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PROBIOTICS AS A TOOL TO IMPROVE MICROBIAL QUALITY OF SOFT CHEESE

(With 4 Figures)

By
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المدعمات الحيوية كأداة لتحسين الجودة الميكروبية للجبن الطري

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تعتبر المدعمات الحيوية عند استخدامها لها تأثير فعال على صحة الإنسان حيث أنها تقلل من نسبة الأمراض المعدية ونسبة حدوث سرطان القولون تقلل من نسبة الكوليسترول. منتجات الألبان أكثر الأغذية أهمية لما تحتويه من عناصر غذائية ضرورية لبناء جسم الإنسان، ويعتبر الجبن الدمياطي أكثرها استهلاكاً لمذاقه المميز وسعره المناسب ونظراً لإنتاج هذه المنتجات بكميات كبيرة وبطرق تصنيع مختلفة وتحت ظروف صحية قد تعرضها لأن تكون سبباً لإصابة المستهلك بالأمراض المختلفة. تم دراسة نمط نمو وتأثير نوعان من سلالات المدعمات الحيوية (لاكتوباسيلاس (1%) والبايفيدوبكتيري (1%)) كل على حده واستخدام مزيج منهما (1:1%) على العد الكلي للميكروبات القولونية والميكروب العنقودي الذهبي للجبن الدمياطي أثناء التصنيع والتخزين في درجة حرارة الثلاجة (4 ±1 م °). وقد أسفرت النتائج على أن المدعمات الحيوية قد خفضت العد الكلي للميكروبات القولونية والميكروب العنقودي الذهبي للجبن الدمياطي بالمقارنة بالجبن الدمياطي التقليدي بنسب متفاوتة. وقد وجد أن الجبن المدعم باللاكتوباسيلاس له القدرة على خفض ميكروب العنقودي الذهبي أكثر من االبايفيدوبكتريا. كما أن المدعمات الحيوية كان عددها في الجبن الدمياطي عند نهاية فترة التخزين في الحدود التي من خلالها تعطي تأثيراً ايجابيا على صحة الإنسان.

SUMMARY

Functional foods incorporating probiotic bacteria such as *bifidobacteria* and *lactobacilli* have a variety of beneficial health effects in human. The objective of the present study was to investigate the survival of *L. acidophilus* and *Bifidobacterium lactis* during manufacture and storage of Damietta cheese stored at 4±1^{o}C in brine and to determine the effects of *Lactobacillus acidophilus* and *Bifidobacterium lactis* on the microbial quality of Damietta cheese. Cheese samples were examined for coliforms and *Staphylococcus aureus* counts. The results revealed a significant ($P > 0.05$) differences in the counts of c*oliforms* and *S.aureus* in the probiotic cheese with those in control cheese. The obtained results verify that using *Lactobacillus acidophilus* and *Bifidobacterium lactis* as probiotics had a greater inhibitory effect on *coliforms* and *Staphylococcus aureus* counts in cheese. *Lactobacillus acidophilus* probiotic cheese is more effective in inhibition of *S.aureus* than either probiotic cheese with *Bifidobacterium lactis* or combination of them ($P > 0.05$). The final numbers of *L. acidophilus* and *Bifidobacterium lactis* were greater than the minimum (10^7 cfu /g), as suggested by Ishibashi and Shimamura (1993), required to produce health benefits claimed for probiotic cheese. The results showed that Damietta cheese can be an effective carrier of probiotics to consumer.

Key words: *Probiotics, S.aureus, Coliforms, Damietta cheese*

INTRODUCTION

There has been an increasing interest in dairy products containing specific bacterial species with potential health improving properties. Probiotic bacteria are defined as living microorganisms which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition (Ross *et al.,* 2002). Health benefits for products containing live probiotic bacteria include alleviation of symptoms of lactose intolerance, treatment of diarrhea, anticarcinogenic properties, reduction in blood cholesterol and improvement in immunity (Shah, 2007). Consumption of high concentration of probiotic bacteria at 10^7 viable cells per gram or per millilitre of product is required to confer health benefits (Ishibashi and Shimamura, 1993).

