# GROWTH AND VIABILITY OF CERTAIN PROBIOTIC STRAINS IN THE PRESENCE OF LACTIC ACID CULTURE IN UNSALTED KAREISH CHEESE

Zommara, M.A.<sup>1</sup> and Azza M. Elbaz<sup>2</sup>

1-Department of Dairy Science, Faculty of Agriculture, Tanta University, Kafr El-Sheikh 33516 Egypt,

2-Animal Production Research Institute, Agriculture Research Center, Egypt

#### **ABSTRACT**

This study was carried out to explore the growth manner and viability of L. acidophilus, B. longum and L. casei used lactic acid culture for the manufacture of unsalted Kareish cheese during 15 days of cold storage at 7°C±1. There were no significant variation in all cheese treatments in there content of fat (4.8-4.9%), total solid (23-25%) and cheese yield (about 24%). Similar curd tension was found in all cheese curd which indicated a complete coagulation resulting in a firm curd. There were no differences in fresh cheese acidity (1.5-1.6%) however, Acidophilus-cheese resulted in a slightly higher acidity (1.9%) at the end of storage period. The curd synersis rate was significantly different among all treatment during 6 hours of drainage in the descending order acidophilus, casei, Control and longum. The cheese TVFA gradually increased in all cheese treatments throughout the storage period, however the acidophilus and longum-cheese had the highest and the lowest TVFA content at the end of storage period. The microbiological analysis showed comparable total, streptococci and lactobacilli bacterial counts among all cheese treatments during storage period. The viability of L. acidophilus and L. casei was higher (109 CFU/g cheese) than B. longum (106 cfu/g cheese) after 15 days of storage. No yeasts or moulds were detected in the fresh cheese or after 5 days of storage. They appeared after 10 days of storage (1.2-2.8 X103 cfu/g) and significantly increased by 2 log cycles (1.1-1.3X10<sup>5</sup> cfu/g) at the end of storage period with no significant differences among all cheese treatments. The sensory evaluation of cheese demonstrated acceptable sensory properties for fresh cheese (0.93-0.96), or after 15 days of storage (0.91-0.95). The Longum-cheese gained the highest organoleptic score in spite of appearance of a slight acetic acid flavour, whereas the acidophilus-cheese gained the lowest score accompanied with a moderate sour taste.

Keywords: Kareish cheese, Probiotics, L. acidophilus, L. casei, B. longum

## INTRODUCTION

Kareish cheese is an Egyptian traditional soft white acidic cheese made from the naturally acid coagulated low fat raw cow or buffalo milk "Laban Rayeb" produced as a by-product after separation of sour cream on the top of milk in an earthenware pots (Matared) at room temperature depending on the earth gravity. Kareish cheese is one of the most popular cheese varieties in Egypt, owing to its high protein (~17%), low fat content (~6%) and low price (El-Gendy, 2001) As Kareish cheese commonly contains a high moisture content (about 70%) it must be consumed within few days after production. Its maximum shelf life does not exceed 12 days at 5°C (Abou Dawood and Gomai, 1977). Many attempts have been done to improve its microbial properties by following hygienic procedures, using

pasteurized skim milk, fermentation with pure lactic acid cultures either alone or mixed with rennet (Fahmi, 1960; Abou Dawood, 2002).

Most bacteria with probiotics properties belong to the genera Lactobacillus and bifidobacteria, which are common but not dominant members of the indigenous microbiota of the human gastrointestinal tract (Sghir et al., 2000 and Walter et al., 2001). The probiotic potential of various Lactobacillus and Bifidobacterium strains has been discussed in numerous reviews and includes well-documented management of intestinal disorders such as lactose intolerance, infant gastroenteritis and rotavirus-associated diarrhoea, antibiotic-associated intestinal symptoms (mainly diarrhoea) and food allergy in babies (Salminen et al., 1998; Isolauri et al., 1999, 2001; Marteau et al., 2001 and Kaur et al., 2002) These disorders and diseases are associated with intestinal microbiota imbalance and increased gut ermeability (Salminen et al., 1996). In addition to these beneficial effects on disturbed intestinal microbiota, probiotics can modulate immune response, lower biomarkers such as harmful fecal enzyme activities, and show positive effects against superficial bladder cancer and cervical cancer (McFarland, 2000). Other potential areas of probiotic nutritional management include alleviation of inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS) symptoms, mucosal vaccines and immunomodulation, infection control and eradication of multidrug-resistant microbes, treatment of candidal vaginitis, prevention of transmission of AIDS and sexually transmitted diseases, lowering cholesterol and blood, and antimutagenic/anticarcinogenic activity (Alvarez-Olmos and Oberhelman, 2001; Kopp-Hoolihan, 2001; Marteau et al., 2001 and Kaur et al., 2002) Owing to their perceived health benefits, lactobacilli and bifidobacteria have been increasingly included in yoghurts and fermented milk during the past two decades (Daly and Davis, 1998).

