VIABILITY OF PROBIOTIC BACTERIA IN KARISH CHEESE DURING STORAGE PERIOD BY FREEZING OR IMMERSING IN VEGETABLE OILS

Tarek M. El-Nemr

Received on: 19/6/2005 *Accepted on: 23/8/2005*

ABSTRACT

Viability of probiotic bacteria in Karish Cheese was assessed during storage (6 months) by freezing (-15°C) and immersing in vegetable oils (sunflower and olive 1:1 v/v) at 20-25°C. The anaerobic conditions created by immersing in oil enhanced the viability of bifidobacteria comparing with freezing during storage period. On the other hand, increases of titrable acidity, acetaldehyde and nitrogen progressing were detected in the immersed Karish cheese rather than in frozen cheese. Both storage treatments were acceptable toward organoleptic properties.

(Key words: viability, probiotic bacteria, Karish Cheese, storage)

INTRODUCTION

Probiotics are living microorganisms incorporated into foods for health benefits (Fuller, 1989) and nutrition (Guarner and Schaafsma, 1998). Most commonly probiotic microorganisms used in dairy fermented industries are *Lactobacillus acidophilus* and bifidobacteria (Daly and Davis, 1998). Foods containing that microorganisms called "functional foods" and described as "foods claimed to have a positive effect on health" (Lee and Salminen, 1995). It should contain 10^6 – 10^7 cfu g^{-1} probiotic microorganisms and should be consumed at level higher than 100 g day^{-1} to have probiotic effects on health (Ishibashi and Shimamura, 1993). The market for probiotic containing products has rapidly grown because of their health benefits (Ross, *et al*, 2002).

Recently, probiotic bacteria have been introduced into many variety of cheese (Dinakar and Mistry 1994; Gomes *et al*, 1995; Blanchette *et al*, 1996; Gobbetti *et al*, 1998; El-Sayed, 1998; Ross *et al*, 2002 and Kasimoğlu *et al*, 2004). Karish cheese; which based on fermenting skimmed milk with lactic culture, is one of the famous and most popular cheese type consumed in Egypt (El-Sadek and Abd El-Mottaleb (1958) and Abou-Donia (1991). On the other hand, bifidobacteria have been incorporated into Karish cheese (Khattab *et al* 1986, Abou-Dawood, 2002, El-Nemr *et al*, 2003 and Fathy *et al* 2005) and recommended as a new product for the Egyptian market.

Karish cheese is mostly consumed and marketed in a fresh form (shelf life 15 days at 5 – 10°C for packed product and 3 days for non packed product). This study focused on the possibility of extending the shelf life of probiotic Karish cheese by freezing and immersing in vegetable oil similar to that used in Labneh preservation in the Middle East area (Turkan *et al* 1999). On the other hand, the viability of probiotic microorganisms was studied during storage period.

MATERIALS AND METHODS

Microorganism

Active mesophilic acidifying and aroma cultures (M. M. series) which contain *Lactococcus lactis* subsp. *lactis*; *Lactococcus lactis* subsp. *cremoris* and *Lactococcus lactis* var. *diacetylactis* obtained from Rhonne-Polulenc, France. Active mixed thermophilic *lactis* cultures containing *Lactococcus acidophilus*; *Bifidobacterium spp.* and *Streptococcus thermophilus* were obtained from Ch. Hansen Als, Denmark.

Culturing milk

Mesophilic acidifying and aroma producing cultures were used directly with an average inoculation level of 2 – 5 U/100 L milk at 25°C/5 hr. (pH 4.5 – 4.8). The thermophilic lactic cultures were added directly to milk with parameters of 50 U/250 L milk 37°C, 5 hr, (pH 4.5 – 4.8)

Bifidus milk

Active thermophilic cultures containing bifidobacteria were cultured in skimmed milk at 37°C for 1 hr before inoculation with the rest of the cultures at a ratio (1:1).

Cheese manufacturing

Karish cheese was manufactured by the conventional method from cow's skimmed milk (Faculty herd), antibiotic free and supplemented with 1.5% skimmed milk powder. Then milk was acted on by lactic acid bacteria, and the formed curd was ladled into wooden frames lined with muslin cloth, 1% salt was dispersed and the curd pressed by suitable weights. The resultant cheese was stored in deep-freezer at -15°C or immersed in vegetable oil at 20-20°C.

