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Effect of probiotics on microbiological and chemical quality attributes of Alexandria semidry sausage

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Abstract

Changes in microbiological and chemical quality of Alexandria semidry sausage were investigated during refrigerated storage where sausage mix was treated with differentsix groups 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 andL.acidophilus M92. Produced sausage was ripened at 20°C in fermentation chamber for 3 days, after that, fermentation was stopped by gradual elevation of temperature followed by cooking to 72°C core temperature then the product was kept under refrigerated storage at 4°C for three months. Product was examined during fermentation and storage periodto assay the changes in both microbiological and chemical quality parameters. Results of microbiological examination revealed that incorporation of different types of probiotics resulted in a significant increase in lactic acid bacteria count, decrease in total yeast and mould and lipolytic bacterial count during fermentationperiod, while, refrigerated storage resulted in decrease in lactic acid bacteria with an increase in yeast and mould count. However, Staphylococcus aureus, Enterobacteriacae and proteolytic bacteria were below the delectable limit. Chemical examination of fermented sausage indicated thatrefrigerated storage resulted in reduction in moisture content with subsequent increase in protein, fat and ash content. Probiotics resulted in a significant and gradual decrease in pH value during fermentationperiod and in the first month of storage followed by significant increase during storage. Bifidobacteriumlactis resulted in the highest pH while mixture of L.caseiand L.acidophilusresulted in the highest acidity at the end of fermentation period.

Key words: Probiotics, fermented sausage, chemical quality, lactic acid bacteria, microbiological quality, starter cultures.

Introduction

Fermentation not only offers a practical means of food preservation but also contributes to a variety of food products with unique characteristics. Fermented sausage is one of fermented meat products which comprised of coarse mixtures of lean meats and fatty tissues combined with salts, nitrite, sugars and spices,stuffed into permeable casing and subjected to fermentation under defined conditions of temperature and relative humidity resulted in reduction of moisture content and water activity which necessary to build-up the typical flavor and texture of the final product**(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 **(Bover-Cid et al., 2001)**. Lactic acid bacteria have a positive effect on the hygienic properties of the fermented sausage by inhibiting pathogenic and spoilage flora either by acidification or by the production of antimicrobials. They also play an important role in the development of texture, colour and flavor of fermented products **(Villani et al., 1994).** During sausage fermentation, there are many biochemical and physical reactions take place resulted in a significant changes in the initial characteristics of the product such as changes in the initial microflora, reduction of nitrates to nitrites, solubilization and gelification of myofibrillar and sarcoplasmic proteins, proteolytic, lipolytic and oxidative phenomena, and dehydration **(Casaburi et al., 2007)**. 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 **(Tharmaraj and Shah, 2003)**. However they have minor side effects only in diseased or immunocompromised patients **(Marteau, 2002; Gueimonde et al., 2006 andVankerckhoven et al., 2008)**. There are many factors affecting the quality and durability of semi dry sausages such as type of starter culture, composition, ground degree of meat and fat, thermal processing, vacuum packaging, temperature and relative humidity during the storage **(Chen et al., 2007)**.Therefore, the aim of the present work was to investigate the impact of treatment of Alexandria semidry sausage withdifferent types of probiotic starter cultures on microbiological and chemical quality which occur during refrigerated storage.

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 (FabbricaAttrezzatureMacchineAlimentrac, 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) Bifidobacteriumlactis Bb-12 (CHR Hansen Denmark),

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

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

Group (D)L.lactisMA16 (Danisco Russia),

Group (E) Mixture of BifidobacteriumlactisBb-12 CHR Hansen Denmark and L.acidophilusM92 (Danisco).

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

Subsequently, the sausages mixture was automatically stuffed into a small diameter (30 mm) cellulose casing (~400 g each) and placed in a fermentation chamber at 20°C and 65-70% relative humidity for 3 days, three samples were taken from each group and examined at each day of fermentation period for microbiological and chemical analysis. After that, sausage was transferred to cooking chamber at 50°C for 1 hour, 60°C for 1 hour, 70°C for 1 hour at 80°C to 72°C core temperature. Fermented sausage was stored at 4°C for further analysis. Then examined at(30, 60, 90 days) of storage period for the previous investigations. Each experiment was repeated three times.