Cheese provides lactic acid bacteria (LAB) conditions that assist both bacterial growth and survival. Many of the LAB found in cheese belong to the same species as probiotics bacteria, including *L. casei, L. paracasei, L. rhamnosus and L. planarum* (Lindberg *et al.*, 1996 and Gardiner *et al.*, 1998, Mehanna *et al.*, 2002). The suitability of cheeses as a carrier of probiotic LAB has been shown both for mature cheeses and fresh cheeses (Gomes *et al.*, 1995; Gardiner *et al.*, 1998; Osman and Abbas, 1999; Vinderola *et al.*, 2000, El-Zayat and Osman, 2001, Shehata *et al.*, 2001, Abou Dawood, 2002).

Use of a supporter culture together with a probiotic culture results in a reduction in spores and contaminants however, the viability of various LAB cultures needs to be considered. The most important issue is that the entire LAB used must be able to grow together without inhibiting each other. According to Lourens-Hatting and Viljoen (2001), the survival of some probiotic bacteria is influenced by the ability of the supporter culture to eliminate oxygen and produce low post-acidification. To benefit the health of the consumer, a probiotic bacterium has to reach its target (gut) alive. The minimum suggested level of viable probiotic cells at the time of consumption is approximately 10<sup>7</sup> cfu/g product, and the suggested daily intake is approximately 10<sup>9</sup> viable cells (around 100 g of product per day) (Kurman & Rasic, 1991 and Ishibashi & Shimamura, 1993).

In the present study three probiotic strains, namely: L. acidophilus, B. longum or L. casei were incorporated with a commercial lactic culture for Kareish cheese production. Some chemical, physical and sensory properties of cheese as well as the probiotics viability during cold storage were evaluated.

## MATERIALS AND METHODS

## Bacterial strains and culture starter

Commercially available lyophilized cultures of Lactobacillus acidophilus (L. acidophilus, La-5) and Lactobacillus casei were obtained from Chr. Hansen. Laboratories Copenhagen, Denmark. Bifidobacterium longum (B. longum) was a gift from the Department of Dairy Science, Faculty of Agriculture, Alexandria University, Egypt. Commercially available lyophilized lactic culture for Direct Vat Set (DVS) FRC-60 was obtained from Chr. Hansen Laboratories, Copenhagen, Denmark.

## Kareish cheese making

Kareish cheese was made as described by Fahmi (1960) with some modification. Fresh buffaloes skim milk (about 1.5%) fat was heated to 65°C for 30 min, cooled to 32°C and divided to 4 equal aliquots with 5 kg each. A portion of milk was inoculated with 2% of Kareish cheese starter (control). The other portions were inoculated separately with a mixture (1:1) of Kareish cheese starter and one of the previously mentioned probiotics. All milk portions were incubated at 32°C till complete coagulation. Curd of each treatment was hoped to drainage under light pressing for 24 hours at room temperature in cylindrical aluminum cups with small holes in the bottom and all around the cups wall, occupied with cheesecloth,. Samples of cheese were examined in the fresh cheese and after 5,10 and 15 days storage at 7c.

## Cheese analysis

Cheese samples were analyzed in triplicate for percentage of fat, moisture and titratable acidity according to the methods described by AOAC (1990). The percent of fat in cheese dry matter (FDM) was calculated.

## Measurement of curd tension

The curd tension (g) was estimated according to Abd El-Salam et al., (1994). The apparatus used consisted of knives of constant weight (5.0g), H-shaped with needle in the middle ending with a hook, and a wire crossing a freely rotating wheel attached to the knife at one end and a pan (5.0g) at the other. The knife was placed in a 100 ml beaker. The prepared milk inoculated with the starter culture (50 ml) was added to the beaker and incubated at 32°C until coagulation. The curd tension was measured, after holding the curd in the refrigerator overnight, as weigh in grams able to remove the knife from the curd.

## Measurement of curd synersis

The curd syners was measured at room temperature as described by Rashed (1982). 100 g portions of the milk inoculated with the starter cultures were put in a deep bowel equipped with a semi-sphere net with a hand. After coagulation at 32°C the net was carefully removed from the bowel and put in a funnel over a 100-ml cylinder. The rest of the curd in the bowel

was determined in order to estimate the weight of curd on the net. The whey drained from the curd into the cylinder was measured at appropriate times and calculated as ml whey per 100 g curd.

## Determination of total volatile fatty acids (TVFAs)

Cheese TVFAs were determined by a direct distillation method according to Kosikowski (1978), and the results were expressed as ml 0.1N-NaOH/100g cheese.

