



## Effect of Probiotics on Biogenic Amines levels in Alexandria Semidry Sausage during Refrigerated Storage

Nermeen, M.L. Malak, Gehan, M.A. Kassem, Mohamed, M.T. Emarra, Nabil, A. Yassien  
Food Hygiene and control Department, Faculty of Vet. Medicine, Cairo University, 12211, Giza, Egypt

### Abstract

Six groups of Alexandria semidry sausage were produced using different types of probiotics, Bifidobacteriumlactis Bb-12, L.casei 01, L.acidophilus M92, L.lactisMA16, Mixture of BifidobacteriumlactisBb-12 and L.acidophilusM92. Mixture of L.casei 01 and L.acidophilus M92. Produced sausage was ripened in fermentation chamber for 3 days, after that, fermentation was stopped by gradual elevation of temperature followed by cooking to core temperature 72°C then the product was kept under refrigerated storage at 4°C for three months. Product was examined periodically for sensory and biogenic amines examination. Results of sensory examination revealed that incorporation of different types of probiotics resulted in generally high sensory panel score for all sensory attributes. While refrigerated storage decreased chewiness, juiciness, odor and flavor attributes while increased cohesiveness and hardness. Using of Bifidobacteriumlactis resulted in more flavor and less acid while mixing with L.acidophilus revealed synergetic effect leading to improvement of acid flavour. Biogenic amines determination showed that incorporation mixture of L.casei and L.acidophilus caused significant reduction in 2-phenylethylamine, tryptamine, putresine, cadaverine and histamine levels during refrigerated storage while L.acidophilus, L.lactis and the mixture of Bifidobacteriumlactis and L.acidophilus resulted in a significant reduction in tyramine level. However, all types of probiotics resulted in slight increase in spermidine level throughout storage period.

**Key words:** Probiotics, Alexandria semidry sausage, biogenic amines, lactic acid bacteria.

### Introduction

Alexandria semidry sausage is considered one of the oldest traditional meat products in Egypt. It was introduced to Egypt by Greek people in the middle of 19<sup>th</sup> century. Production of Alexandria semidry sausage is an art transmitted from one generation to another and was based on using of back inoculum of fermentative bacteria. Nowadays production of such popular meat products in Egypt is facing many problems and subjected to different forms of adulteration and producing facilities which determine its quality as well as safety. Fermented sausage resulted from biochemical, microbiological, physical and sensorial changes occurring in a meat mixture during ripening under defined conditions of temperature and relative humidity. During sausage fermentation, complex biochemical and physical reactions take place that result in a significant change in the initial characteristics (Casaburi et al., 2007) which may be accompanied with undesirable characteristics such as production of biogenic amines and mold growth. Probiotics are living, health-promoting microbes that have a beneficial effect on human health when taken in adequate amount such as improvement of intestinal transit and digestion, improvement of symptoms of lactose intolerance, increase in immune response, reduce of diarrhea episodes, prevent of colon cancer and lower of blood cholesterol (Tharumaraj and Shah, 2003). Concerning probiotics side effects, they are a

minor and seen only in diseased or immunocompromised patients (Marteau, 2002; Reid, 2005 and Gueimonde et al., 2006). They can be used as starter culture for production of fermented sausage production (Chuayana et al., 2003). Meat starter cultures are preparations which contain living or resting microorganisms that develop desired metabolic activity in the meat. They are facultatively heterofermentative strains, which produce lactic acid from hexoses, such as glucose and lactose (Hammes, 1996). Lactic acid bacteria represent the most important group of starter organisms used in fermented sausage. They adapted well to the meat fermentation environment and changes which occur during ripening process. They reduce the pH, having a preservative effect and facilitating the drying process, development of the curing color, and cohesion of dry sausages. Moreover lactic acid bacteria are able to produce amino acid decarboxylase that prevents the accumulation of biogenic amines in the product (Bover-Cid et al., 2001a). Fermented sausages are suitable for the incorporation of probiotic bacteria as they need mild or no heat treatment so it provides suitable conditions required for the survival of probiotics (Khan et al., 2011), but at the same time can potentially support the accumulation of biogenic amines due to intrinsic and extrinsic factors. High amounts of proteolytic activity during ripening which provide the precursors for decarboxylase activity of starter cultures and wild microflora is

one of the major intrinsic factors (Suzzi and Gardini, 2003), While using poor quality raw materials or environmental contamination and inappropriate conditions during processing and storage is constituted the extrinsic factors (Latorre-Moratalla et al., 2010a). Lactobacillus, Bifidobacterium genera and some yeast have been considered safe for human consumption and used as probiotic (Reid, 2005). Biogenic amines are organic bases with aliphatic, aromatic or heterocyclic structures that can be found in several foods which produced by microbial decarboxylation of amino acids (Leroy,

#### Materials and methods

##### Preparation of sausage

Alexandria semidry sausage was manufactured at Food Hygiene and Control Department, Faculty of Veterinary Medicine, Cairo University. The sausage formulation included (850 g/kg beef chuck meat, 130 g/kg beef fat, 16 g/kg salt, 1.6 g/kg monosodium glutamate, 0.002 g/kg Nitrite, 0.5 g/kg ascorbic acid, 0.7 g/kg lactose, 3 g/kg sucrose, liquid smoke 1 g/kg, quantum sufficient of spices including ginger, nutmeg, coriander and clove powdered extracts). Imported deep frozen beef chuck was purchased from a local market within the first time of its shelf life. Beef fat was purchased from El Bassatine slaughter house after carcass preparation and kept frozen until use. Beef chuck and fat was firstly flaked and then minced at 5 mm using Fama (Fabbrica Attrezzature Macchine Alimentar, Rimini, Italy). All ingredients were mixed in mixer for few minutes. After that the meat mix divided into six batches (10 kg each) and 1 g of appropriate starter culture (dissolved in 250 mg full cream milk) added to the following distribution:

Group (A) Bifidobacterium lactis Bb-12 (CHR Hansen Denmark),

Group (B) L.casei 01 (CHR Hansen Denmark),

Group (C) L.acidophilus M92 (Danisco Russia),

Group (D) L.lactis MA16 (Danisco Russia),

Group (E) Mixture of Bifidobacterium lactis Bb-12 CHR Hansen Denmark and L.acidophilus M92 (Danisco).

Group (F) Mixture of L.casei 01 CHR Hansen Denmark and L.acidophilus M92 Danisco Russia.

Subsequently, the sausages mixture was automatically stuffed into a small diameter cellulose (30 mm) casing (~400 g each) and placed in a fermentation chamber at 20°C and 65-70% relative humidity for 3 days. After the end of fermentation period, sausage was transferred to cooking chamber at 50 °C for 1 hour, 60 °C for 1

2006). Consumption of biogenic amines can be of health concern (Gueimonde et al., 2006). They may cause toxic effects especially in consumers with amino oxidase deficiency (Arihara, 2006).

The reasons for the determinations of biogenic amines in fermented sausages are their potential toxicity and the possibility of using them as food quality indicators (Onal, 2007). Therefore, the aim of the present work was to investigate the impact of different types of probiotics in Alexandria semidry sausage on biogenic amines accumulation levels and sensory characteristics during refrigerated storage.

hour, 70°C for 1 hour at 80°C to 72°C core temperature. Fermented sausage was stored at 4°C for further analysis. At each sampling period (days 1, 30, 60, 90) three sausages samples from each batch were taken for sensory analysis and determination of biogenic amines. The remainder of the sampled sausages was stored at 4°C for further analysis. Each experiment was repeated three times.

##### 1- Sensory evaluation (Massimiliano et al., 2009)

Sensory analysis was performed by a panelists team (9) from the staff members of Food Hygiene and Control, Faculty of Veterinary Medicine, Cairo University. Prior to the analysis, panelists were subjected to training session on how to evaluate the sensory attributes of fermented sausage. Reference standards which used in sensory examination include a list of 15 attributes with definitions was used by the panel: 3 for appearance, 2 for texture by fingers, 2 for texture by mouth, 4 for odor and 4 for flavor. Each attribute was rated on a scale from one (absence of perception) to four (very intense perception). The sausage slices (0.5 cm thick three samples per section) served at room temperature on white plastic dishes. Water and unsalted crackers were provided to cleanse the palate between samples.

##### 2- Determination of biogenic amines: Biogenic amines were extracted according to (Eerola, 1996): Sample extraction:

Two grams of fermented sausage were extracted with 20 ml of 0.4 M perchloric acid solution (Merck, 519). Sample extracts (1 ml) were derivatised with addition of 200 µl 2 N sodium hydroxide solution (EKA, 040980), 300 µl saturated sodium bicarbonate (Merck, 6346) and 2 ml of dansyl chloride solution (10 mg dansyl chloride (Serva) in 1 ml acetone). The reaction mixture was incubated at 40°C for 45 min. After incubation the residual dansyl chloride was removed by addition of 100 µl ammonia (Merck,

5432). After 30 min the sample was adjusted to 5 ml with acetonitrile (Rathburn, HPLC-grade), centrifuged and filtered with 0.45 µm filter (Millipore, SJHVL04NS).

**Liquid chromatographic separation:** was performed with HP 1090 liquid chromatography using a Spherisorb ODS2 column (125 × 4 mm, 5 µm) and gradient elution program with a mixture of 0.1 M ammonium acetate (Merck, 1116) and acetonitrile. The flow rate was 1.0 ml/min and the column effluent was monitored at 254 nm.

### Results And Discussion

#### Sensory examination:

There are many factors affecting sensory characteristics of fermented sausage such as raw meat materials, types of starter cultures used for fermentation and processing technologies (Toldra, 2002). Sensory examination of fermented sausage revealed that there was no significant effect ( $P < 0.05$ ) of using different type of probiotics on (color non uniformity, fat/lean demarcation and fat/lean ratio) immediately after preparation. While refrigerated storage significantly increased ( $P < 0.05$ ) color non uniformity regardless to types of probiotics used. Texture examination by finger showed that using of *L.lactis* resulted in a significant increase ( $P < 0.05$ ) in cohesiveness degree at 60 day. Refrigerated storage resulted in significant increase ( $P < 0.05$ ) in cohesiveness and hardness degree and significant decrease in chewiness and juiciness which may be due to loss of moisture content during the storage time. Also there were no significant effect ( $P < 0.05$ ) of different probiotics on cohesiveness (deformation degree before breaking the slice), hardness (Strength required to break a slice with fingers), chewiness and juiciness except while refrigerated storage lead to significant increase ( $P < 0.05$ ) in cohesiveness and hardness degree and significant decrease ( $P < 0.05$ ) in chewiness and juiciness (Figure 1). These results agree with those obtained by Ruiz et al. (2014) and Ruiz et al. (2010) who found that fermented sausage treated with *Bifidobacteriumlactis* had texture score lower than fermented sausage treated with *L.lactis* which result in lowering overall acceptability by panalists. As fermented sausage treated with *Bifidobacteriumlactis* had water activity lower than fermented sausage treated with *L. acidophilus*, leading to dehydration and toughness of the product Ruiz et al. (2014).