As in the case of any probiotic food, in order to exert their health benefits on the consumer's body, probiotic bacteria incorporated in cheese must be able to grow and/or proliferate in the human intestine and therefore should be able to survive during the passage through the gastrointestinal tract (GIT), which involves exposure to hydrochloric acid in the stomach and bile in the small intestine (Stanton *et al.,* 2003).

The majority of probiotic foods already in the market, such as fermented milk and yoghurt are fresh products and are generally consumed within days or weeks of manufacture. Cheese may offer certain advantages over yoghurt-type products in terms of delivery of viable probiotics, such as the higher pH of the cheese, the higher fat content and more solid consistency of cheese may offer protection to the probiotics in the gastrointestinal tract (Stanton *et al.*, 1998).

Studies showed that functional foods incorporating probiotic bacteria such as *bifidobacteria* and *lactobacilli* have a variety of beneficial health effects in human. Due to the repeated therapeutic effects of these organisms, Japan and several European countries have been actively involved in the development and application of *bifidobacteria* (Gomes and Malcata, 1999). Such products are gaining more widespread popularity and acceptance throughout the world, and predictions are that the global market for this type of food will grow rapidly in the coming years (Ross *et al.*, 2000).

In fact, cheese provides a valuable alternative to fermented milks and yogurts as a food vehicle for probiotic delivery, due to certain potential advantages. It creates a buffer against the high acidic environment in the gastrointestinal tract, and thus creates a more favorable environment for probiotic survival throughout the gastric transit, due to higher pH. Moreover, the dense matrix and relatively high fat content of cheese may offer additional protection to probiotic bacteria in the stomach (Ross *et al.*, 2002; Bergamini *et al.*, 2005). This finding was confirmed by Sharp *et al.* (2008).

Damietta cheese is a soft white salty cheese made primarily in Egypt and other Middle Eastern countries. It is typically made from buffalo milk, cow milk, or a mixture. Unlike Feta and other white cheeses, salt is added directly to the milk, before rennet is added.

The development of Damietta cheese containing *Bifidobacteria* was demonstrated by Effat (2000); El-Zayat and Osman (2001) and Shehata *et al.* (2001) and reported that the resultant cheese had the highest scores for organoleptic properties. Mehanna *et al.* (2002) prepared probiotic soft cheese with highly acceptable qualities and the authors considered soft cheese to be a good source for delivering these probiotic bacteria to consumers.

A number of studies have addressed the development of probiotic cheeses including fresh cheese (Gomes and Malcata, 1998; Gomes *et al.*, 1998), semi hard cheese (Bergamini *et al.*, 2006) and white-brined cheese (Özer *et al.*, 2009). These studies have demonstrated that cheeses have a great potential as a carrier to deliver probiotic bacteria to the consumer. Most publications concerning incorporation of probiotic bacteria into cheeses have focused only on their survival during manufacture and storage. So, this study was planned to declare the effect of probiotics in improving the microbial quality of soft cheese.

MATERIALS and METHODS

2.1. Cultures

L. acidophilus and Bifidobacterium lactis were obtained from the Faculty of Agriculture, Alexandria University. *L. acidophilus* was activated by growing two times at 30 ^{o}C overnight in 120 mg/ml sterile reconstituted skim milk (RSM) containing 20 mg/ml glucose and 12 mg/ml yeast extract. *Bifidobacterium lactis* was sub-cultured similarly using 10 ml/ml inocula in sterile RSM supplemented with 0.5 mg/ml L-cysteine hydrochloride (Sigma–Aldrich, St. Louis,MO, USA).