#### Microbiological analysis

Total bacterial count for cheese samples was enumerated on Nutrient agar media (Oxoid, Hanpshire, England). Streptococci were enumerated on modified Chalmers medium according to Vanos and Cox (1986). Lactobacilli, L. acidophilus, L. casei and bifidobacteria were enumerated according to Tharmaraj and Shah (2003). MRS (de Mann, Rogase and Sharpe) media was used to enumerate Lactobacilli after incubation for 48h at 37°C. L. acidophilus was enumerated using basal MRSmaltose agar media prepared by the addition of 10 ml membrane sterilized 20% maltose to 90 ml heat sterilized MRS agar. MRS-NaCl (4% NaCl) agar media was used for counting L. casei after incubation at 37°C for 72 h. MRS-L-cysteine HCI-Lithium chloride agar media was used for the enumeration of bifidobacteria by incubation under anaerobic condition for 72 h at 37°C. Yeasts and moulds were counted in Potato dextrose agar medium (PDA) (Difco, 1974) amended with 25mg/ml streptomycin sulfate to eliminate bacterial contamination. Yeasts and moulds plates were counted after incubation at 28°C for 2 and 7 days, respectively.

## Sensory evalution

Cheese samples were evaluated by 7 staff-members at the Department of dairy Science and Department of Food Science and Technology according to Bodyfelt et al., (1988). Cheese samples were evaluated for flavour (50 points), body and texture (40 points) and appearance (10 points) when fresh and after 15 days of storage at 7°C±1.

## Statistical analysis

Data are expressed as mean ± SE. Significant variations were determined by Duncan's multiple range test (Duncan, 1955).

# **RESULTS AND DISCUSSIONS**

## Cheese analysis and yield

Analysis and yield of Kareish cheese are shown in table (1). There were no significant differences among all cheese treatments in their moisture (75.4-76.8%) and fat (4.8-4.9%) content and accordingly in the percent of fat in dry matter (20-20.8%). These findings are renflected in cheese yield, that was comparable among all cheese treatments (23.7-24%).

Table(1). Analysis, cheese yields and curd tension of fresh Kareish cheese manufactured with different strains of probiotic bacteria.

Cheese Variety	Moisture (%)	Fat FDM (%)		Cheese yield (%)	
Control cheese	75.4 ± 0.2	4.9 ± 0.1	20.0 ± 0.13	23.7 ± 0.28	
Acidophilus-cheese	$76.5 \pm 0.5$	4.8 ± 0.1	20.4 ± 1.13	$23.9 \pm 0.28$	
Longum-cheese	$76.2 \pm 0.5$	4.9 ± 0.1	20.2 ± 0.75	24.2 ± 0.78	
Casei-cheese	$76.8 \pm 0.6$	4.8 ± 0.1	20.8 ± 0.88	23.9 ± 0.28	

Data are means ± SD for 3 replicates

#### Titratable acidity

Changes in cheese titratable acidity during cold storage are shown in Table 2. All cheese varieties had almost comparable acidity when fresh (1.5-1.6%). After 15 days of storage the Acidophilus-cheese had slightly higher (1.9%), but significant, acid concentration than the other cheese (1.7-1.8%). It is well known that *L. acidophilus* is an acid tolerant bacteria. The acidity development in cheese might be attributed to the presence of Kareish cheese starter derived of lactic acid bacteria in all cheese varieties.

Table(2). Acidity (%) of Kareish cheese manufactured with different

Storage Period (day)	Control cheese	Acidophilus- cheese	Longum- cheese	Casei- cheese
Fresh*	1.52 ± 0.02 a	$1.63 \pm 0.0^{a}$	$1.55 \pm 0.05^{a}$	$1.53 \pm 0.03^{a}$
5	1.63 ± 0.02 <sup>A, b</sup>	1.71 ± 0.03 <sup>B, b</sup>	1.68 ± 0.00 AB, a	1.61 ± 0.03 <sup>A, b</sup>
10 15	1.72 ± 0.05°	1.76 ± 0.02 <sup>b</sup>	1.76 ± 0.02 <sup>5</sup>	$1.70 \pm 0.02^{c}$
15	1.77 ± 0.03 <sup>A, C</sup>	1.88 ± 0.02 <sup>B, c</sup>	1.73 ± 0.02 <sup>A, b</sup>	1.70 ± 0.01 <sup>A, c</sup>

<sup>\*</sup> After overnight cooling. Data are means ± SE for 4 replicates. Means with unlike superscript capital letters (between groups at the same storage period) and small letters (within group at different storage period) are significantly different at p<0.05.

#### **Curd synersis**

The rate of curd synersis at room temperature during the first 6 hours of drainage is shown in figure (1). Among all Kareish cheese, the Acidophiluscheese had the highest rate of curd synersis followed by the Casei-cheese and the control. However the cheese mad with *B. longum* had the lowest rate of curd synersis. These results maight be explained by the acidity development in the curd depending on the bacterial strains in the starter cultures used in cheese making. Different rate of acidity development in the curd may affect its shrinkage, and accordingly the rate of curd synersis (Kaytanli et al., 1993). In spite of these findings, the amount of whey drained from all cheese samples was almost comparable among all cheese after 12 hours.