Sensory panel scores for odor showed that storage period had negative effect on the sausage where spices, sour and sweet odor were significantly

#### Statistical analysis

Each analysis was run in three replicates, and collected data were analyzed using SPSS statistics 17.0 for windows. Results were recorded as mean  $\pm$  SE. Analysis of variance was performed by ANOVA procedure to compare results among the different trials and different cooking temperatures by the least significant (LSD) and significance was defined at  $P < 0.05$ .

decreased ( $P < 0.05$ ), while rancid odor was significantly increased ( $P < 0.05$ ) by storage. There were no significant effect ( $P < 0.05$ ) on different probiotics on spices, sour, rancid and sweet odor (Figure 2). Results of flavor attributes indicate that *L. acidophilus* and mixture of *L.casei* and *L.acidophilus* resulted in a significant improvement ( $P < 0.05$ ) in acid flavor when compared with other types of probiotics. Storage resulted in a significant increase ( $P < 0.05$ ) in salt and mold flavor and decrease in sweet flavor. Using *L. casei*, mixture of *Bifido.lactis* and *L.acidophilus* and mixture of *L.casei* and *L.acidophilus* resulted in high acid flavor remained till 90 day (Figure 2). These results may be due to different effect of probiotics on fermented sausage as the ability of microorganisms to degrade amino acids to aroma compounds is highly strain dependent which depend on enzymatic and chemical changes including flavor generation as carbohydrate fermentation, lipolylysis, proteolysis, lipid oxidation and amino acid catabolism (Lucke, 2000; Toldra et al., 2001 and Talon et al., 2002). Furthermore, these results may be referred that lactic acid production by *Bifidobacteriumlactis* is lower than other lactic acid producing bacteria which can affect the sensory quality of fermented sausage (Meile et al., 1997).

#### Determination of biogenic amines levels:

Biogenic amines formation in fermented sausages are affected by several factors, such as ingredients and additives (sugar, curing agents and spices), diameter of sausage and technological ripening conditions (temperature and relative humidity). It can also influenced by the aminogenesis, including microbial growth, acidification, proteolysis, and activity of decarboxylases (Latorre-Moratalla et al., 2012).

There was a significant reduction ( $P < 0.05$ ) in 2-phenylethylamine level during storage period using different types of starter cultures. Using a mixture of *L.casei* and *L. acidophilus* resulted in the highest significant reduction ( $P < 0.05$ ) in its

level followed by *L. acidophilus*, *L.lactis* and mixture of *Bifidobacteriumlactis* & *L. acidophilus* (Table 1). These results agree with results detected by (Shalaby, 1993) and (Suzzi and Gardini, 2003) who found that the level of 2-phenylethylamine (1.5-81 mg/kg) and (0-25.2 mg/kg) respectively. There were no sample of experimentally produced fermented sausage exceeded the toxicity level of 30 mg/kg suggested for 2-phenylethylamine (Gardini et al., 2001). Various species of Enterococci, Gram positive Cocci and *Lactobacillus* resulted in phenylalanine decarboxylation leading to formation of 2-phenylethylamine in fermented sausages (Bover-Cid et al., 2001a and Ansorena et al., 2002). It also can be associated with the presence of high amount of tyramine in the product because the microorganisms which had strong tyrosine decarboxylase activity also have moderate capacity to decarboxylate phenylalanine (Joosten, 1987). Mixture of *L.casei* and *L.acidophilus* lead to the highest significant reduction ( $P<0.05$ ) in tyramine level at the first day (0.18 mg/kg). Moreover all types of starter cultures cause a significant reduction in its level to reach undetectable limit. These results obtained to the fact that addition of starter culture or combination of more than one starter culture lead to competition between decarboxylating microorganisms with subsequent reduction in biogenic amine content. Mixture of *Lactobacillus casei* and *Lactobacillus acidophilus* resulted in a significant reduction ( $P<0.05$ ) in putresine level. Moreover all types of starter cultures cause a significant reduction ( $P<0.05$ ) in its level at 30 and 90 day of refrigerated storage (Table 1). These results agreed with Papavergou et al. (2012) who found that concentrations of putresine ranging from 0 to 505 mg/kg (median: 96.5 mg/kg). However, Ayhan et al. (1999) found high putresine concentrations (159.4 mg/kg) in spontaneously fermented sausages produced in Italy. The same author found high levels of putresine (more than 400 mg/kg) in turkish soudjoucks sausage which were produced without starter culture. Putresine formation is mainly attributed to the microbial decarboxylation of ornithine. It may be also be produced from the decarboxylation of arginine and subsequent deamination of the intermediately produced agmatine (Bover-Cid et al., 2001b). Significant amounts of putresine are produced by various Enterobacteriaceae strains (Durlu-öz kaya et al., 2001) which may be present in early steps of sausage production. Some *Lactobacillus*