Microbiological Examinations: Sausages samples from each batch were collected aseptically, transferred to sterile plastic pouches, homogenized for 90 seconds with sterile quarter-strength Ringer's Solution (Oxoid BR 52) using a Stomacher (Lab blender 400, Sweard lab. Model No. AB 6021). After that 10-fold dilutions were prepared using sterile quarter-strength Ringer's Solution (Oxoid)**(APHA, 1992).** Lactic acid bacteria were enumerated on deManRogosa SharpeAgar (MRS Oxoid) in anaerobic conditions after 48 hours at 30°C**(ISIRI, 1998)**; yeasts and molds on Sabauroud's dextrose agar with Chloramphenicol (Oxoid) after 5 days at25°C**(Cruickshank et al., 1975)**, Enterobacteriaceaeon Violet Red Bile Glucose Agar (VRBG, Oxoid CM 485) after 24 hours at 30°C**(ICMSF, 1978)**, Staphylococcus aureus on Baird-Parker agar plates (Oxoid CM 145) incubated at 37°C for 48 hours**(Bailey and Scott, 1982)**,proteolytic bacteria on skim milk agar (Defico, 232100) at 30°C for 48 hours **(Lee and Kraft, 1992)** and lipolytic bacteria on tributyrin agar plates (Oxoid PM 4) at 30°C for 3 days**(Smith and Haas, 1992).**

Chemical examinations

Proximate analysis: Percentages of moisture, protein, fat and ash contents were determined according to**(AOAC, 2000)**,where moisture content was determined using the direct water distillation method in hot air oven, fat content was determined with the Soxhlet method and the protein content with the Kjeldahl method. The total ash content was determined by igniting the charred sample in a muffle furnace at 525°C until a constant weight was reached.

Measurement of pH value: Five grams from each of samples were homogenized with 20 ml distilled water for 10-15 seconds. The pH was measured using pH meter (LovibondSenso Direct) with a probe type electrode (Senso Direct Type, 330) **(Honikel et al., 1981).**

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 ± 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 $P<0.05$.

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Results and discussion

Microorganisms gain access into sausage from meat, spices and other ingredients, environment, equipment and handlers during processing steps. Lower initial microbial load of sausage mix and maintenance of adequate temperature during storage would improve the microbiological quality and enhance the shelf life of sausage **(Siriken et al. 2006)**.Microbiological examination of Alexandria semidry sausage revealed that there was a significant increase ($p<0.05$) in lactic acid bacteria during the fermentation phase till reach 7 log CFU/g in the third day then it decreasedlinearly throughout refrigeration storage period **(Table 1)**.Sausage treated with L. acidolactishad the highest significant increase ($p<0.05$) in lactic acid bacteriaduring fermentation however sausages treated by bifidobacteriumlactis had the highest count at the end of storage period. These results agree with results obtained by **(Živković et al. 2012)** who found that lactic acid bacteria markedly increased in the next two days of fermentation by around one logarithmic unit/day, reaching approximately 7 log cfu/g on the third day.This increase in lactic acid bacteria during fermentation period as a result of the combined effects of lowering the pH, increasing the brine content and decreasing the water activity due to drying **(Flores and Bermel, 1996)**.