#### **Curd tension**

All cheese curd expressed comparable curd tensions. The curd tension values (g) expressed as mean ± SE were 10.6±1.24, 11.7±1.31, 12.2±1.41 and 11.8±1.6 for control, acidophilus, longum and casei-cheese, respectively. These results indicate the occurrence of complete and firm coagulation of milk by the starter cultures.

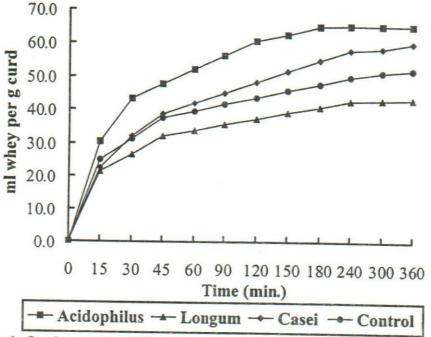


Fig. 1: Curd synersis of Kareish cheese containing different strains of probiotics

# Total volatile fatty acids (TVFAs)

Table (3) shows TVFA of Kareish cheese made with different probiotics during 15 days of cold storage. TVFA of All cheese treatments increased gradually during storage period except for that made with B. longum.

Table 3: Total volatile fatty acids of Kareish cheese manufactured with different strains of probiotic bacteria during cold storage

Storage Period Control (day) cheese		Acidophilus- cheese	Longum- Case Cheese Chee		
Fresh*	5.4 ± 0.2 A, a	$7.0 \pm 0.6^{B, a}$	9.2 ± 0.4 <sup>C, a</sup>	4.9 ± 0.6 <sup>A, a</sup>	
5	8.1 ± 0.3 <sup>A, b</sup>	8.6 ± 0.4 <sup>A, b</sup>	11.4 ± 0.2 <sup>B, b</sup>	8.5 ± 0.5 <sup>A, b</sup>	
10	$8.6 \pm 0.4^{A, b}$	11.8 ± 0.2 <sup>8, c</sup>	11.2 ± 0.4 <sup>B, b</sup>	11.8 ± 0.5 <sup>B</sup> .0	
15	11.2 ± 0.4 <sup>A, c</sup>	12.8 ± 0.0 <sup>8, c</sup>	8.2 ± 0.2 <sup>C, a</sup>	10.9 ± 0.3 <sup>A. G</sup>	

<sup>\*</sup> After overnight cooling. Data are means ± SE for 4 replicates. Means with unlike superscript capital letters (between groups at the same storage period) and small letters (within group at different storage period) are significantly different at p<0.05.

The Longum-cheese resulted in increased TVFAs up to the 10<sup>th</sup> day of storage followed by a reduction at the end of storage. On the other hand, the acidophilus and the longum-cheese had significantly higher TVFA compared to the control either when fresh or together with casei-cheese after

10 days of storage. At the end period of storage, the acidophilus and the longum-cheese resulted in the highest and the lowest significant content of TVFA respectively, compared to the control and the casei-cheese. The increase of TVFA maight be dedicated to the progress of fermentation process. The elevated TVFA in longum-cheese when fresh maight be attributed to the fact that bifidobacteria produces 1.5 moles of acetic acid as well as 1 mole of lactic acid as end products of the fermentation process of 1 mole of glucose (Tamime et al., 1995). However, the reduction in the TVFA in longum-cheese after 15 days of storage may attributed to the reduction in the viability of B. longum as shown in table (7).

## Total bacterial count (TBC)

Table (4) shows total bacterial counts (TBC) (log cfu/g) of Kareish cheese made with different probiotics during 15 days of cold storage. No significant differences were found among all cheese varieties throughout the storage period. The TBC significantly increased in the Acidophilus-cheese after 5 days of storage with no change up to the end of storage period.

Table 4: Total bacterial count (log cfu/gm) of Kareish cheese manufactured with different strains of probiotic bacteria

during cold storage

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Storage Period (day)	Control cheese	Acidophilus- cheese	Longum- cheese	Casei- cheese
Fresh*	9.9 ± 0.31 ab	9.2 ± 0.50°	9.9 ± 0.36	10.2 ± 0.57
5	10.6 ± 0.41°	10.8 ± 0.29 <sup>b</sup>	10.0 ± 0.23	10.2 ± 0.37
10	$10.7 \pm 0.40^{\circ}$	10.4 ± 0.35 ab	10.2 ± 0.64	10.2 ± 0.23
15	9.1 ± 0.41°	10.6 ± 0.43ab	10.1 ± 0.42	10.0 ± 0.53

\* After overnight cooling. Data are means ± SE for 4 replicates. Means within group at different storage period with unlike superscript letters are significantly different at p<0.05. Streptococci count

Table (5) shows streptococci count (log cfu/g) of Kareish cheese made with different probiotics during 15 days of cold storage. All cheese varieties had comparable streptococci count up to the 10<sup>th</sup> day of storage (2.1-19X10<sup>11</sup> cfu/g). After 15 days of storage, the longum and casei-cheese resulted in higher streptococci count than the other cheese. The effect of storage period was more in the control and the acidophilus-cheese, which had significantly reduced number of streptococci after 15 days of storage although their count was relatively high (3.9-17X10<sup>9</sup> cfu/g).