species (Suzzi and Gardini, 2003) and faecal Enterococci (Ansorena et al., 2002) have also the potential to produce putresine in fermented sausages. All types of starter cultures resulted in a significant reduction ( $P<0.05$ ) in cadaverine level at 1 day of production followed by another significant reduction ( $P<0.05$ ) in its level at 60 and 90 day of storage. Moreover mixture *L. casei* and *Lactobacillus acidophilus* cause a significant reduction ( $P<0.05$ ) in cadaverine level throughout storage period (Table 1). This results may be due to addition of sodium nitrite which was able to reduce putrescine and cadaverine accumulation (Cantoni et al., 1994). Mixture of *Lactobacillus casei* and *Lactobacillus acidophilus* cause a significant reduction ( $P<0.05$ ) in histamine level at the first day in contrast with that of raw meat (0.466 mg/kg) used. Moreover, all types of starter culture caused a significant reduction during storage (Table 1). These results were agreed with results obtained by (Papavergou, 2012) who found that histamine concentration in fermented sausage ranged from 0 to 514 mg/kg. Histamine formation in meat products may occur through the activity of specific histidine decarboxylase positive microorganisms belonging mainly to the Enterobacteriaceae family (Roig-Sagués et al., 1996). Some *Lactobacillus* and *Pseudomonas* species (Roig-Sagués et al., 1996) and some species belonging to the genera *Micrococcus* and *Staphylococcus* (Suzzi and Gardini, 2003) have also been identified as histamine producers. Considerable accumulation of histamine in fermented sausages can be resulted from extended ripening times, high initial microbial load of raw materials due to non-appropriate storage, inadequate decrease of pH at the beginning of ripening period (Suzzi and Gardini, 2003 and Miguélez-Arrizado et al., 2006). However (Cantoni et al., 1994) observed that addition of sodium nitrite in Italian dry sausages was able to increase level of histamine concentration to three levels.

Table (1) showed that *L.acidophilus*, *L.lactis* and the mixture of *Bifidobacteriumlactis* and *L.acidophilus* resulted in a significant reduction ( $P<0.05$ ) in tyramine content. Low tyramine level may be due to using of bacterial starter cultures with limited tyrosine decarboxylating activity (Ansorena et al., 2002 and Komprda et al., 2009). Tyramine formation during fermented sausage production is mainly linked to the tyrosine decarboxylating activity of various fermenting or contaminant lactic acid bacteria

including several *Lactobacillus* and *Enterococcus* strains which may be present in them (Bover-Cid and Holzapfel, 1999; Bover-Cid et al., 2001a and Guerrini et al., 2007). Latorre-Moratalla et al. (2010b) found that *L. acidophilus* showed the highest tyramine content among fermented products

Results in (Table 1) showed that all types of starter culture resulted in a slight increase in spermidine level. These results agreed with results

**Conclusion**

Incorporation of some types of probiotics generally improved sensory attributes of fermented sausage. While *L. acidophilus*, *L. casei* and mixture of *L. casei* and *L. acidophilus* and *Bifidobacterium. L. lactis* and *L. acidophilus* were the highest acid flavor score through storage period. The results of biogenic

amines analysis revealed that using different types of probiotics in significant reduction in biogenic amines content throughout refrigerated storage period where of mixture of *Lactobacillus casei* and *Lactobacillus acidophilus* cause a significant reduction in 2-phenylethylamine, tryptamine, putresine, cadaverine and histamine levels during refrigerated storage.

amine analysis revealed that using different types of probiotics in significant reduction in biogenic amines content throughout refrigerated storage period where of mixture of *Lactobacillus casei* and *Lactobacillus acidophilus* cause a significant reduction in 2-phenylethylamine, tryptamine, putresine, cadaverine and histamine levels during refrigerated storage.

Figure (1): Effect of different probiotics on appearance and texture of Alexandrian semidry sausage during refrigerated storage at 4°C