Immersing oil

Mixture of sunflower oil (Sunny, local product, Egypt) and olive oil (Janaklis, local product, Egypt) 1:1 were used for immersing the cheese during storage period as described by Turkan *et al*, (1999).

Media

Bifidobacterium was counted on lithium chloride galactose agar according to Lapierra *et al*, (1992) using double layered plates. The rest of cultures were counted by the standard plate colony count (SPC)

Department of Dairy Science and Technology, Faculty of Agriculture (Shatby), Alexandria University, Alexandria,

according to American Public Health Association APHA (1992).

Chemical Analysis

Total acidity was determined as the percentage equivalent of lactic acid (Desjardins *et al*, 1990). Acetaldehyde contents of acetaldehyde as ppm were determined using the basic fuchsin reagent according to the method of Robinson *et al*, (1977). While Formol and Shilovich numbers.

Formol and Shilovich numbers were measured by the method described by Twab and Hofi (1966).

Organoleptic evaluation

Karish cheese treatments were tasted for aroma and overall taste by a taste – panel of 15 expert members from Dairy Science and Technology Department. Samples were evaluated for Karish cheese flavor intensity, bitterness or other detectable flavor as strong, moderate and weak.

RESULTS AND DISCUSSIONS

Probiotic Karish cheese (43.7% Total solids) was stored by freezing -20°C, and by immersing in vegetable oils (sunflower and olive 1:1 v/v) 20 – 25°C (Fig. 1). Physico-chemical properties, organoleptic and the viability of probiotic bacteria were assessed during storage period (6 months).

Physico-chemical properties of stored probiotic Karish cheese

Fig. 1 shows the storing of the cheese by immersing in vegetable oil. While data in Fig. 2 reveal changes in pH, acidity during storage period of probiotic Karish cheese stored by freezing or immersing in vegetable oils. Comparing with the immersing cheese while showed more decreasing in the pH value specially at the end of the storing at 25°C which may be due to the storage temperature is more suitable to the growth of the starter used. On the other hand, titrable acidity of all treatments showed increasing acidity during storage period, it reached 1.48 and 1.80% for freezed and immersed cheese, respectively. Acetaldehyde contents (ppm) presented in (Fig. 3) was nearly double for immersed Karish cheese compared with freezed cheese during storage period. After 30 days of storage, both cheeses gradiently decreased in acetaldehyde content. Changes of acetaldehyde contents as a result of bacterial action in fermented dairy products particularly probiotic products were previously observed by Akalina, 1996; Helmy *et al*, 2000 and El-Nemr *et al*, 2003. Acetaldehyde is the major flavor component in dairy fermented product (Hild, 1979). Bifidobacteria have been reported to produce 3:1 moles of acetic: lactic acid during fermentation (Dave and Shah, 1997). Nitrogen content progressing (soluble nitrogen) was expressed as formol and shilovich numbers (Fig. 4). Values generally increased during storage, due to protein breakdown which agree with Abdou and Dawood, 1977. Elevation in soluble nitrogen values were noticed for probiotic Karish cheese stored in vegetable oils comparing with cheese stored by freezing. This is due to increasing of proteolytic activity under anaerobic conditions which are desired to *Bifidobacterium spp.* These findings are consistent with that of El-Nemr *et al*, 2003 and Helmy *et al*, 2005.

Microbiological properties of stored probiotic Karish cheese

Viable counts of *Bifidobacterium spp.* and Karish cheese starter bacteria are shown in Fig. 5. Bifidobacteria remained viable in both stored Karish cheese with the level of probiotic action (10^6 cfu g^{-1}) as referred by Kurmann, 1988 and Mattila – Sandholm *et al*, 2002. Viable counts of bifidobacteria were higher in immersed Karish cheese rather than the freezed one, that may be due to anaerobic conditions (Dave and Shah, 1997) which created by oil. On the other hand, freezing or immersing in oil hold the rest of lactic acid bacteria viable during storage period. Enhancing of the viability of probiotic bacteria in fermented dairy products by different procedures was studied by many researchers (Dave and Shah, 1997 and 1998; Gomes *et al*, 1998; Vinderola *et al*, 2002; Gooda *et al*, 2002 and El-Nemr *et al*, 2003 and 2004). Most of that researches based on using Oxygen scavenger (Ascorbic acid and Cystein hydrolysates) or prebiotics. Oxygen content and redox potential have been shown to be important factors for the viability of Bifidobacteria during storage of fermented milk product (Brunner *et al* 1993).