2.2. Cheese preparation (Fahmi and Sharara, 1950):

Damietta white cheese was manufactured in the pilot plant (Department of Food Hygiene; Faculty of Veterinary Medicine; Alexandria University). Eighty liters antibiotic-free whole buffalo milk (pH 6.8) was obtained from the Veterinary Medicine Practice Farm of Alexandria University. Milk was pasteurized at 72^{o}C for 2 min. After the milk was cooled to 32^{o}C, $Cacl_2$ (0.02%) were added into the milk. Milk was transferred to stainless steel cheese vats, each containing 20 L of milk. The first vat, the probiotic cheese, milk was inoculated with *L. acidophilus* (1ml/100ml), the second one was inoculated with *Bifidobacterium lactis* (1ml/100ml), the third vat was inoculated with combination of *L. acidophilus* (1ml/100ml) and *Bifidobacterium lactis* (1ml/100ml) while the last one was used as a control. Chymosin was added to each cheese vat at a level sufficient to coagulate the milk in 60 min (50 ml/20 L). The coagulum was cut with a sterile knife into small cubes and the curds were allowed to rest in the whey for 5–10 min. The curds (pH 6.4) were transferred to stainless-steel moulds lined with cheesecloth. The surfaces of the cheeses were covered with cheesecloth, drained without pressure for 20 min, and pressed (40 kg weights for 100 L milk) for 3 h. The weights were removed, the cheese cloths opened and the cheese mass cut into cubic pieces. The cubes were brine-salted (5%, w/w, NaCl) for 13 h. After brine salting, each batch was stage of manufacture. Then each batch stored at 4^{o}C for 60 days. held at room temperature (20^{o}C) for 6 h; this is described as the resting

2.3. Sampling

Raw milk, fresh curd and cheese samples were examined for c*oliforms*, *S.aureus*, *L. acidophilus* and *Bifidobacterium lactis* bacteria.

Cheese samples were taken for bacterial enumeration at 10, 20, 30, 40, 50 and 60 days of storage.

2.4. Microbiological analysis

From each cheese type, 10 g cheese were transferred into a sterile bag under aseptic conditions and homogenized in 90 ml of sterile Trisodium citrate solution 2% for 2 min using a Lab blender 400 stomacher. Serial dilutions were prepared by adding l ml to 9ml sterile peptone water (0.1 %). All samples were tested for:

1 - Counts of *L. acidophilus* and *Bifidobacterium lactis* MRS agar (pH 5.4) (Oxoid) and incubated anaerobically (GasPak System, Oxoid) at 37^{o}C for 3 days.

2 - *Coliforms* count (MPN/g) was done according to Harrigan (1998).

3 - *Staphylococcus aureus* count was done according to ICMSF (1986).

2.5. Statistical analysis

All samples were analyzed in duplicate and the experiment was repeated three times by using SPSS 10 statistical software (SPSS, 1999).

RESULTS

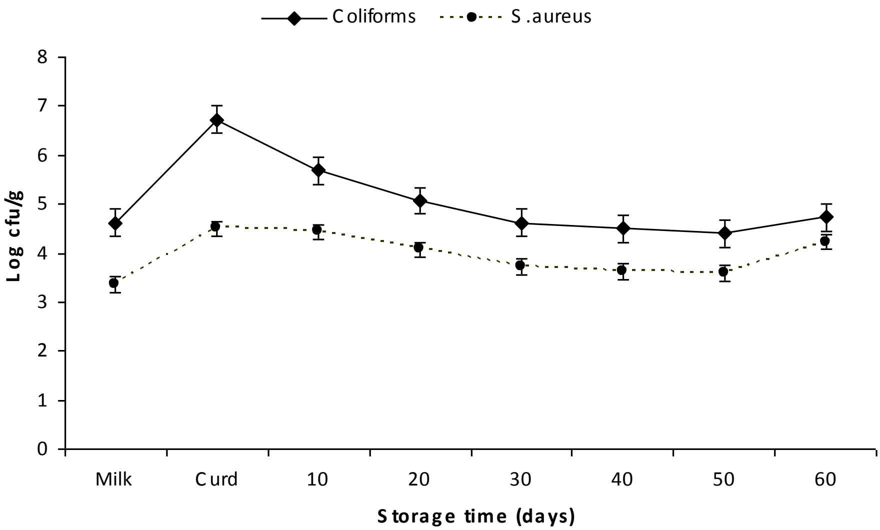

Fig. 1: The growth patterns of coliforms and Staphylococcus aureus in Damietta cheese samples stored at 4˚C