Table (1): Lactic acid bacteria in Alexandria semidry sausage

	Fermentation period			Storage period		
Types of probiotics	1 day	2 day	3 day	30 day	60 day	90 day
Bifidobacteriumlactis	$_{i}5.64^{a}\pm0.26$	$_{ii}7.22^{a}\pm0.29$	$_{ii}7.55^{a}\pm0.38$	$_{i,iii}4.89^{ab}\pm0.04$	$_{iii}4.04^{a}\pm0.36$	$_{iv}2.43^{a}\pm0.30$
L. casei	$_{i}6.86^{a}\pm0.06$	$_{i}6.99^{a}\pm0.17$	$_{i}7.41^{a}\pm0.45$	$_{ii}4.80^{ab}\pm0.03$	$_{iii}2.48^{ac}\pm1.24$	$_{iv}<2^{b}$
L. acidophilus	$_{i}5.65^{a}\pm0.35$	$_{ii}7.14^{a}\pm0.23$	$_{ii}7.47^{a}\pm0.31$	$_{i}5.19^{a}\pm0.34$	$_{iii}0.83^{bc}\pm0.83$	$_{iii}<2^{b}$
L.lactis	$_{i,ii}6.04^{a}\pm0.85$	$_{i}7.19^{a}\pm0.36$	$_{i}7.81^{a}\pm0.04$	$_{ii}4.70^{ab}\pm0.09$	$_{iii}2.25^{ab}\pm1.17$	$_{iii}<2^{b}$
Bifido.lactis+L.acidophilus	$_{i}6.41^{a}\pm0.63$	$_{i}7.12^{a}\pm0.34$	$_{i}7.46^{a}\pm0.25$	$_{ii}4.20^{b}\pm0.36$	$_{iii}<2^{b}$	$_{iii}<2^{b}$
L.casei +L.acidophilus	$_{i,ii}6.26^{a}\pm0.41$	$_{i}6.76^{a}\pm0.64$	$_{i}7.59^{a}\pm0.31$	$_{ii}4.59^{ab}\pm0.35$	$_{iii}<2^{b}$	$_{iii}<2^{b}$

a-c: Means with different subscript within the same row for each parameter differ significantly ($P<0.05$).
i-v: Means with different superscript within the same column for each parameter differ significantly ($P<0.05$)

Table (2): Total yeast and mold in Alexandria semidry sausage

	Fermentation period			Storage period		
Types of probiotics	1 day	2 day	3 day	30 day	60 day	90 day
Bifidobacteriumlactis	$_{i}4.63^{a}\pm0.19$	$_{ii}3.16^{a}\pm0.24$	$_{iii}<2^{a}$	$_{iiii}6.18^{a}\pm0.08$	$_{i,iv}5.27^{a}\pm0.22$	$_{i,iv}5.05^{ab}\pm0.08$
L. casei	$_{i}4.89^{a}\pm0.18$	$_{i,ii}3.43^{a}\pm0.61$	$_{ii}<2^{a}$	$_{i,ii}2.49^{b}\pm1.32$	$_{i}4.03^{bc}\pm0.53$	$_{i}4.62^{a}\pm0.36$
L. acidophilus	$_{i,iv}4.50^{a}\pm0.30$	$_{ii}2.51^{a}\pm0.30$	$_{iii}<2^{a}$	$_{i,ii}2.76^{b}\pm1.40$	$_{iv}5.28^{a}\pm0.04$	$_{iv}5.55^{bc}\pm0.44$
L.acidolactis	$_{i}4.03^{a}\pm0.51$	$_{ii}1.77^{a}\pm0.96$	$<2^{a}{}_{ii}$	$_{ii}1.00^{b}\pm1.00$	$_{i}3.83^{bc}\pm0.29$	$_{i}5.37^{abc}\pm0.25$
Bifido.lactis+L.acidophilus	$_{i}5.50^{a}\pm0.35$	$_{ii,iii}1.73^{a}\pm0.87$	$_{ii}<2^{a}$	$_{iii}2.49^{b}\pm1.25$	$_{i,iii}3.65^{b}\pm0.27$	$_{i}5.10^{ab}\pm0.05$
L.casei +L.acidophilus	$_{i}5.02^{a}\pm0.37$	$_{ii}2.46^{a}\pm0.09$	$_{iii}<2^{a}$	$_{iv}4.06^{ab}\pm0.11$	$_{i}4.91^{ac}\pm0.52$	$_{v}6.10^{c}\pm0.10$

a-c: Means with different subscript within the same row for each parameter differ significantly ($P<0.05$).
i-v: Means with different superscript within the same column for each parameter differ significantly ($P<0.05$)

