function of clove and cinnamon levels.

Additives		Mean Difference (I – J)	Standard Error	Signification
(I)	(J)			
Control	0.5% Cloves	3.4704*	0.343	0.0000
	1.0% Cloves	6.5833*	0.343	0.0000
	1.5% Cloves	9.0589*	0.343	0.0000
	0.5 Cinnamon	0.4389	0.343	0.9850
	1.0% Cinnamon	4.5744*	0.343	0.0000
	1.5% Cinnamon	6.0144*	0.343	0.0000

The mean difference is significant at 0.05 level

Table 5: Pair wise Comparison of Tyramine content in sausage samples as a function of Clove and Cinnamon levels.

Additives		Mean Difference (I – J)	Standard Error	Signification
(I)	(J)			
Control	0.5% Cloves	1.5444*	0.280	0.0000
	1.0% Cloves	4.0044*	0.280	0.0000
	1.5% Cloves	7.1011*	0.280	0.0000
	0.5 Cinnamon	1.5056*	0.280	0.0000
	1.0% Cinnamon	1.7333*	0.280	0.0000
	1.5% Cinnamon	3.6244*	0.280	0.0000

The mean difference is significant at 0.05 level

Fig. (1) : Influence of Clove on Histamine Content Of Fresh Sausage Stored at 4°C

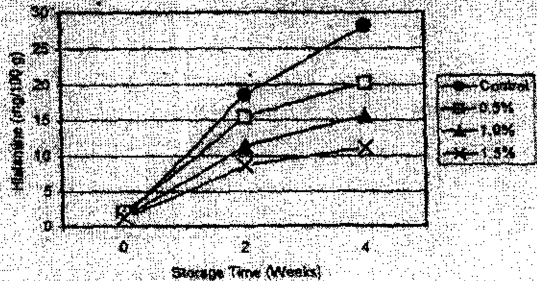


Fig. (3) : Influence of Clove on Tyramine Content of Fresh Sausage Stored at 4°C

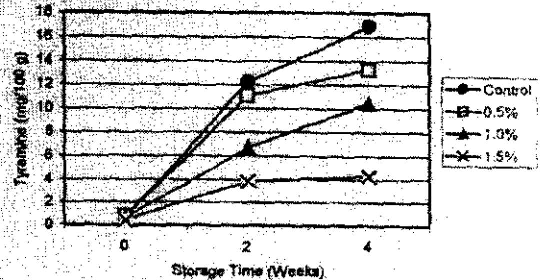


Fig. (2) : Influence of Cinnamon on Histamine Content Of Fresh Sausage Stored at 4°C

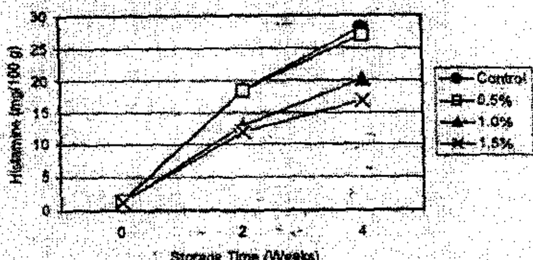
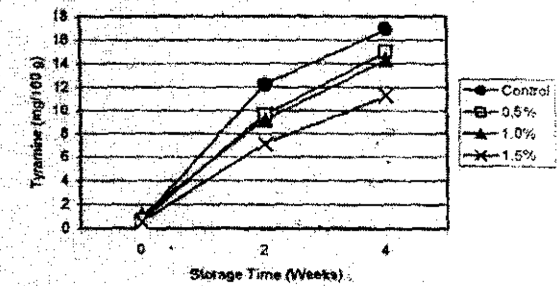


Fig. (4) : Influence of Cinnamon on Tyramine Content of Fresh Sausage Stored at 4°C



DISCUSSION

Histamine and tyramine content (mg/100g) in some meat products
Histamine content:

From data in Table 1 histamine concentration is higher in dry sausage than that in luncheon. With respect to tyramine a relatively high level is detected in dry sausage samples. However, in the other investigated samples, tyramine is very low. From the data of Table 1 also dry sausage contained the highest level of histamine and tyramine among the investigated meat products. This may be related to the long fermentation period by the action of natural micro-flora of such product (Naila *et al.,* 2010). During sausage ripening, histamine concentration increased Ten fold during the first three days of ripening, while the concentration of histidine was correspondingly decreased (Eerola *et al.,* 1997).