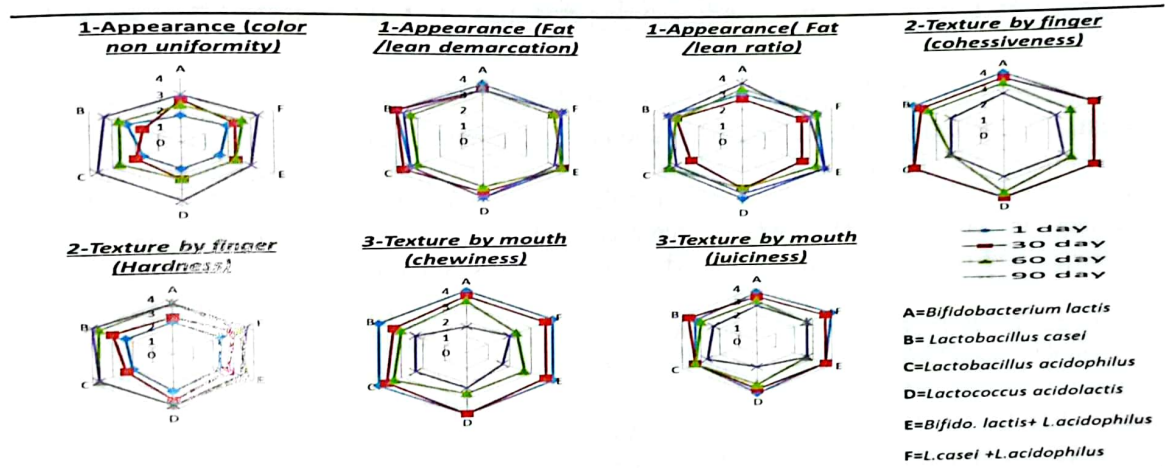
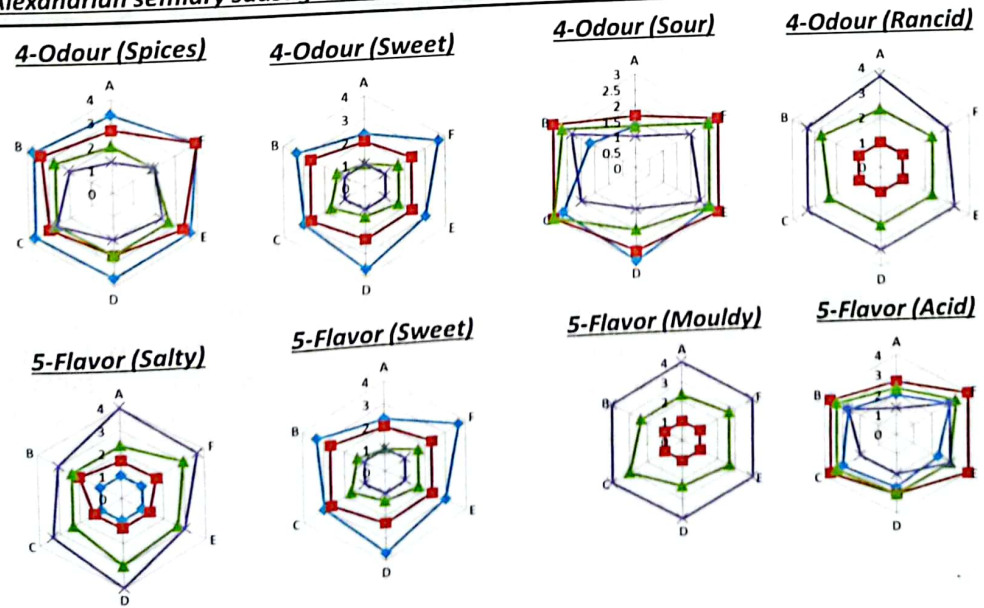


Figure (2): Effect of different probiotics on odor and flavor of experimentally produced Alexandrian semidry sausage during storage at 4°C:



**Table (1) Effect of different probiotics on biogenic amines content of experimentally produced fermented sausage during storage at 4°C:**

	2-phenylethylamine (mg/kg)				tryptamine (mg/kg)				Putresine (mg/kg)				Cadaverine (mg/kg)			
	1 day	30 day	60 day	90 day	1 day	30 day	60 day	90 day	1 day	30 day	60 day	90 day	1 day	30 day	60 day	90 day
A	2.97 <sup>a</sup>	5.61 <sup>a</sup>	0.11 <sup>a</sup>	0.16 <sup>a</sup>	2.67 <sup>a</sup>	2.03 <sup>a</sup>	ND	ND	0.59 <sup>a</sup>	0.88 <sup>ab</sup>	0.37 <sup>ab</sup>	0.33 <sup>a</sup>	0.05 <sup>a</sup>	0.60 <sup>a</sup>	0.24 <sup>a</sup>	0.13 <sup>a</sup>
B	4.55 <sup>b</sup>	5.51 <sup>a</sup>	0.10 <sup>a</sup>	0.16 <sup>a</sup>	1.85 <sup>a</sup>	1.86 <sup>a</sup>	ND	ND	0.53 <sup>a</sup>	0.64 <sup>bc</sup>	0.21 <sup>a</sup>	0.34 <sup>a</sup>	0.06 <sup>a</sup>	0.46 <sup>ab</sup>	0.09 <sup>b</sup>	0.26 <sup>b</sup>
C	5.36 <sup>b</sup>	4.25 <sup>ab</sup>	0.13 <sup>a</sup>	0.04 <sup>b</sup>	2.19 <sup>a</sup>	1.71 <sup>a</sup>	ND	ND	0.53 <sup>a</sup>	0.85 <sup>ab</sup>	0.29 <sup>ab</sup>	0.14 <sup>b</sup>	ND	0.41 <sup>b</sup>	0.19 <sup>a</sup>	0.06 <sup>a</sup>
D	5.05 <sup>b</sup>	3.50 <sup>b</sup>	0.11 <sup>a</sup>	0.08 <sup>ab</sup>	2.31 <sup>a</sup>	2.82 <sup>a</sup>	ND	ND	0.62 <sup>a</sup>	0.92 <sup>a</sup>	0.32 <sup>ab</sup>	0.29 <sup>ab</sup>	0.12 <sup>b</sup>	0.11 <sup>c</sup>	0.06 <sup>b</sup>	0.05 <sup>c</sup>
E	5.44 <sup>b</sup>	3.65 <sup>b</sup>	0.17 <sup>a</sup>	0.05 <sup>b</sup>	2.42 <sup>a</sup>	1.21 <sup>a</sup>	ND	ND	0.56 <sup>a</sup>	0.73 <sup>b</sup>	0.35 <sup>ab</sup>	0.33 <sup>a</sup>	0.49 <sup>c</sup>	0.17 <sup>a</sup>	0.10 <sup>b</sup>	0.07 <sup>a</sup>
F	0.25 <sup>c</sup>	1.71 <sup>c</sup>	0.27 <sup>b</sup>	0.03 <sup>b</sup>	0.18 <sup>b</sup>	2.40 <sup>a</sup>	ND	ND	0.25 <sup>b</sup>	0.52 <sup>c</sup>	0.43 <sup>b</sup>	0.18 <sup>ab</sup>	0.05 <sup>a</sup>	0.12 <sup>c</sup>	0.17 <sup>a</sup>	0.04 <sup>c</sup>