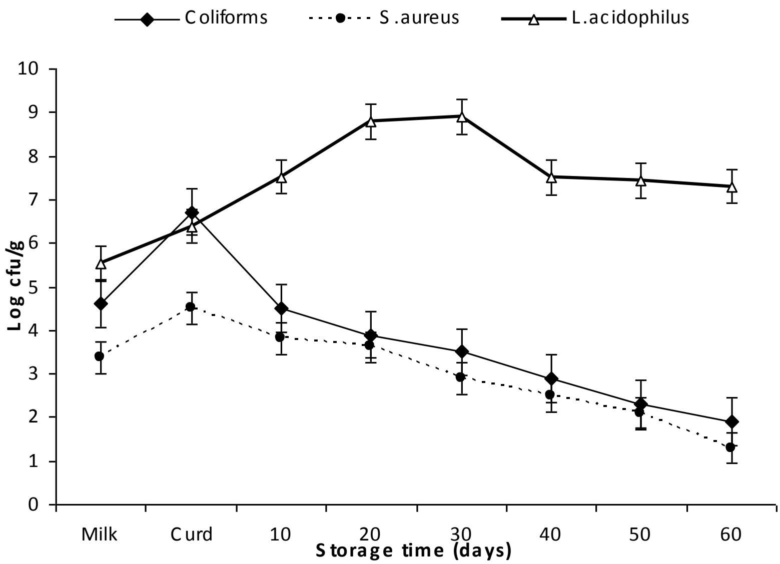

Fig. 2: The growth patterns of coliforms and Staphylococcus aureus in Probiotic Damietta cheese samples with L. acidophillus stored at 4˚C

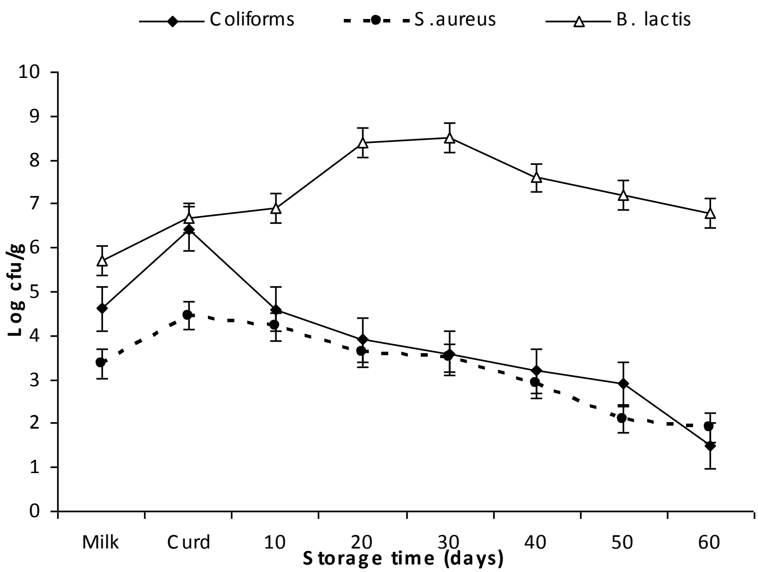

Fig. 3: The growth patterns of coliforms and staphylococcus aureus in probiotic Damietta cheese samples with B. lactis stored at 4˚C

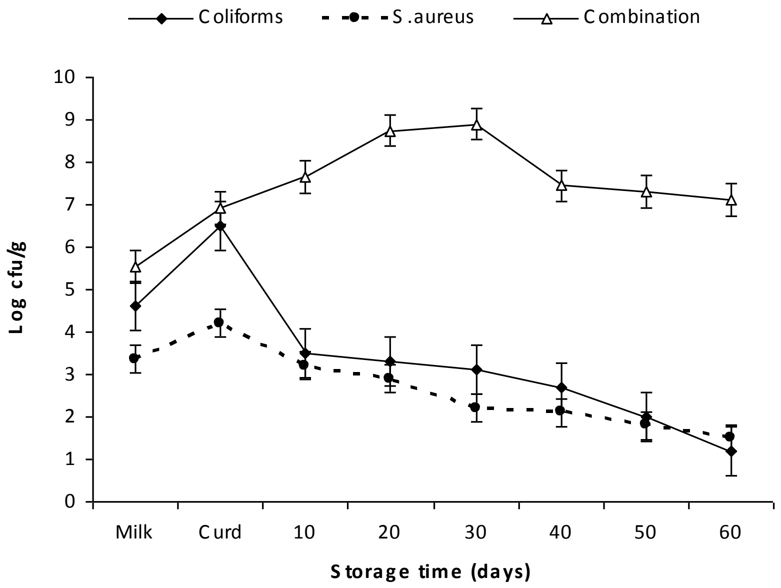

Fig. 4: The growth patterns of coliforms and Staphylococcus aureus in probiotic Damietta cheese samples with combination of L. acidophillius and B.lactis stored at 4˚C