Sensory evaluation of stored probiotic Karish cheese

Generally, probiotic Karish cheese which stored by freezing and immersing in vegetable oils gave an acceptable results along the storage period (Table 1). Freezed probiotic cheese has higher scores particularly at the end of storage period.

CONCLUSION

The current study showed that probiotic Karish cheese could be stored by freezing or immersing in vegetable oils up to 6 months and holds the level of probiotic action (10^7 cfu g^{-1}). The anaerobic conditions which created by immersing cheese in oil enhanced the viability of probiotic bacteria rather than storage by freezing.

Fig. 1: Probiotic Karish cheese stored by immersing in vegetable oils (Sunflower and olive 1: 1 v/v) at 20 – 25°C.

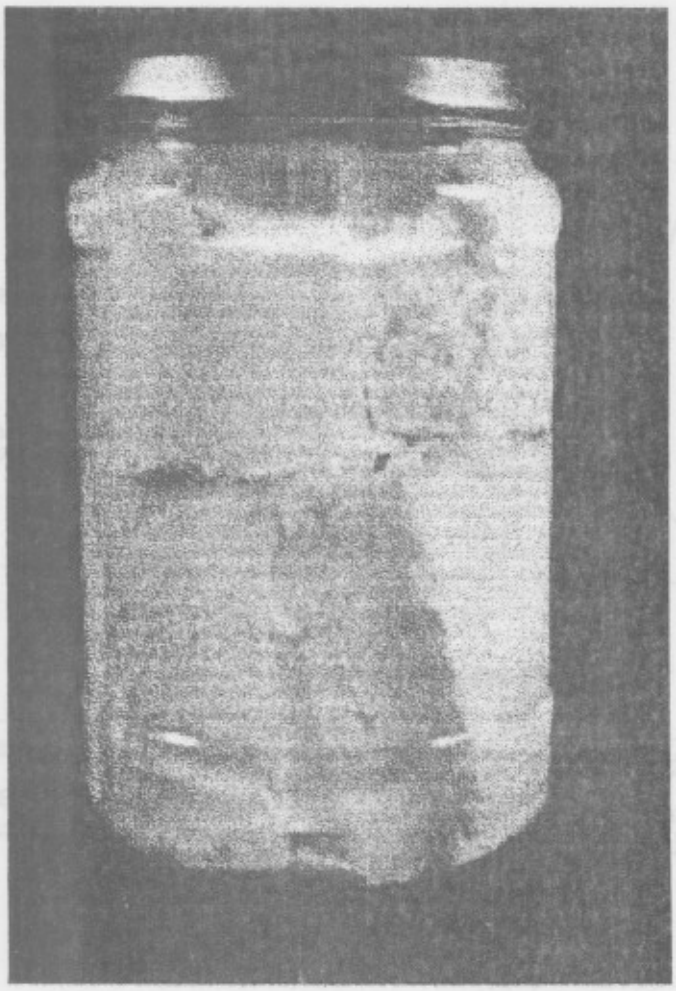

Fig. 2: Changes in titrable acidity (% lactic acid) and pH in probiotic Karish cheese stored by freezing and immersing in vegetable oil during storage period (Average of 3 replicates).