Using of different types of probiotics significantly decreased ($P<0.05$) total yeast and mould count **(Table 2)** during fermentation phase followed by a significant increase ($P<0.05$) throughout storage period. L.casei had the strongest effect in reduction but mixture of L.casei and L.acidophilus had the weakest effect on reduction of total yeast and mould count at the end of storage period. These results agree with those obtained by **Casaburi et al. (2007)** who found that count of yeast and molds increased after ripening period; and **Urso et al. (2006)** who found that the count of yeast count decreased toward the end of fermentation period. While **Sachindra et al. (2005)** stated that cooking process was effective in reducing the yeasts and moulds counts substantially in sausage.Incorporation of different types of probiotics resulted in a significant reduction ($p<0.05$) in lipolytic bacteria count from the first day of fermentation till the end of storage period **(Table 3)**. Moreover,Staphylococcus aureus, Enterobacteriacae and proteolytic bacteria were below the delectable limit either during fermentation or during storage period. These results may be due to the antimicrobial activity of starter cultures metabolite such as organic acids, hydrogen peroxide and bacteriocins which give a protective effect **(Lücke, 2000 ;Ammor and Mayo, 2007)**.These results also agree with those obtained by **Ruiz et al. (2014)**who found that Bifidobacteriumlactis and L.acidophilus strains have the ability to reduce the population of pathogenic bacteria by the production of acids, hydrogen peroxide and bacteriocin (lactacin B). Moreover **Schillingerand Lücke, (1989)** found that enterobacteria and Staph. aureuswas reduced below the detection limit in the early phase of

fermentation due to a rapid drop in pH to below 5.3.

Table (3): Lipolytic bacteria in Alexandria semidry sausage

	Fermentation period			Storage period		
Types of probiotics	1 day	2 day	3 day	30 day	60 day	90 day
Bifidobacteriumlactis	$_{i}3.34^{a}\pm0.21$	$_{ii}2.00^{a}\pm1.01$	$_{iii}<2^{a}$	$_{iii}<2^{a}$	$_{iii}<2^{a}$	$_{iii}<2^{a}$
L. casei	$_{i}4.37^{a}\pm0.70$	$_{ii}1.77^{a}\pm0.91$	$_{iii}<2^{a}$	$_{iii}<2^{a}$	$_{iii}<2^{a}$	$_{iii}<2^{a}$
L. acidophilus	$_{i}2.43^{a}\pm1.23$	$_{ii}0.67^{a}\pm0.67$	$_{ii}<2^{a}$	$_{ii}<2^{a}$	$_{ii}<2^{a}$	$_{ii}<2^{a}$
L.lactis	$_{i}1.87^{a}\pm0.94$	$_{i}1.43^{a}\pm0.72$	$_{i}<2^{a}$	$_{i}<2^{a}$	$_{i}<2^{a}$	$_{i}<2^{a}$
Bifido.lactis+L.acidophilus	$_{i}2.10^{a}\pm1.06$	$_{i,ii}1.53^{a}\pm0.77$	$_{ii}<2^{a}$	$_{ii}<2^{a}$	$_{ii}<2^{a}$	$_{ii}<2^{a}$
L.casei +L.acidophilus	$_{i}1.77^{a}\pm0.96$	$_{ii}<2^{a}$	$_{ii}<2^{a}$	$_{ii}<2^{a}$	$_{ii}<2^{a}$	$_{ii}<2^{a}$

a-c: Means with different subscript within the same row for each parameter differ significantly ($P<0.05$).
i-v: Means with different superscript within the same column for each parameter differ significantly ($P<0.05$)

Table (4): Moisture content in Alexandria semidry sausage

	Fermentation period			Storage period		
Types of probiotics	1 day	2 day	3 day	30 day	60 day	90 day
Bifidobacteriumlactis	$_{i}61.28^{a}\pm0.41$	$_{i,ii}58.25^{a}\pm1.04$	$_{ii}51.71^{a}\pm4.27$	$_{iii}42.16^{a}\pm0.92$	$_{iii,iv}38.80^{a}\pm3.80$	$_{iv}34.52^{a}\pm2.27$
L. casei	$_{i}60.21^{a}\pm1.37$	$_{i}59.92^{a}\pm0.32$	$_{ii}51.66^{a}\pm2.89$	$_{ii,iii}49.03^{a}\pm1.65$	$_{iii,iv}42.40^{a}\pm4.59$	$_{iv}35.87^{a}\pm1.62$
L. acidophilus	$_{i}59.16^{a}\pm0.26$	$_{i,iii}52.23^{b}\pm2.75$	$_{ii,iii}47.33^{a}\pm4.67$	$_{ii,iii}42.83^{a}\pm5.89$	$_{ii}40.84^{a}\pm4.65$	$_{ii}39.15^{a}\pm1.46$
L.lactis	$_{i}60.10^{a}\pm4.14$	$_{i,iv}56.91^{ab}\pm0.22$	$_{ii,iv}47.27^{a}\pm1.83$	$_{ii,iii,iv}44.98^{a}\pm2.11$	$_{ii,iii}39.41^{a}\pm0.54$	$_{iii}34.90^{a}\pm7.82$
Bifido.lactis+L.acidophilus	$_{i}58.96^{a}\pm0.31$	$_{i,iii}57.43^{ab}\pm0.47$	$_{i,iii,iv}48.82^{a}\pm3.05$	$_{ii,iii}46.49^{a}\pm7.36$	$_{ii,iv}41.80^{a}\pm0.88$	$_{ii}36.72^{a}\pm4.32$
L.casei +L.acidophilus	$_{i}62.43^{a}\pm0.99$	$_{i}58.28^{a}\pm0.88$	$_{ii}46.94^{a}\pm2.10$	$_{ii,iv}44.47^{a}\pm4.94$	$_{iii,iv}38.87^{a}\pm1.27$	$_{iii}29.28^{a}\pm1.35$