	Histamine (mg/kg)				Tyramine (mg/kg)				Spermidine (mg/kg)				Spermine (mg/kg)			
	1 day	30 day	60 day	90 day	1 day	30 day	60 day	90 day	1 day	30 day	60 day	90 day	1 day	30 day	60 day	90 day
A	0.49 <sup>ab</sup>	0.63 <sup>a</sup>	0.2 <sup>a</sup>	0.23 <sup>a</sup>	0.06 <sup>a</sup>	0.58 <sup>a</sup>	0.13 <sup>a</sup>	0.25 <sup>a</sup>	0.23 <sup>a</sup>	0.17 <sup>a</sup>	0.19 <sup>a</sup>	0.4 <sup>a</sup>	1.64 <sup>a</sup>	1.25 <sup>a</sup>	0.57 <sup>a</sup>	0.94 <sup>ab</sup>
B	0.38 <sup>bc</sup>	0.72 <sup>a</sup>	0.21 <sup>a</sup>	0.29 <sup>a</sup>	ND	0.13 <sup>b</sup>	0.08 <sup>a</sup>	0.14 <sup>ab</sup>	ND	0.05 <sup>a</sup>	0.21 <sup>a</sup>	0.2 <sup>b</sup>	1.72 <sup>a</sup>	1.59 <sup>a</sup>	0.76 <sup>bc</sup>	1.41 <sup>a</sup>
C	0.54 <sup>ab</sup>	0.55 <sup>b</sup>	0.2 <sup>a</sup>	0.15 <sup>a</sup>	ND	0.30 <sup>b</sup>	0.12 <sup>a</sup>	0.08 <sup>b</sup>	0.12 <sup>b</sup>	0.10 <sup>a</sup>	0.20 <sup>a</sup>	0.2 <sup>b</sup>	1.10 <sup>a</sup>	1.21 <sup>a</sup>	0.87 <sup>bc</sup>	0.28 <sup>bc</sup>
D	0.63 <sup>b</sup>	0.67 <sup>a</sup>	0.3 <sup>a</sup>	0.06 <sup>b</sup>	0.15 <sup>b</sup>	0.28 <sup>bd</sup>	0.24 <sup>ab</sup>	0.03 <sup>b</sup>	0.16 <sup>b</sup>	0.13 <sup>a</sup>	0.22 <sup>a</sup>	0.2 <sup>b</sup>	1.15 <sup>a</sup>	1.17 <sup>a</sup>	1.41 <sup>bc</sup>	0.13 <sup>c</sup>
E	0.47 <sup>ab</sup>	0.20 <sup>c</sup>	0.2 <sup>a</sup>	0.17 <sup>a</sup>	0.13 <sup>b</sup>	0.79 <sup>c</sup>	0.33 <sup>b</sup>	0.05 <sup>b</sup>	0.16 <sup>b</sup>	0.15 <sup>a</sup>	0.20 <sup>a</sup>	0.2 <sup>b</sup>	0.76 <sup>a</sup>	2.29 <sup>a</sup>	1.98 <sup>b</sup>	0.30 <sup>b</sup>
F	0.32 <sup>c</sup>	0.07 <sup>c</sup>	0.37 <sup>a</sup>	0.15 <sup>a</sup>	0.05 <sup>a</sup>	0.37 <sup>d</sup>	0.13 <sup>a</sup>	0.35 <sup>c</sup>	0.17 <sup>a</sup>	0.21 <sup>a</sup>	0.16 <sup>a</sup>	0.19 <sup>b</sup>	1.71 <sup>a</sup>	1.67 <sup>a</sup>	2.74 <sup>b</sup>	0.22 <sup>bc</sup>

**References**

Ansorena, D.; Montel, M.C.; Rokka, M.; Talon, R.; Enrola, S.; Rizzo, A.; Raemaekers, M. and Demeyer, D. (2002): Analysis of biogenic amines in northern and southern European sausages and role of flora in amine production. *Meat Sci.*, 61: 141-147.

Arihara, K. (2006): Strategies for designing novel functional meat products. *Meat Sci.*, 74: 219-229.

Ayhan, K.; Kolsarici, N. and Ozkan, G.A. (1999): The effects of a starter culture on the formation of biogenic amines in Turkish soudjoucks. *Int J Food Microbiol.*, 53: 183-188.

Bover-Cid, S. and Holzapfel W. (1999): Improved screening procedure for biogenic amine production by lactic acid bacteria. *Int J Food Microbiol.*, 53: 33-41.

Bover-Cid, S.; Hugas, M.; Izquierdo-Pulido, M. and Vidal-Carou, M.C. (2001a): Amino acid-decarboxylase activity of bacteria isolated from fermented pork sausages. *Int J Food Microbiol.*, 66: 185-189.

Bover-Cid, S.; Izquierdo-Pulido, M. and Vidal-Carou, M. C. (2001b): Changes in biogenic amine and polyamine contents in slightly fermented sausages manufactured with and without sugar. *Meat Sci.*, 57: 215-221.

Cantoni, C.; Bersani, C.; Damenis, L. and Comi, G. (1994): Ammine biogenenegli insaccaticrudistagio nati. *Industrie Alimentari.*, 23: 1239-1243.