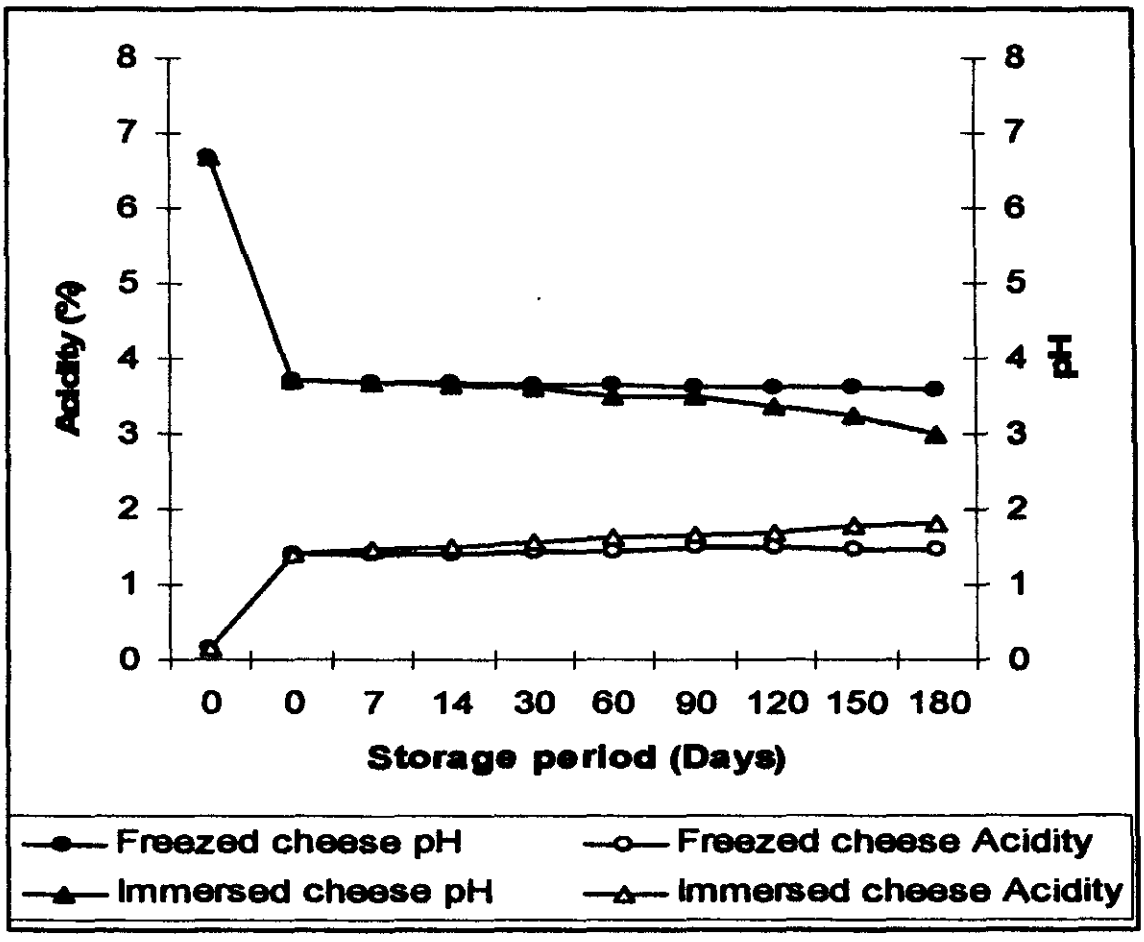

Fig. 3: Acetaldehyde contents (ppm) of probiotic Kariah cheese stored by freezing and immersing in vegetable oil during storage period (Average of 3 replicates).