Table 5: Streptococci count (log cfu/gm) of Kareish cheese manufactured with different strains of probiotic bacteria

during cold storage

Storage Period (day)	Control cheese	Acidophilus- cheese	Longum- cheese	Casei- cheese
Fresh*	11.5 ± 0.45°	11.5 ± 0.21°	11.5 ± 0.35	11.5 ± 0.24
5	11.0 ± 0.32°	10.6 ± 0.57 <sup>ab</sup>	10.7 ± 0.36	11.6 ± 0.24
10	10.2 ± 0.62 <sup>ab</sup>	10.3 ± 0.11b	10.4 ± 0.52	11.7 ± 0.61
15	9.44 ± 0.25 A. B	9.94 ± 0.26 AB. b	$10.5 \pm 0.30^8$	11.6 ± 0.31

<sup>\*</sup> After overnight cooling. Data are means ± SE for 4 replicates. Means with unlike superscript capital letters (between groups at the same storage period) and small letters (within group at different storage period) are significantly different at p<0.05.</p>
Lactobacilli count

Table (6) shows lactobacilli count (log cfu/g) of Kareish cheese made with different probiotics during 15 days of cold storage. Comparable lactobacilli counts were found among all cheese treatments when fresh (8.6-62X10<sup>10</sup> cfu/g) and after 10 days of storage (7.9-22.9X10<sup>10</sup> cfu/g). Duration of storage had no significant effect on lactobacilli counts. A slight reduction of lactobacilli count were observed after 15 days of storage (1.8-9.7X10<sup>9</sup> cfu/g) in all cheese varieties.

Table 6: Lactobacilli count (log cfu/gm) of Kareish cheese manufactured with different strains of probiotic bacteria during cold storage

Storage Period (day)	Control cheese	Acidophilus- cheese	Longum- cheese	Casel- Cheese
Fresh*	10.9 ± 0.53	10.6 ± 0.58	10.8 ± 0.22	$11.2 \pm 0.50^{a}$
5	10.5 ± 0.82	$10.0 \pm 0.48$	10.1 ± 0.44	10.8 ± 0.30 <sup>ab</sup>
10	10.3 ± 0.59	10.0 ± 0.62	10.4 ± 0.51	10.4 ± 0.66 <sup>ab</sup>
15	8.83 ± 0.37	9.16 ± 0.31	9.44 ± 0.38	9.36 ± 0.36 <sup>b</sup>

<sup>\*</sup> After overnight cooling. Data are means ± SE for 4 replicates. Means within group at different storage period with unlike superscript letters are significantly different at p<0.05.</p>

## L. acidophilus, B. longum, and L. casei bacterial counts

Table (7) shows *L. acidophilus*, *B. longum*, and *L. casei* bacterial counts (log cfu/g) in Kareish cheese made with these probiotics during 15 days of cold storage. *L. acidophilus* count in the acidophilus-cheese showed no significant changes during 10 days of storage (about 4.5X10<sup>10</sup> cfu/g), followed by a slight reduction to reach 2.8X10<sup>9</sup> cfu/g after 15 days of storage. In longum-cheese, the count of *B. longum* gradually decreased during storage from 9.7X10<sup>11</sup> cfu/g when fresh to 5.5X10<sup>8</sup> cfu/g after 10 days to reach 5.8X10<sup>6</sup> cfu/g at the end of storage period. The growth manner of *L. casei* in Casei-cheese during storage period was similar to that found for *L. acidophilus*. The bacterial count slightly decreased during the first 10 days of storage from an average of 4.2X10<sup>11</sup> cfu/g to reach 5.4 X10<sup>9</sup> cfu/g at the end of storage period. In this respect, Kareish cheese starter derived-LAB may act as a supporter culture for the used probiotics through providing suitable condition for its growth and viability (Lourens-Hatting and Viljoen, 2001).