Casaburi, A.; Aristoy, M.; Cavella, S.; Di Monaco, S.; Ercolini, D.; Toldra, F. and Villani, F. (2007): Biochemical and sensory characteristics of traditional fermented sausages of Vallo di Diano (Southern Italy) as affected by the use of starter cultures. *Meat Sci.*, 76: 295-307.

Chuayana, J.E.L.; Ponce, C.V.; Rivera, C.V. and Cabrera E.C. (2003): Antimicrobial activity of probiotics from milk products. *Phil J. Microbiol. Infect. Dis.*, 32: 71-74.

Durlu-özkaya, F.; Ayhan, K. and Vural, N. (2001): Biogenic amines produced by Enterobacteriaceae isolated from meat products. *Meat Sci.*, 58: 163-166.

Eerola, S.; Maijala, R.; Sagues, A.X.R.; Salminen, M. and Hirvi T. (1996): Biogenic amines in dry sausages as affected by starter culture and contaminant amine positive Lactobacillus. *J. Food Sci.*, 61: 1243-1246.

Gardini, F.; Martuscelli, M.; Caruso, M.C.; Galgano, F.; Crudele, M.A.; Favati, F.; Guerzoni, M.E. and Suzzi, G. (2001): Effects of pH, temperature and NaCl concentration on the

- growth kinetics, proteolytic activity and biogenic amine production of *Enterococcus faecalis*. *Int. J. Food Microbiol.*, 64, 105-117.
- Guelmonde, M.; Frias, R. and Ouwehand, A. C. (2006): Assuring the continued safety of lactic acid bacteria used as probiotics. *Biologia*, 61: 755-760.
- Guerrini, S.; Mangani, S.; Franci, O. and Vincenzini, M. (2007): Biogenic amine producing capability of bacterial populations isolated during processing of different types of dry fermented sausages. *Ital J Anim Sci.*, 6: 688-690.
- Hammes, W. (1996): Qualitätsmerkmale von Starterkulturen., In: Buckenhußkes, H., (Ed.), 2. Stuttgarter Rohwurstforum. Gewürzmußler, Stuttgart, pp. 29-42.
- Joosten, H. M. L. J. (1987): Conditions allowing the formation of biogenic amines in cheese: 3. Factors influencing the amounts formed. *Neth. Milk Dairy J.*, 41: 329-357.
- Kalač, P. and Krausová, P. (2005): A review of dietary polyamines: Formation, implications for growth and health and occurrence in foods. *Food Chem.*, 90: 219-230.
- Khan, M.I.; Arshad, M.S.; Anjum, F.M.; Sameen, A.; Aneeq-ur-Rehman, Gill, W.T. (2011): Meat as a functional food with special reference to probiotic sausages. *Food Res Int.*, 44: 3125-3133.
- Komprda, T.; Sládková, P. and Dohnal V. (2009): Biogenic amine content in dry fermented sausages as influenced by a producer, spice mix, starter culture, sausage diameter and time of ripening. *Meat Sci.*, 83: 534-542.
- Latorre-Moratalla, M.L.; Bover-Cid, S.; Talon, R.; Garriga, M.; Zanardi, E.; Ianieri, A.; Fraqueza, M.J.; Elias, M.; Drosinos, E.H. and Vidal-Carou, M.C. (2010a): Strategies to reduce biogenic amine accumulation in traditional sausage manufacturing. *LWT-Food Sci. Technol.*, 43: 20-25.
- Latorre-Moratalla, M. L.; Bover-Cid, S.; Talon, R.; Garriga, M.; Aymerich, T.; Zanardi, E.; Ianieri, A.; Fraqueza, M. J.; Elias, M.; Drosinos, E. H.; Lauková, A. and Vidal-Carou, M. C. (2010b): Distribution of aminogenic activity among potential autochthonous starter cultures. *J. Food Prot.*, 73: 524-525.
- Latorre-Moratalla, M.L.; Bover-Cid, Sara.; Veciana-Nogués, M.T. and Vidal-Carou, M.C. (2012): Control of biogenic amines in fermented sausages: role of starter cultures. *Front in Microbiol.*, 3: 1-9.
- Leroy, F.; Verluyten, J. and Vuyst, L. (2006): Functional meat starter cultures for improved sausage fermentation. *Int J Food Microbiol.*, 106: 270-285.
- Lucke, F.K. (2000): Utilisation of microbes to process and preserve meat. *Meat Sci.*, 56: 105-115.
- Marteau, P. (2002): Probiotics in clinical conditions. *Clin Rev. Allergy Immunol.*, 22, 255-273.
- Massimiliano, S.; Manuela, D. and Mara L. S. (2009): Changes of physicochemical, microbiological, and textural properties during ripening of Italian low-acid sausages. Proteolysis, sensory and volatile profiles. *Meat Sci.*, 81: 77-85.
- Meile, L.; Ludwig, W.; Rueger, U.; Gut, C.; Kaufmann, P.; Dasen, G.; Wenger, S. and Teuber, M. (1997): *Bifidobacterium lactis* sp. nov.: a moderately oxygen tolerant species isolated from fermented milk. *Syst Appl Microbiol.*, 20: 57-64.
- Miguélez-Arrizado, M. J.; Bover-Cid, S.; Latorre-Moratalla, M. L. and Vidal-Carou, M. C. (2006): Biogenic amines in Spanish fermented sausages as a function of diameter and artisanal or industrial origin. *J Sci Food Agric.*, 86: 549-557.
- Onal, A. (2007): A review: current analytical methods for the determination of biogenic amines in foods. *Food Chem.*, 103: 1475-1486.
- Papavergou, E.J.; Savvaidis, I.N. and Ambrosiadis, I.A. (2012): Levels of biogenic amines in retail market fermented meat products. *Food Chem.*, 135: 2750-2755.
- Reid, G. (2005): The importance of guidelines in the development and application of probiotics. *Current Pharmaceutical Design*, 11: 11-16.
- Roig-Sagués, A.X.; Hernández-Herrero, M.; López-Sabater, E.I.; Rodríguez-Jerez, J.J. and Mora-Ventura, M.T. (1996): Histidine decarboxylase activity of bacteria isolated from raw and ripened Salsichon, a Spanish cured sausage. *J. Food Protec.*, 59: 516-520.
- Ruiz, J.N.; Villanueva, N.D.M. and Contreras-Castillo, C.J. (2010): Sensory evaluation of Italian salami with probiotic properties. International conference of food innovation October 25-29-2010 in Valencia Spain Research Institute of Food Engineering and Development Polytechnic University of Valencia.
- Ruiz, J. N., Villanueva, N. D. M., Favaro-Trindade, C. S., Contreras-Castillo, C. J., (2014): Physicochemical, microbiological and sensory assessments of Italian salami sausages with probiotic potential. *Sci. Agric.*, 71: 204-211.
- Shalaby, A.R. (1993): Survey on biogenic amines in Egyptian foods: sausages. *J. Sci. Food Agric.*, 62: 219-224.