DISCUSSION

The results presented in Figure 1 verify the growth pattern of *coliforms* and *Staphylococcus aureus* in Damietta cheese samples during storage at 4^oC as their counts in milk used in manufacture were 4.61 and 3.36 log cfu/g, respectively. Their counts increased gradually to reach maximum number at the 20^{th} day of storage period followed by a gradual decrease in count by the end of storage period reaching 4.74 and 4.23 log cfu/g, respectively. These results were substantiated with those obtained by Aiad (2002) and Amer and Ewina (2003).

Coliforms, in dairy products, played an important role in microbiological analysis on accounting of their significance as indicator organisms for pinpointing the unhygienic condition during processing, handling and distribution. The presence of *S.aureus* in soft cheese may originate from skin, mouth and/or nose of workers handled the food. *S. aureus* is a good indicator of the personal hygiene of workers with respiratory infection and suppuration (Kamat *et al.,* 1991).

The growth of *S.aureus* in food products is a potential public health hazard since many strains, in favorable growth conditions produce thermostable enterotoxins which cause intoxication food poisoning if ingested as well as neurotoxins which act on vomiting centers in the brain via the vagus nerve (Adams and Moss, 2000). However, Luca *et al.* (1997) reported that *S.aureus* was the leading cause of foodborne intoxication, and the minimum infection dose was 10^5-10^7/g or 1-20µg enterotoxin /person.

The numbers of *L. acidophilus* in the probiotic cheese during storage are shown in Figure 2. Initial numbers of *L. acidophilus* inoculated into the milk were 5.54 log cfu/ml, but they grew rapidly during the first 10 days of storage and reached to 7.52 log cfu/ g in cheese. Rapid growth of *L. acidophilus* might be due to the fermentation of lactose. It is well known that lactobacilli grow best under acidic conditions (Kandler and Weiss, 1986). High moisture and low salt content in the centre of the cheese might support *L. acidophilus* for 10 days.

The viable cell numbers of *L. acidophilus* began to decrease after 30 days of storage, because of the decrease in moisture level, subsequently increase in salt content, and the low storage temperature (4^{o}C). Although *L. acidophilus* decreased until the end of the storage period, it did not decrease below 7.3 log cfu /g cheese. As indicated earlier, it is necessary to maintain the viability of *L. acidophilus* at 7 log cfu /g of cheese, to call the cheese probiotic (Ishibashi and Shimamura, 1993). Also, it was stated that a minimal concentration of 10^7cfu/g or ml of food should be present at the moment of intake to assure a favorable impact of probiotics on a consumer's health (De Vuyst, 2000).

The present results are similar to the results obtained by Bergamini *et al.* (2005) who found a viability of 10^9 cfu/g in a semi-hard Argentinean cheese produced with *Lactobacillus paracasei* in a substrate composed of milk and milk fat on day 60. Our results are also in accordance with previous studies conducted for Turkish Beyaz cheese (Akgün, 1995; Kasimoglu *et al.*, 2004).

The results in Figure 2 revealed that a gradual decrease in numbers of *coliforms* and *S.aureus* occurred during the prolonged shelf-life of Damietta cheese kept at 4$\pm$1^{o}C. *Coliforms* and *S.aureus* achieved the minimum counts on the 60th day of storage reaching 1.9 and 1.3 log cfu/g, respectively. There were significant (P >0.05) differences in the counts of *Coliforms* and *S.aureus* in the probiotic cheese with those in control cheese.

Data revealed in that Damietta cheese (control) showed high *Staphylococcus aureus* count than that probiotic one. These results suggested that probiotic bacteria incorporated in the manufacture of Damietta cheese reducing the growth of *S.aureus* in cheese is due to facultative anaerobic conditions created by probiotic bacteria in fermented food (Batish *et al.,* 1997), or due to their lactic and organic acids production, decreasing the pH of the growth environment (Caplice and Fizgerald, 1999).