a-c: Means with different subscript within the same row for each parameter differ significantly ($P<0.05$).
i-v: Means with different superscript within the same column for each parameter differ significantly ($P<0.05$)

Proximate chemical analysis of fermented sausages depends on many factors such as diameter of the sausage, type and level of additives, fermentation temperature, relative humidity and the air speed during fermentation as well as the presence or absence of lactic acid bacteria and their nature **(Zara et al., 2007).** Moisture content of experimentally produced sausage **(Table 4)**was significantly decreased ($p<0.05$) during fermentation and storage period. These results may be due to loss of water during fermentation and storage in which pH reach to isoelectric point, this low pH which occur during fermentation resulted in coagulation of protein so it permanently loss its ability to hold up more water. Mixture of L.casei and L.acidophilus resulted in the highest significant ($p<0.05$) moisture loss followed by L.acidolactis at the end of storage peroid. These results agree with results obtained by **Casaburi et al. (2007)** who found that moisture content at the end of ripening was reduced to 23% and 34%. **Živković et al., (2012)**recorded similar results that fermented sausages at the end of ripening period were characterized by decreased in moisture content by 25.11% to 27.89%.

Significant increases ($p<0.05$) in protein, fat and ash content were recorded in Alexandria semidry sausage **(Tables 5, 6, 7)** during fermentation and storage period as a result of dryness and moisture loss. Mixture of L.caseiandL.acidophilus resulted in the highest protein, fat and ash content at the end of storage period as a result of highest moisture loss. These results agree with **Vesković et al. (2013)** who found that increasing amount of fat and protein content as a result of increase the amount of water loss during fermentation.

Table (5): Protein content in Alexandria semidry sausage

	Fermentation period			Storage period		
Types of probiotics	1 day	2 day	3 day	30 day	60 day	90 day
Bifidobacteriumlactis	$_{i}19.15^{a}\pm0.85$	$_{i}19.32^{a}\pm0.57$	$_{i}19.75^{a}\pm0.20$	$_{ii}26.38^{a}\pm0.81$	$_{ii}27.22^{a}\pm0.59$	$_{ii}28.19^{a}\pm0.27$
L. casei	$_{i}19.35^{a}\pm0.16$	$_{i}18.55^{a}\pm0.66$	$_{i}19.39^{a}\pm0.21$	$_{ii}25.89^{a}\pm0.45$	$_{iii}27.53^{a}\pm0.67$	$_{iii}28.45^{ab}\pm0.42$
L. acidophilus	$_{i}20.57^{a}\pm0.27$	$_{i}19.29^{a}\pm64$	$_{i}19.85^{a}\pm0.33$	$_{ii}25.77^{a}\pm0.89$	$_{ii,iii}26.64^{a}\pm0.99$	$_{iii}27.79^{a}\pm0.23$
L.lactis	$_{i}19.58^{a}\pm0.78$	$_{i}18.18^{a}\pm91$	$_{i}18.37^{b}\pm0.55$	$_{ii}25.67^{a}\pm0.84$	$_{ii,iii}27.15^{a}\pm0.37$	$_{iii}28.44^{ab}\pm0.15$
Bifido.lactis+L.acidophilus	$_{i}20.02^{a}\pm0.48$	$_{i}19.05^{a}\pm0.45$	$_{i}19.30^{ab}\pm0.11$	$_{ii}26.35^{a}\pm0.64$	$_{iii}27.56^{a}\pm0.80$	$_{iii}27.94^{a}\pm0.08$
L.casei +L.acidophilus	$_{i}19.92^{a}\pm0.55$	$_{i}19.21^{a}\pm0.26$	$_{i}20.21^{a}\pm0.30$	$_{ii}25.63^{a}\pm0.93$	$_{iii}28.15^{a}\pm0.20$	$_{iii}29.05^{b}\pm0.16$