Table 7: L. acidophilus, B. longum, and L. casei bacterial counts (log cfu/gm) of Kareish cheese manufactured with different strains of probiotic bacteria during cold storage

Storage Period	Acidophilus- cheese	Longum - cheese	Casei- cheese
(day)	L. acidophilus	B. longum	L. casei
Fresh*	10.4 ± 0.38 <sup>a</sup>	$10.1 \pm 0.32^{a}$	$11.3 \pm 0.49^{a}$
5	9.8 ± 0.41 <sup>ab</sup>	$9.3 \pm 0.53^{ab}$	11.0 ± 0.45°
10	10.0 ± 0.47 <sup>ab</sup>	8.5 ± 0.34 <sup>b</sup>	10.9 ± 0.30°
15	8.6 ± 0.64 <sup>b</sup>	$6.6 \pm 0.27^{c}$	$9.6 \pm 0.24^{\circ}$

<sup>\*</sup> After overnight cooling. Data are means ± SE for 4 replicates. Means within group at different storage period with unlike superscript letters are significantly different at p<0.05.</p>

#### yeasts and moulds counts

Table (8) shows yeasts and moulds counts (log cfu/g) of Kareish cheese made with different probiotics during 15 days of cold storage. No yeasts or moulds were detected in all cheese varieties when fresh or after 5 days of storage. Yeasts and moulds appeared after 10 days of storage (1.2-2.8 X10<sup>3</sup> cfu/g) and significantly increased by 2 log cycles (1.1-1.3X10<sup>5</sup> cfu/g) at the end of storage period with no significant differences among all cheese varieties.

Table 8: Effect of storage period on Yeasts and moulds count (log cfu/gm) of Kareish cheese manufactured with different strains of prohigic bacteria during cold storage

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Storage Period (day)	Control cheese	Acidophilus- cheese	Longum- cheese	Casei- cheese	
Fresh*	$0.00 \pm 0.00^{a}$	$0.00 \pm 0.00^{a}$	$0.00 \pm 0.00^{a}$	$0.00 \pm 0.00^{a}$	
5	$0.00 \pm 0.00^{a}$	$0.00 \pm 0.00^{a}$	$0.00 \pm 0.00^{a}$	$0.00 \pm 0.00^{\circ}$	
10	2.96 ± 0.24 <sup>b</sup>	$2.90 \pm 0.42^{b}$	2.92 ± 0.44 <sup>b</sup>	2.95 ± 0.26 <sup>b</sup>	
15	4.86 ± 0.27°	$4.91 \pm 0.26^{c}$	$4.95 \pm 0.42^{c}$	$4.92 \pm 0.20^{\circ}$	

<sup>\*</sup> After overnight cooling. Data are means ± SE for 4 replicates. Means within group at different storage period with unlike superscript letters are significantly different at p<0.05.</p>

#### Sensory evaluation of cheese

The sensory evaluation of cheese when fresh and after 15 days of cold storage is shown in Table (9). All cheese treatments had acceptable sensory properties when fresh (0.93-0.96). or after 15 days of storage (0.91-0.95). The longum-cheese gained the highest score when fresh (0.96) and after 15 days of storage (0.95) characterized with a slight acetic acid flavour, whereas the acidophilus-cheese gained the lowest score when fresh (0.93) and after storage (0.91). The low score of acidophilus-cheese was accompanied with a moderate sour flavour formed in this cheese.

Table 9: sensory properties of Kareish cheese manufactured with different strains of probiotic bacteria when fresh and after 15 days of cold storage.

Parameter	Score	Cheese	Control	Acidophilus- cheese	Longum- cheese	Casei- cheese
Flavour	(50)		48	46	48	47
Body &Texture	(40)	7	38	38	39	39
Appearance	(10)	Fresh	9	9	9	9
Total	(100)		95	93	96	94
Flavour	(50)	S O D	48	45	47	46
<b>Body &amp;Texture</b>	(40)	After days cold	37	36	38	38
Appearance	(10)	000	9	9	10	10
Total	(100)	10 5	94	91	95	94

As a conclusion, the present study shows the possibility of using of probiotics *L. acidophilus*, *B. longum*, *L. casei*, along with lactic acid culture in the production of good quality unsalted Kareish cheese with acceptable probiotic cell viability and sensory properties when fresh or for 15 days for

Kareish cheese made with L. acidophilus and L. casei and 10 days for that made with B. longum when stored at 7°C±1.