The high antimicrobial activity of Lactobacilli is associated with the production and synergistic activity of organic acids and hydrogen peroxide, whereas the antagonistic activity of Lactobacilli against Gram-negative and Gram –positive bacteria dependent on the fermentation group of Lactobacilli (Annuk *et al.,* 2003). Axelsson *et al.* (1989) and Chung *et al.* (1989) reported that some strains of Lactobacilli produced β–hydroxylpropion aldehyde induced from glycerol which had a wide antimicrobial spectrum against Gram-negative and Gram –positive bacteria. This may be explaining the present findings.

The survival of the *Bifidobacterium lactis in* Damietta cheese over 60 days of storage was studied. It was seen that, in the first 10 days, an initial increase in numbers, was observed to reach the maximum numbers after 30 days of storage 8.5 log cfu/g and then in the period 40-60 days it declines reaching 6.78 log cfu/g (Figure, 3). Given a consumption of a nominal one serving (30 g) of cheese/day, the intake of each *Bifidobacterium* would be between 10^9 and 10^{10}/day that is well above the levels suggested as providing therapeutic benefits (Boylston *et al.,* 2004).

The data presented in Figure 3 showed the growth pattern of *coliforms* and *S.aureus* counts occurred during the prolonged shelf-life of Damietta cheese kept at 4 ± 1^{o}C. *Coliforms* and *S.aureus* reached lowest counts on the 60^{th} day of storage reaching 1.5 and 1.9 log cfu/g, respectively. There were significant ($P > 0.05$) differences in the counts of *coliforms* and *S.aureus* in the probiotic cheese than those in control cheese.

Bifidobacteria produce bacteriocins which are active against *S. aureus* (Cotter *et al.,* 2005). Many *Bifidobacteria* produce bacteriocins with rather broad spectra of inhibition and several

Bifidobacteria bacteriocins offer potential applications for food preservation (Marshall and Tamime, 1997; Galvez *et al.,* 2007).

It is evident from Figure 4 that *coliforms* and *S. aureus* counts were decreased in probiotic cheese with combination of *Lactobacillus acidophilus* and *Bifidobacterium lactis* (1:1%) stored at (4 ± 1^oC) reaching 1.2 and 1.5 log cfu/g, respectively, by the end of storage period. There were significant (P >0.05) differences in the counts of *coliforms* and *S.aureus* in the probiotic cheese with combination of *Lactobacillus acidophilus* and *Bifidobacterium lactis* (1:1%) than those in control cheese.

The obtained results verified that combination of *Lactobacillus acidophilus* and *Bifidobacterium lactis* (1:1%) had a greater inhibitory effect on coliforms count in cheese than on using each one alone. *Lactobacillus acidophilus* probiotic cheese is more effective in inhibition of *S.aureus* than either probiotic cheese with *Bifidobacterium lactis* or combination of both (P >0.05).

The results show that cheeses can be an effective carrier of probiotic to consumer. Cheeses also have a number of advantages over fresh fermented products such as yoghurt as a delivery system for viable probiotics to consumer (Ong *et al.,* 2006). Cheeses have higher pH, a more solid consistency, a higher fat content and a higher buffering capacity than yoghurt; thus would offer more protection to probiotic organisms during storage and in the gastrointestinal tract (Kailasapathy and Chin, 2000; Vinderola *et al.*, 2002). Addition of 5 g cheese to 10 ml of gastric juice increased the pH from 2.00 to 4.74, whereas 5 g of yoghurt increased the pH to only 3.65 (Gardiner *et al.,* 1998). Buriti *et al.* (2005) tested the supplementation of Minas fresh cheese with *L. paracasei.* The cheeses studied by the authors presented populations above 10^6 cfu/g during cheese production, and population increased during the whole storage, reaching 10^8 cfu/g after 21 days.

It could be concluded that, this inhibitory effect of probiotic and potentially probiotic strains can be helpful for the use as biopreservatives in the production of cheeses. These strains, besides contributing together with the starter culture for the production of organic acids, may also produce other antimicrobial compounds, including hydrogen peroxide, alcoholic compounds, diacetyl, and bacteriocins. This inhibitory activity creates a hostile environment for pathogens and spoilage organisms.

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