The detected histamine level in dry sausage ranged from 2.68 to 16.47 mg/100g (Table 1) which is higher than the level of (0.75 to 10.50 mg/100g), which was reported by Ali (1985) but lower than the level of 7.5 to 40.5mg/100g in dry sausage stated by Shalaby (1995). The detected histamine level in luncheon ranged from ND to 3.65 mg/100g, which is lower than the level of (9 to 23.4 mg/100g), which reported by Ali (1985).

Tyramine content:

Tyramine level detected in dry sausage is in the range of ND to15.32 mg/100g, which is higher compared with the level of 2.55 to 7.00 mg/100g, which was reported by Ali (1985) but lower than the level of 9.5 to 52.8 mg/100g in dry sausage determined by Shalaby and Rahman (1995). The detected tyramine level in luncheon ranged between 0.11 to 0.82, which is lower than the level of ND to 7.65 mg/100g that reported by Ali (1985).

The variation noticed in the level of biogenic amines may be related to the variation in ripening process, quality of raw materials Maihala *et al.,* 1995 and the natural of micro flora responsible for fermentation that may be variable and differ on their decarboxylase activity leading to variations in the production of biogenic amines (Suzzi and Gardini, 2003). Moreover, the concentration of nitrite and nitrate or other preservatives may be also responsible (Shalaby, 1997).

As for tyramine, none of the investigated samples reached to level 6mg, which was reported to be a dangerous dose for patients receiving MAOIs (Yongmei *et al.,* 2009). In sausage a level of 100mg histamine/100g sausage is considered the maximum level necessary to induce a toxic response in human (Gardini *et al.,* 2009; Stadnik; Dolatowski, 2010).

Influence of clove and cinnamon on histamine content of fresh sausage during storage at 4°C.

The results in Table 2 and Figs 1&2 revealed that expanding the storage time gradually increased histamine content in control samples was 1.44 ± 0.05 mg/100g increased gradually to 18.47 ± 0.3 mg/100g after 2 weeks and to 28.20 ± 1.00 mg/100g after 4 weeks. Addition of clove at various concentrations (0.5, 1.0 and 1.5%) resulted in a dose dependent reduction of histamine level reaching 20.20 ± 0.68, 15.57 ± 0.06 and 11.11 ± 0.31 mg/100g, respectively at the end of storage period. The highest reduction percentage of histamine (60.60%) was found in samples stored for 4 weeks with 1.5% cloves. Cinnamon also has a dose dependent reduction effect on the level of histamine. The highest effect obtained at 1.5% cinnamon the histamine content of the sausage was reduced by 40.25% after 4 weeks. The same effect of clove and cinnamon was detected by (Shakila *et al.,* 1996) who pointed out that Indian mackerel treated with clove and cinnamon at 3% and stored for up 24 hours at 30°C showed a significant inhibitory action of histamine, putrescine and tyramine. It was also reported that cloves and cinnamon were found to be the most affective spices against bacterial growth and biogenic amine formation than other studied (sage, black pepper, allspice, cumin, chill and nutmeg) (Wendakoon and Sakaguchi, 1992).

It may notice from the results that all the sausage samples contained histamine below 100 mg/100g. The limit that has been suggested to be the minimum level necessary to induce toxic response in humans. As well as the maximum mean level of detected histamine was 28.20 mg/100g, so consumption of 335 g of this sample could reach the toxic limit (100mg/100g).

Influence of clove and cinnamon on tyramine content of fresh sausage during storage at 4°C.