## REFERENCES

- Abd El-Salam, M. H.; El-shibiny, S.; Mahfouz, M. B.; El-Dein, H. F.; El-Atriby, H. and Antila, V. (1991). Preparation of whey protein concentrate from fresh salted whey and its use in yoghurt. J. Dairy Res., 58: 503-509.
- Abou Dawood, A. E. and Gomai, A. Y. (1977). The use of high toatal solids reconstituted skim milk in Kareish cheese making, Egyptian J. Dairy Sci., 5: 229-234.
- Abou Dawood, S. A. I. (2002). Survival of nonencapsulated and encapsulated Bifidobacterium bifidum in probiotic Kareish cheese. Egyptian J. Dairy Sci., 30: 43-52.
- Alvarez-Olmos, M and Oberhelman, R. A. (2001). Probiotic agents and infectious diseases: a modern perspective on a traditional therapy. Clin. Infect. Dis., 32: 1567-1576.
- AOAC. Official Methods of Analysis, 15th ed., Helrich, K. Ed. (1990). Association of Official Analytical Chemists. Washington D.C, USA.
- Bodyfelt, F. W; Tobias, J. and Tourt, G. M. (1988). The sensory evaluation of dairy products. pp. 227-270, Von Nostrand Reinhold, New York.
- Daly, C. and Davis, R. (1998). The biotechnology of lactic acid bacteria with emphasis on application in food safety and human health. Agric. Food Sci. Finland, 7: 251-265.
- Difco. 1974. Manual of dehydrated cultured media and reagents for microbiological and chemical laboratory procedures. 9<sup>th</sup>.ed., Dfico Lab. Incorporated, Detroit, Washington 48201, USA.
- Duncan, D. B. (1955). Multiple range and Multiple F tests. Biometrics, 11: 1-42.
  El-Gendy, Sh. M. (2001). Some traditional fermented dairy products in Egypt.
  Proc. 8th Egyptian Conference for Dairy Science and Technology,
  Cairo, Egypt, papers II. pp. 465-479.
- El-Zayat, A. I. And Osman, M. M. (2001). The use of probiotics in Tallaga cheese. Egyptian J. Dairy Sci., 29: 99-106.
- Fahmi, A. H. (1960). Kareish cheese. J. Agric. Sci. Cairo, Egypt, 13, 1 (In Arabic).
- Gardiner, G.; Ross, R. P.; Collins, J. K.; Fitzgefald, G. and Stanton, C. (1998). Development of probiotic cheddar cheese containing human-derived lactobacillus paracasei strains. Appl. Environ. Microbiol., 64: 2192-2199.
- Gomes, A. M. P.; Malcata, F. A.; Klaver, M. and Grande, H. G. (1995). Incorporation and survival of Bifidobacterium sp. Strain Bo and Lactobacillus acidophilus strain Ki in a cheese product. Neth. Milk Dairy J., 49: 71-95.
- Ishibashi, N. and Shimamura, S. (1993) Bifidobacteria: Research and development. In Japan. Food Technol. 47: 126-135.
- Isolauri, E.; Salminen, S. and Mattila-Sandholmt (1999). New functional foods in the treatment of food allergy. Ann. Med., 31: 299-302.

- Isolauri, E.; Sutas, Y. Kankäänpaa, P.; Arvilommi, H. and Salminen, S. (2001). Probiotics: effect on immunity. Am J. Clin. Nutr., 73 (suppl): 444S-450S.
- Kaytanli, M.; Erdem, Y. K. and Tamer, I. M. (1993). Factors affecting whey drainage rate of renneted skim milk gels. Egyptian J. Dairy Sci., 21: 185-196
- Kaur, I. P.; Chopra, K. and Saini, A. (2002). Probiotics: potential pharmaceutical application. Eur. J. Pharmac. Sci., 15: 1-9.
- Kopp-Hoolihan, L. (2001). Prophylactic and therapeutic uses of probiotics: a review. J. Am. Diet Assoc., 101: 229-238.
- Kosikowiski, F.V. (1978) Cheese and Fermented Milk Food .2nd Ed., Published by the author, Cornell Univ., Ithaca, New York, USA.
- Kurmann, J. A. and Rasic, J. L. (1991). The health potential of products containing bifidobacteria. Pages, 117-158, In Therapeutic Properties of Fermented Milks. Robinson, R. K. (ed.), Elsevier Applied Food Science series, London, United Kingdum.
- Lindberg, A.; Christiansson, A.; Rukke, E.; Eklund, T and Molin, G. (1996). Bacterial flora of Norwegian and Swedish semi-hard cheeses after ripening, with special references to Lactobacillus. Neth. Milk Dairy J., 50: 563-572.
- Lourens-Hatting, A. and Viljoen, B. C. (2001). Review: yoghurt as probiotic carrier food. Int. Dairy J., 11: 1-17.
- Marteau, P. R.; De Verese, M.; Cellier, C. J. and Schrezenmeir, J. (2001). Protection from gastrointestinal diseases with the use of probiotics. Amr. J. Clin. Nutr., 73 (2suppl): 430S-436S.
- McFarland, L. V. (2000). A review of the evidence of health claims for biotherapeutic agents. Microbial. Ecol. Health Dis., 12: 65-76.
- Mehanna, N. Sh; Sharaf, O. M.; Ibrahim, C. A. and Tawfik, N. F. (2002). Incorporation and viability of some probiotic bacteria in functional dairy food. 1. Soft cheese. Egyptian J. Dairy Sci., 30: 217-229.
- Osman, M. M. and Abbas, F. M. (1999). Bifidus Karish cheese. J. Agric. Sci. Mansoura Univ. 23: 5043-5053.
- Rashed, M. A. (1982). The influence of storage time and temperature on the characteristics of the raw milk. Ph.D. Thesis, Univ. of Agric. Sci. Godollo, Hungary.
- Salminen, S.; Isolauri, E. and Salminen, E. (1996). Probiotics and stabilization of the gut mucosal barrier. Asia Pacific J. Clin. Nutr., 5: 53-56.
- Salminen, S.; Ouwehanda, A. C. and Isolauri, E (1998). Clinical applications of probiotic bacteria. Int. Dairy J.,8: 563-572.
- Sghir, A.; Gramet, G.; Suau, A.; Rochet, V.; Pochart, P. and Dore, J. (2000). Quantification of bacterial groups within human fecal flora by oligonucleotide probe hybridization. Appl. Environ. Microbiol., 66: 2263-2266.
- Shehata, A. E.; El-Nawawy, M. A.; El-Kenany, Y. M. and Aumara, I. E. M. (2001). Production of soft cheese with health benefits. Proc. 8th Egyptian Conference for Dairy Science and Technology, Cairo, Egypt, papers II, pp.609-623.