- Suzzi, G. and Gardini F. (2003):**Biogenic amines in dry fermented sausages: a review. *Inter. J Food Microbiolo.*,88: 41-54.
- Talon, R.; Leroy-Setrin, S. and Fadda S. (2002):**Bacterial starters involved in the quality of fermented meat products. In *Research Advances in The Quality of Meat and Meat Products*, F. Toldra, editor. Research Signpost, Kerala, India.,pp 175-191.
- Tharmaraj, N. and Shah, N.P. (2003):**Selective enumeration of *Lactobacillus delbrueckii* ssp. *Bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Bifidobacteria*, *Lactobacillus casei*, *Lactobacillus rhamnosus*, and *Propionibacteria*. *J Dairy Sci.*, 86: 2288-2296.
- Toldra, F. (2002):**Dry-cured meat products Trumbull. ph.d. instituto de agroqufmica y tecnologiad e alimentos (csic). po box 73 46100 burjassot (valencia) spain. ct: food & nutrition press. 1-238.
- Toldra, F.; Sanz, Y. and Flores, M. (2001):**Meat fermentation technology. In *Meat Science and Applications*, Y.H. Hui, Shorthose, R., Young, O., Koochmarai, M. and R. Rogers, editor. Marcel Dekker, Inc, New York, USA., pp. 537-561.

### الملخص العربي

تأثير البروبيوتك على مستويات الأمينات الحيوية في السجق الإسكندراني شبه الجاف أثناء الحفظ بالتبريد

نرمين مكرم لويس- جيهان محمد عبد العزيز- محمد محمد طلعت عماره- نبيل عبد الجابر ياسين

قسم الرقابة الصحية على الأغذية- كلية الطب البيطري - جامعة القاهرة

تم تصنيع ست مجموعات من السجق الإسكندراني شبه الجاف باستخدام أنواع مختلفة من البروبيوتك وقد تم تخمير الأنواع المختلفة من السجق في حجرة تخمير لمدة ثلاث أيام وبعد ذلك تم إيقاف التخمير بواسطة الارتفاع المتدرج للحرارة ثم تم تسوية المنتج حتى درجة حرارة 72° درجة مئوية داخل المنتج وبعد ذلك تم حفظ المنتج في الثلجة عند درجة حرارة 4 درجة مئوية لمدة ثلاث شهور وقد تم فحص المنتج بصفه دوريه لكل من الخواص الحسيه و محتواها من الأمينات الحيوية ولقد أظهرت النتائج الفحص الحسى ان استخدام الأنواع المختلفة من البروبيوتك أدى إلى ارتفاع الخواص الحسيه بكاملها بينما أدى الحفظ في الثلجة إلى تقليل درجات المضغ و العصيرية والرائحة والنكهة وزيادة التماسك والصلابة و لقد أدى استخدام البيفيدوباكتريم لأكتس إلى تحسن أكثر للنكهة و درجة حموضة أقل بينما اظهر الخليط من البيفيدوباكتريم لأكتس و الاكتوباسيلس اسيدوفيلس إلى تأثير تعاضدي و الذي بدوره أدى إلى تحسن النكهة الحمضية. و لقد أظهرت نتائج تحديد الأمينات الحيوية ان استخدام خليط من الاكتوباسيلس كازي و الاكتوباسيلس اسيدوفيلس أدى إلى تناقص واضح في مستوى كل الأمينات الحيوية بينما أدى إضافة الاكتوباسيلس اسيدوفيلس و الاكتوباسيلس اسيدولاكتس و خليط من البيفيدوباكتريم لأكتس و و الاكتوباسيلس اسيدوفيلس إلى تناقص واضح في مستوى التيرامين. ولقد أدى إضافة كل أنواع البروبيوتك إلى زيادة طفيفة في مستوى الاسبرميدين أثناء الحفظ بالتبريد