Results in Table 3 and Figs 3&4 showed that the tyramine content in control samples was 0.65 ± 0.07 mg/100g increased gradually during storage reaching 16.9 ± 1.47 mg/100g after 4 weeks. While in samples contained cloves at the investigated levels (0.5, 1.0 and 1.5%), there was a significant reduction in the tyramine content due to addition of cloves in a dose dependent manner. The highest level of cloves (1.5%) reduced the level of tyramine formed 74.79 % after 4 weeks of storage. The same sequence was observed due to addition of cinnamon. Since the concentration of tyramine was reduced by 11.12%, 15.33% and 33.25% after 4 weeks of storage at the three studied levels 0.5, 1.0 and 1.5%, respectively.

Concerning tyramine, the three studied levels of both spices showed a significant effect on the reduction of tyramine during storage period. Most samples contained tyramine level higher than the hazard limit 6mg with exception of sausage samples containing 1.5% cloves. It could be concluded that, although clove and cinnamon resulted in a significant reduction effect on histamine and tyramine in sausage samples, however tyramine still above the hazard level (6mg). There was no significant effect of cinnamon at 0.5% level on the concentration of histamine, while there was highly significant effect of cinnamon at 1.0 and 1.5% levels and cloves at the three studied levels on the reduction of histamine. It could be observed from the data of Tables 2 and 3 that the degree of effectiveness of clove was higher than of cinnamon on the reduction of both amines.

Results in Table 4 revealed that there is no significant effect of cinnamon at 0.5% level on the concentration of histamine, while there is highly significant effect of cinnamon at 1.0 and 1.5% levels and cloves at the three studied levels on the reduction of histamine. Concerning tyramine, the studied levels of both spices showed a significant effect on the reduction of tyramine during storage period (Table 5).

The results in Tables 4 and 5 showed that significant reduction in both histamine and tyramine levels due to addition of clove or cinnamon in fresh sausage stored at 4°C for 4 weeks. This could be attributed to the highly inhibitory effect of such spices to bacterial growth (Zaika, 1988).

The major antimicrobial compound in clove and cinnamon are known to be eugenol and cinnamic aldehyde that inhibit the extra-cellular enzymes of bacteria (Thoroski *et al.,* 1989) and may have an inhibitory action on the synthesis of decarboxylases, which might be a probable for the inhibition of amine production (Wendakoon and Sakaguchi, 1992).

Control

The existing method for controlling biogenic amines in food is refrigeration. However, since some bacteria that form biogenic amines can grow below 5°C, refrigeration alone is not always controlling biogenic amines and thus emerging control measures need to be considered (Niala *et al.,* 2010). Emerging control measures for delaying biogenic amine formation include high hydrostatic pressure (HHP), irradiation, packaging, microbial modeling and the use of food additives or preservatives. These methods only delay biogenic amines formation in food primarily through the inhibition of bacteria or the decarboxylase enzyme activity responsible for amine production. Application of sufficient heat or freezing storage can prevent further development of biogenic amines, although product needs to be protected from recontamination in the case of heat and from thawing in case of freezing (Stadnik and Dolatowski, 2010).

In Conclusion:

The choice of starter culture is fundamental to guarantee the quality of final products in relation to their biogenic amines content. For this reason, the inability to form biogenic amines, to survive during ripening and storage and to possess amine oxidase activity should be relevant criteria to be taken into consideration in the selection of starter cultures for the fermentation of dry sausages (Suzzi and Gardini, 2003; Karovičová and Kohajdová, 2005). The selection of lactic acid bacteria with application in meat fermentation has to take in consideration the various, specific requirements of the fermentation process (Roig-Sagués and Eerola, 1997). The importance of using measures focused on the hygienic quality of both raw material and processing units to avoid the development of aminogenic contaminant bacteria and in turn, to reduce biogenic amines content, is well known. However, proper hygiene may not be enough to avoid some biogenic amines formation and other technological measures must be applied (Latorre-Moratalla *et al.,* 2010).

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