Tamime, A. Y.: Marshall, V. M. and Robinson, R. K. (1995). Microbiological and Technology aspects of milks fermented by bifidobacteria. J. Dairy Res. 62: 151-187.

Tharmarai, 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

Vanos, V. and Cox, L. (1986). Rapid routine method for the detection of lactic acid bacteria among competitive flora. Food Microbiol., 3: 223-234.

Vinderola, C. G.; Prosello, W.; Ghiberto, T. D. and Reinheimer, J. A. (2000). Viability of probiotics (Bifidobacterium, Lactobacillus acidophilus and Lactobacillus casei) and noncrobiotic microflora in Argentinian Frescocheese, J. Dairy Sci., 83: 1905-1911.

Walter, J.: Hertel, C.: Tannock, G. W. Lis, C. M.: Munro, K. and Hammes, W. P. (2001). Detection of Lactobacillus, Pediococcus, Leuconostoc and Weissella species in human feces using group-specific PCR primers and denaturing gradient gel electrophoresis. Appl. Enveron. Microbiol., 67: 2578-2585.

نمو وحيوية بعض أنواع من بكتريا البروبيوتك عند استخدامها مع مزرعة من بكتريا حمض اللكتيك في جين القريش غير المملح

محسن عبد العزيز زمارة \* و عزة محمد الداز \*\*

\* قسم الألبان - كلية الزراعة - كفر الشيخ - جامعة طنطا

\*\* معهد بحوث الانتاج الحيواني مركز البحوث الزراعية - مصر

لاتفاض حيويه البختريا المحتلفة في محتواها الكلسي من البكتريا و كدنك بكتريا السالم وجود المتلافات ملموطة بين أصناف الجبن المختلفة في محتواها الكلسي من البكتريا وكدنك بكتريا السالم المتلفظة الكلسي من البكتريا Streptococci والسحت النسائج السضا احتفاظ البكتريا Lactobacill بعيويتها حتى نهاية مدة التخزين لتصل إلى حوالى ١٠ خلية جم جبن، بينما انخفضت حيوية البكتريا B. longum التفرين ليسلم التفرين للم من التخزين لتصل في نهاية التخزين السي تتوجد الفطريات والخمائر سواء في الجبن الطازجة أو بعد ٥ إلم من التخزين لتصل في نهاية التخزين السي حوالى ١٠ خلية أجم في أصناف الجبن المختلفة. أوضح التحكيم الحسى للجبن المختلفة عدم وجود أي طعوم غوية ما المحالة المحتوية المحتوية المحالة الم غريبة وتم قبولها جميعاً بصفة عامة مع حصول الجبن المصنعة ببكترياً B. longum على أعلى السدرجات على الرغم من وجود طعم ضعيف لجامض الخليك، تلاها جبن المقارنة والجسين المسصنعة بالبكتريسا casei ثم الجبن المصنع ببكتريا L. acidophilus التي تميزت بارتفاع حموضتها قليلا مقارنة بالجبن

الكتري. توضح النتائج المتحصل عليها في هذه الدراسة إمكانية استخدام بكتريب البروبيوتك خاصـة L. وacidophilus و Casei و B. Longum مع بادئ بكتريا حامض اللاكتيك في صناعة الجبن القريش الغير مملح ويشترط في ذلك حفظها تحت تبريد لمدة لاتزيد عن ١٥ يوم في حالة استخدام النوعيين الأولـين و ١٠ أيام بالنسبة للنوع الثالث حتى نضمن وجودها بأعداد مناسبة في الجبن الناتج (١٠-١٠ خليـة/جـم) بحيث تصل إلى الأمعاء في حالة نشطة وبأعداد مناسبة الإظهار خصائصها الصحية.