a-c: Means with different subscript within the same row for each parameter differ significantly ($P<0.05$).
i-v: Means with different superscript within the same column for each parameter differ significantly ($P<0.05$)

Table(6): Ether extractable fat in Alexandria semidry sausage:

Types of probiotics	Fermentation period			Storage period		
	1 day	2 day	3 day	30 day	60 day	90 day
Bifidobacteriumlactis	$_{i}24.42^{a}\pm0.77$	$_{ii}17.28^{ab}\pm0.85$	$_{i}23.07^{a}\pm2.09$	$_{iii}31.81^{a}\pm0.44$	$_{iii}33.70^{a}\pm2.42$	$_{iii}33.86^{a}\pm1.71$
L. casei	$_{i}24.51^{a}\pm0.21$	$_{ii}16.65^{a}\pm0.71$	$_{i}27.15^{a}\pm0.73$	$_{i,iii}27.75^{a}\pm0.90$	$_{iii}31.39^{a}\pm2.62$	$_{iv}38.95^{a}\pm1.04$
L. acidophilus	$_{i,ii,iii}24.91^{a}\pm0.13$	$_{ii}19.63^{b}\pm1.42$	$_{iii}28.38^{a}\pm3.03$	$_{iii}30.20^{a}\pm3.94$	$_{iii}30.96^{a}\pm1.69$	$_{iii}31.16^{a}\pm2.58$
L.lactis	$_{i,ii}20.18^{b}\pm1.51$	$_{i}18.13^{ab}\pm1.33$	$_{ii,iv}26.61^{a}\pm22.76$	$_{iii,iv}29.30^{a}\pm1.62$	$_{iii,iv}32.22^{a}\pm0.42$	$_{iii}35.62^{a}\pm5.61$
Bifido.lactis+L.acidophilus	$_{i,ii}21.37^{b}\pm0.45$	$_{i}16.78^{ab}\pm0.38$	$_{ii,iii}26.13^{a}\pm1.91$	$_{ii,iii}26.71^{a}\pm4.31$	$_{iii}28.85^{a}\pm1.25$	$_{iii}32.43^{a}\pm1.26$
L.casei +L.acidophilus	$_{i}20.80^{b}\pm0.52$	$_{i}17.37^{ab}\pm0.33$	$_{ii}26.57^{a}\pm1.20$	$_{ii,iii}29.10^{a}\pm2.48$	$_{iii}33.08^{a}\pm0.99$	$_{iv}38.98^{a}\pm1.79$

a-c: Means with different subscript within the same row for each parameter differ significantly (P<0.05).
i-v: Means with different superscript within the same column for each parameter differ significantly (P<0.05)