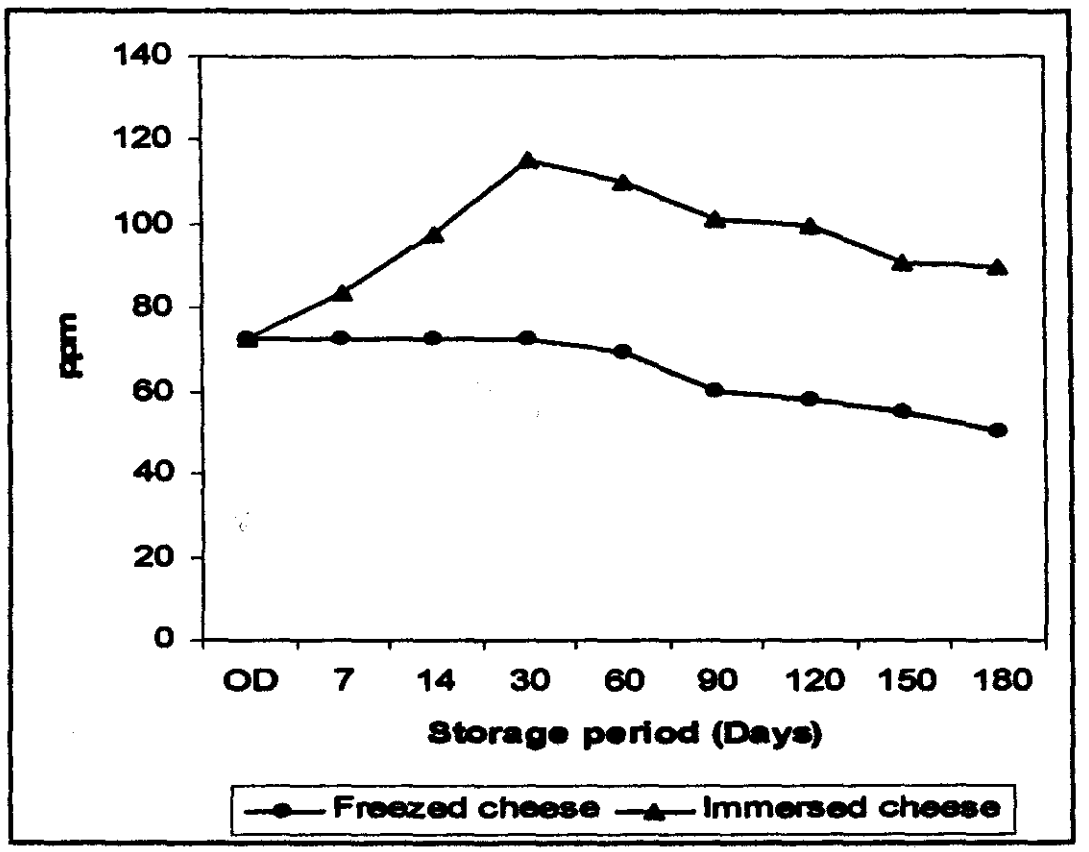

Fig. 4: Nitrogen progressing (Formol and Shilovich No) of probiotic Karish cheese stored by freezing and immersing in vegetable oil during storage period (Average of 3 replicates).

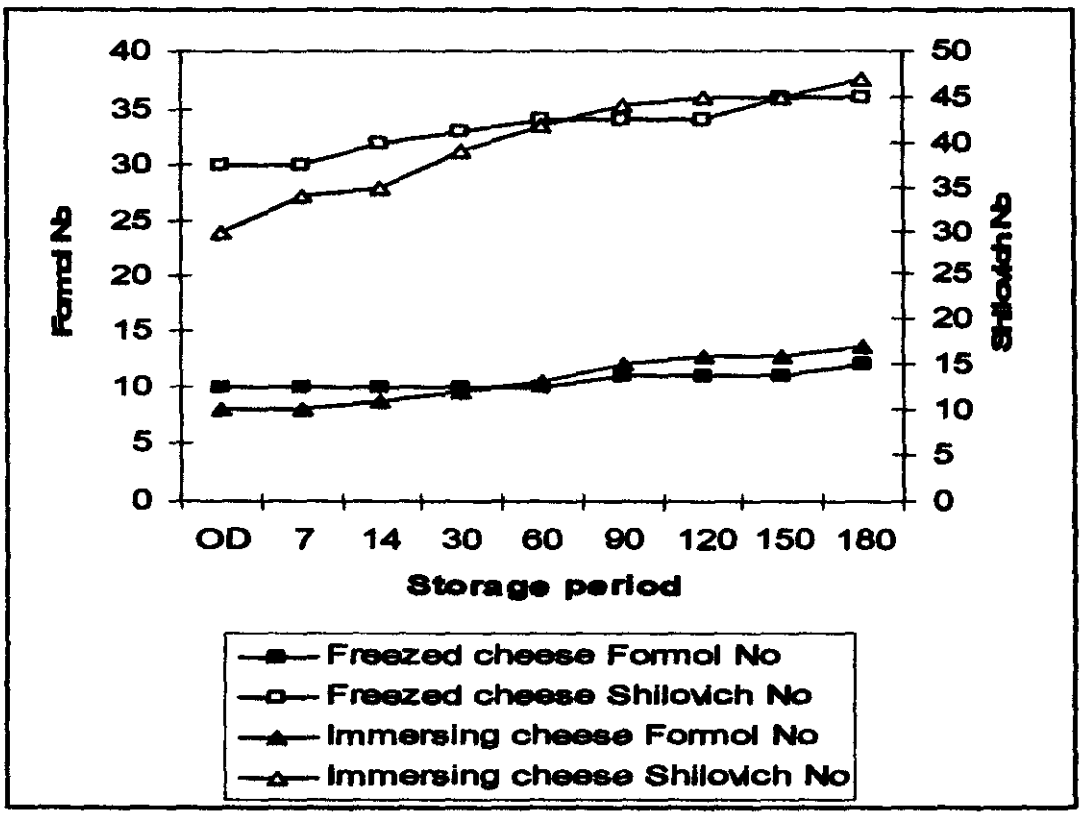

Fig. 5: Viable counts (cfu/g) of probiotic Kariah cheese stored by freezing and immersing in vegetable oil during storage period (Average of 3 replicates).