Table (7): Ash content in Alexandria semidry sausage

Types of probiotics	Fermentation period			Storage period		
	1 day	2 day	3 day	30 day	60 day	90 day
Bifidobacteriumlactis	$_{i}2.58^{a}\pm0.19$	$_{i}2.59^{ab}\pm0.27$	$_{i,ii}3.19^{a}\pm0.11$	$_{i,ii}3.09^{a}\pm0.07$	$_{ii}3.32^{a}\pm0.20$	$_{ii}3.53^{a}\pm0.32$
L. casei	$_{i,iv}2.64^{a}\pm0.03$	$_{i}2.45^{ab}\pm0.04$	$_{i,ii}2.86^{a}\pm0.04$	$_{ii,iv}3.01^{a}\pm0.16$	$_{ii,iii}3.31^{a}\pm0.21$	$_{iii}3.60^{a}\pm0.30$
L. acidophilus	$_{i}2.57^{a}\pm0.23$	$_{i}2.94^{b}\pm0.29$	$_{i}2.78^{a}\pm0.30$	$_{i}3.04^{a}\pm0.64$	$_{i}3.39^{a}\pm0.43$	$_{i}3.54^{a}\pm0.22$
L.lactis	$_{i,ii}2.53^{a}\pm0.29$	$_{i}2.34^{a}\pm0.15$	$_{ii,iii}3.15^{a}\pm0.04$	$_{ii,iii}3.08^{a}\pm0.23$	$_{iii}3.38^{a}\pm0.18$	$_{iii}3.52^{a}\pm0.25$
Bifido.lactis+L.acidophilus	$_{i,ii}2.60^{a}\pm0.13$	$_{i}2.36^{a}\pm0.09$	$_{ii,iii}3.12^{a}\pm0.14$	$_{ii,iii}3.19^{a}\pm0.50$	$_{iii}3.39^{a}\pm0.15$	$_{iii}3.41^{a}\pm0.07$
L.casei +L.acidophilus	$_{i}2.11^{a}\pm0.08$	$_{i}2.30^{a}\pm0.04$	$_{ii}3.13^{a}\pm0.24$	$_{ii}3.17^{a}\pm0.20$	$_{ii}3.28^{a}\pm0.20$	$_{ii}3.60^{a}\pm0.15$

a-c: Means with different subscript within the same row for each parameter differ significantly (P<0.05).
i-v: Means with different superscript within the same column for each parameter differ significantly (P<0.05)

Measurement of pH in experimentally produced fermented sausage **(Table 8)** indicated that treatment of Alexandra semidry sausage with different types of starter cultures resulted in a significant gradual decrease (p<0.05) in pH in the three days of fermentation and in the first month of storage followed by significant increase (p<0.05) at the second and third month of storage. Fermented sausage treated with Bifidobacteriumlactis resulted in the highest pH through fermentation and during storage period. Using of mixture of L.caseiand L.acidophilusresulted in the highest acidity at the end of fermentation period whileL.casei treated fermented sausage resulted in the lowest pH. These results were in agreement with **Ruiz et al. (2014)** who observed that pH of the fermented sausage treated with Bifidobacteriumlactiswas higher than that treatedL. acidophilus, indicating that Bifidobacteriumlactisproduced less lactic acid than other types of starter cultures. The results also agreed with **Salgado et al. (2005)** who found that initial pH dropped in interval of 0.16 to one pH unit or more in different treatments of fermented sausage due to production of lactic acid by lactic acid producing bacteria. After the pH drop, there was an increase in pH values during storage period which can be attributed to bacterial activity resulted in liberation of alkaline metabolites **Reddy and Rao (2000)** or due to the growth of spoilage bacteria **Ahmad and Srivastava (2007). (Salgado et al., 2005)** added that increasing of pH appears to be more related to the decrease in lactic acid content than to the formation of low molecular weight nitrogen compounds.

Table (8) Measurement of pH value in Alexandria semidry sausage:

Types of probiotics	Fermentation period			Storage period		
	1 day	2 day	3 day	30 day	60 day	90 day
Bifidobacteriumlactis	$_{i,ii}5.89^{ab}\pm0.01$	$_{i,ii}5.88^{a}\pm0.01$	$_{i}5.58^{a}\pm0.01$	$_{i}5.47^{a}\pm0.01$	$_{i,ii}5.93^{a}\pm0.39$	$_{ii}6.32^{a}\pm0.16$
L. casei	$_{i}5.87^{ac}\pm0.01$	$_{i,ii}5.73^{b}\pm0.01$	$_{ii}5.51^{a}\pm0.02$	$_{iii}4.51^{b}\pm0.01$	$_{iii}4.63^{b}\pm0.19$	$_{iii}4.76^{b}\pm0.07$
L. acidophilus	$_{i}5.91^{b}\pm0.00$	$_{i,ii}5.84^{a}\pm0.00$	$_{i,ii}5.20^{b}\pm0.02$	$_{ii}4.74^{c}\pm0.12$	$_{i,ii}5.19^{bc}\pm0.07$	$_{i,ii}5.75^{ab}\pm0.91$
L.lactis	$_{i}5.86c\pm0.01$	$_{ii}5.05^{c}\pm0.03$	$_{ii}4.97^{c}\pm0.00$	$_{ii}4.94^{d}\pm0.01$	$_{iii}5.31^{c}\pm0.10$	$_{i}5.90^{ab}\pm0.10$
Bifido.lactis+L.acidophilus	$_{i}5.88^{ac}\pm0.01$	$_{ii}5.52^{d}\pm0.03$	$_{iii}5.25^{b}\pm0.06$	$_{iv}4.53^{b}\pm0.02$	$_{iii}5.12^{bc}\pm0.08$	$_{ii}5.45^{ab}\pm0.08$
L.casei +L.acidophilus	$_{i}5.87^{ac}\pm0.01$	$_{ii}5.47^{d}\pm0.04$	$_{iii,iv}4.64^{d}\pm0.01$	$_{iii}4.53^{b}\pm0.01$	$_{iv}4.87^{bc}\pm0.01$	$_{v}6.60^{a}\pm0.26$