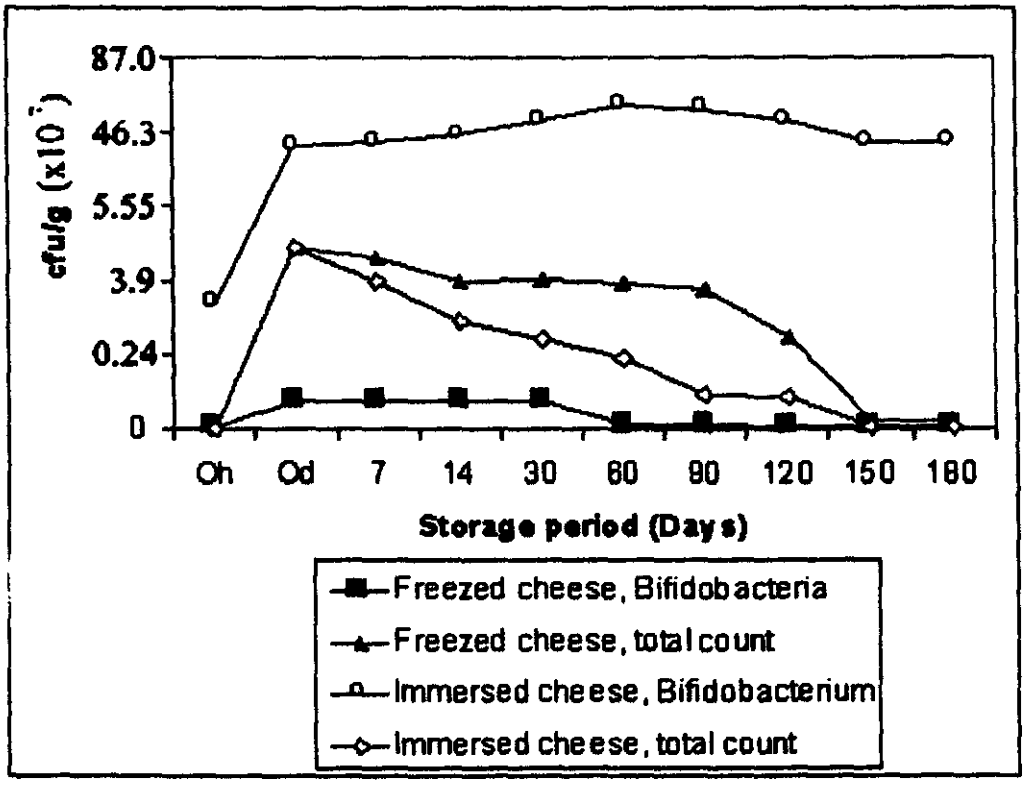

Table 1: Organoleptic scoring of probiotic Karish cheese stored by freezing and immersing in vegetable oil during storage period (Average of judgers).

Storage period (Days)	Freezed cheese			Immersed cheese		
	Acid taste	Texture	No. Bitterness	Acid taste	Texture	No. Bitterness
Od	9.5	10	10	9.5	10	10
7	10	9	10	9	9	10
14	10	9	10	9	9	10
30	10	9	10	9	8	10
60	9.5	8	10	8.5	8	10
90	9	8	10	8.5	8	9
120	9	8	10	8	8	9
150	9	8	10	7	7	8.5
180	8.5	7	10	7	7	8

REFERENCES

Abdou, M. S. and Dawood, A. H. M. (1977). Effect of heat treatment of skim milk on yield, quality and chemical composition of skim milk cheese (Karish). Egyptian J. Dairy Sci. 5: 1.

Abou Dawood, S. A. I. (2002). Survival of nonecapsulated and encapsulated *Bifidobacterium bifidum* in probiotic Karish cheese. Egyptian J. Dairy Sci, 30: 43.

Abou-Donia, S. A. (1991) Manufacture of Egyptian soft, pickled cheeses. In: "Feta and Related cheeses" R. K. Robinson and A. T. Tamime (E. d.), Ellis Horwood, London.

Akalina, A. S. (1996). L^+ D^- lactic acid contents and aroma profiles in bioghurt, bifighurt, biogard in comparison with yoghurt. Egyptian J. Dairy Sci. 24: 227.

American Public Health Association (APHA) 1992. "Methods for the Microbiological Examination of Foods" 3rd ed. Carl Vanderzant and Don F. Splittstoesser (Ed), Washington D. C. 2005.

Blanchette, L.; Roy, D.; Belanger, G. and Gowthier, S. F. (1995). Production of cottage cheese using dressing fermented by Bifidobacteria. J. Dairy Sci. 81: 37.

Brunner, J. C. Spillmanns, H. and Puhan, Z. (1993). Changes in pH, free SH groups, Oxygen and redox potential during fermentation of milk with *Bifidobacterium longum*. Milchwirtschaftliche-Forschung, 22: 26.

Dave, R. I. and N. P. Shah (1977). Viability of yoghurts and Probiotic Bacteria in yoghurts made from commercial starter cultures. Int. Dairy Journal 7: 31.

Dave, R. I. and Shah, N. P. (1997). Effectiveness of ascorbic acid as an Oxygen Scavenger in improving viability of probiotic bacteria in yoghurts made with commercial starter cultures. International Dairy J. 7: 435.

Dave, R. I. and Shah, N. P. (1998). Ingredient supplementation effects on viability of probiotic bacteria in yoghurt. J. Dairy Sci. 81: 2804.

Desjardins, M.; Roy, D. and Goulet, J. (1990). Growth of *Bifidobacterium* and their enzyme profiles. J. Dairy Sci. 73: 299.

Dinakar, P. and Mistry, V. V. (1991). Growth and viability of *Bifidobacterium bifidum* in cheddar cheese. J. Dairy Sci. 77: 2854.

Doly, C. and Davis, R. (1998). The biotechnology of lactic acid bacteria with emphasis on applications in food safety and human health. Agricultural Food Science, Finland 7: 219.

El-Nemr, T. M.; Awad, S. A. and Ali, A. H. (2003). Increasing of probiotic and therapeutic action in Karish cheese using Tolue Balsam extract. Egyptian J. of Food Sci., 31(1-2): 213.

El-Nemr, T. M.; Awad, S. A. and Ali, A. H. (2004). Cheese whey and skimmed milk as a base for probiotic dairy fermented products supplemented with some herb oils. The 9th Egyptian Conf. for Dairy Sci. & Tech. "Milk and Dairy Products for a healthy Future", Dokki, 9 – 11 October, Cairo, Egypt.

El-Sadek, G. M. and Abd El-Mottaleb, L. (1958). The effect of heat treatment and temperature of storage on the yield, quality and chemical composition of Karish cheese. J. Dairy Res., 25, 480-485.

El-Sayed, E. M. (1998). Growth and survival of bifidobacteria in Ras cheese for use as a probiotic food. Annals of Agric. Sci. Moshtohar, 36: 2381.

Fathy, F. A.; W. I. El-Kholy and N. F. Tawfik (2005). Survival of micro capsulated *Bifidobacterium bifidum* and *Propionibacterium freudenreichii* subsp. *shermanii* in Karish cheese. Egyptian J. Dairy Sci., 33: 65-7.

Fuller, R. (1989). Probiotics in man and animals. J. Appl. Bacteriology, 66: 365.

Gomes, A. M. P., Malcata, F. X.; Klaver, F. A. M. and Grande, H. J. (1995). Incorporation and survival of *Bifidobacterium sp.* strain BO and *Lactobacillus acidophilus* strain K: in a cheese product. Neth. Milk Dairy J. 49: 71.

Gomes, A. M. P.; Vieira, M. M. and Malcota, F. X. (1998). Survival of probiotic strains in cheese during ripening: Simulation of rates of salt diffusion and microorganism survival. J. of Food Engineering, 36: 281 – 301.

Gooda, E.; El-Nemr, T. and A. H. Malak (2002). Viability of *Bifidobacterium sp.*in ice milk product enhanced by some herb oil. J. Agric. Sci. Mansoura Univ. 27(5): 3313.