a-c: Means with different subscript within the same row for each parameter differ significantly (P<0.05).
i-v: Means with different superscript within the same column for each parameter differ significantly (P<0.05

Conclusion

Using of different types of probiotics resulted in a significant increase in lactic acid bacteria count, decrease in total yeast and mould and lipolytic bacterial count during fermentation period, while, refrigerated storage resulted in decrease in lactic

acid bacteria with an increase in yeast and mould count. However, Staphylococcus aureus, Enterbacteracae and proteolytic bacteria were below the delectable limit. Chemical examination of fermented sausage indicated thatrefrigerated storage resulted in reduction in moisture content with subsequent increase in protein, fat and ash content. Probiotics resulted in a significant and gradual decrease in pH value during fermentation period and in the first month of storage followed by significant increase during storage.

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الملخص العربى

تأثير البروبيوتك على مستويات الامينات الحيويه فى السجق الاسكندرانى شبه الجاف اثناء الحفظ بالتبريد

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تم دراسة التغيرات الميكربيولوجيه و الكيميائيه فى السجق الاسكندرانى شبه الجاف و الذى تم تصنيعه بأنواع مختلفه من البروبيوتك فقد تم تصنيع ست مجموعات من السجق باستخدام ستة انواع مختلفه من البربيوتك وقد تم تخميره فى حجرة تخمير عند درجة حراره 20° درجه منويه لمدة ثلاث ايام وبعد ذلك تم ايقاف التخميربواسطه الارتفاع المتدرج للحراره ثم تم تسويه المنتج حتى درجه حراره 72° درجه منويه داخل المنتج وبعد ذلك تم حفظ المنتج فى الثلاجه عند درجة حراره 4° درجه منويه لمدة ثلاث شهور وقد تم فحص المنتج بصفه اثناء فترة التخمر و كذلك اثناء فترة التخزين لفحص التغيرات الميكربيولوجيه و الكيمائيه. ولقد اظهرت النتائج ان استخدام الانواع المختلفه من البروبيوتك ادت الى ارتفاع الحمل البكتيرى اثناء فترة التخمر لبكتيريا حمض اللاكتيك وانخفاض العد الكلى للخمائر و العفن و كذلك البكتيريا المحلله للدهون بينما ادى التخزين بالتبريد الى انخفاض الحمل البكتيرى لبكتيريا حمض اللاكتيك و زياده فى العد الكلى للخمائر و العفن بينما لم يتم عزل كل من بكتيريا مكور العنقود الذهبى والبكتيريا الامعائيه و البكتيريا المحلله للبروتين.أظهر الفحص الكيميائى ان الحفظ بالتبريد ادى الى انخفاض نسبة الرطوبه وبالتالى ارتفاع نسبة البروتين والدهون والرماد. و قد ادى استخدام الانواع المختلفه من البروبيوتك الى انخفاض ملحوظ فى الاس الهيدروجينى خلال فترة التخمر و الشهر الاول من التخزين ثم ارتفاع خلال الفتره الاخيره من التخزين. استخدام البيفيددوبكتريم لاكتس ادى الى اعلى رقم للاس الهيدروجينى بينما استخدام اللاكتوباسيلس كازى الى اعلى نسبة حموضه بكاملها بينما اد فى نهاية فترة التحمر.