Guarner, F. and Schaafsma, G. J. (1998). Probiotics. International J. of Food Microbiology, 39: 237.

Helmy, A. H.; El-Nemr, T. M. and Attia, I. A. (2000). Caraminative bio – yoghurt enrichment of bifido – yoghurt with some herbs oils. J. Agric. Mansoura Univ. 25(7): 4389.

Helmy, A. M.; El-Nemr, T. M.; Awad, S. A. and Attia, I. A. (2005). Interaction between probiotic labneh and probiotic whey enhanced with some herb oils and different packaging materials. Alexandria J. Agric. Res. 50(1): 63.

Hild, J. (1979). Quantitative bestimung einiger wichtiger aromakomonenten. Milchwissenschaft, 34: 281.

Ishibashi, N. and Shimamura, S. (1993). Bifidobacteria research and development in Japan. Food Technology, 47: 126.

Kasimoğlu, A. Göncüoğlu, M. and Akgün, S. (2004). Probiotic white cheese with *Lactobacillus acidophilus*. International Dairy Journal, 14: 1067.

Khattab, A. A.; Zaky, Nadia and Shoukry, YMR (1986). The use of *Lactobacillus acidophilus* and *Bifidobacterium adolscentis* in Karish cheese making. Comm. In Sci. & Development Res., 15: 118.

Kurmann, J. A. (1988). Starters with selected intestinal bacteria. Pages 41 – 55 in IDF Bull. No. 227. Ind. Dairy Fed., Brussels, Belgium.

Lapierra, L.; Undeland, P. and Cox, L. J. (1992). Lithium chloride-sodium propionate agar for the enumeration of *Bifidobacterium* in fermented dairy products. J. Dairy Sci. 75(5): 1192.

Lee, T. K. and Salminen, S. (1995). The coming of age of probiotics. Trends in Food Science and Technology, 6: 241.

Mattila – Sandholm, T.; Myllärinen, P.; Crittenden, R.; Megensen, G.; Fondèn, R. and Saarela, M. (2002). Technological challenges for future probiotic foods. International Dairy J. 12: 173.

Robinson, R. K.; Gamine, A. and Chobb, I. (1977). Acetaldehyde as an indicator of flavor intensity in yoghurt. Milk industry, 79: 4.

Ross, R. P.; Fitzgerald, G.; Collins, K. and Stanton, C. (2002). Cheese delivering biocultures – probiotic cheese. Australian J. of Dairy Technology, 57: 71.

Tawab, G. and Hofi, A. (1966). Testing cheese ripening by rapid chemical techniques. Indian J. Dairy Sci. 19: 39.

Turkan, K.; Robinson, R. K. and Gordon, M. H. (1999). The role of olive oil in the preservation of yoghurt cheese (Labneh anbaris). International J. of Dairy Technology, 52(2): 68.

Vinderola, C. G.; Prosello, W.; Ghilberto, D. and Reinheimer, J. A. (2002). Viability of probiotic (*Bifidobacterium, Lactobacillus acidophilus* and *Lactobacillus casei*) and nonprobiotic microflora in Argentinean Fresco cheese. J. of Dairy Science, 83: 1905.

الملخص العربى

حيوية البكتيريا الداعمة حيوياً في الجبن القريش خلال فتره تخزينها باستخدام التجميد و النقع في مخلوط زيتي لفترة طويلة

طارق مراد النمر
قسم علوم و تكنولوجيا الألبان، كلية الزراعة (الشاطبي)
جامعة الإسكندرية، جمهورية مصر العربية

تم تقدير معدلات البقاء بحيوية للبكتيريا الداعمة حيوياً في الجبن القريش خلال فترة تخزينها لمدة طويلة (٦ أشهر) باستخدام التجميد(- ١٥°م) و كذلك في مخلوط زيت نباتي على حرارة الغرفة؛ و التي تراوحت بين ٢٠ – ٢٥°م. هذا، و لقد أتاحت الظروف اللاهوائية للغمر في الزيت الى تحسين كلاً من الحيوية لبكتيريا البيفيدو و كذلك زيادة معدلات الحموضة و محتويات الأسيتالدهيد و التسوية بالمقارنة مع طريقة التجميد. بينما حققت كلا الطريقتين تقبلاً تجاه الخواص الحسية طوال فترة التخزين.