J Food Sci Technol (February 2016) 53(2):996–1003
DOI 10.1007/s13197-015-2159-7

ORIGINAL ARTICLE

Physicochemical, microbiological and spoilage analysis of probiotic processed cheese analogues with reduced emulsifying salts during refrigerated storage

Sheida Ehsannia[1] · **Mohammad Reza Sanjabi**[2]

Revised: 23 November 2015 / Accepted: 22 December 2015 / Published online: 6 January 2016

Abstract Microbial quality of low-salt processed cheeses supplemented with *Bacillus coagulans* spores (10^7–10^8 CFU/g) relying on their physicochemical characteristics during 60 day-cold storage was evaluated. A reduction in moisture content, water activity and pH value and a significant enhancement in proteolytic index of control and probiotic samples were obtained by prolonging storage time. Survival rate of the probiotic cells significantly decreased up to day 30, while total count of the viable cells increased by increasing storage time. A 20 and 67 % increase in total counts of coliforms and mold-yeast of the control sample were respectively observed after 60 days of cold storage. A considerable decrease in the total counts of coliforms and mold-yeast was also found in the processed cheeses containing probiotic supplement. According to the macroscopic and sensory assessment, off-odors and off-flavors in the control sample were diagnosed after day 1 of cold-storage. Noticeably, the resistance to spoilage was more prominent in samples containing the probiotic cells.

Keywords *Bacillus coagulans* spores · Cheese safety · Probiotic cheese · Low sodium · Antimicrobial potential · Spoilage

✉ Mohammad Reza Sanjabi
mr.sanjabi@hotmail.com; msanjabii@gmail.com

[1] Department of Food Science and Technology, Islamic Azad University, Pharmaceutical Sciences Branch (IAUPS), No 99, Yakhchal, Gholhak, Dr. Shariati, P.O. Box 19395-6466, Tehran, Iran

[2] Department of Animal and Poultry Science and Fisheries, Agricultural Research Institute, Iranian Research Organization for Science and Technology (IROST), TehranP.O. Box 3353-5111, Iran

Introduction

Processed cheese is a heat-treated cheese with smooth, homogeneous and stable mass which is made in a discontinuous procedure by heating a mixture of natural cheeses with emulsifying salts under lower pressure and constant stirring until a homogenous mass of desired properties is formed (Guinee 2009; Bubelová et al. 2015). Prolonging the shelf life of natural cheese and finding the additional ways to apply unsold natural cheese are the main reasons for developing processed cheese (Solowiej et al. 2014). Use of emulsifying salts in the cheese structure has some functions in the development of a stable dairy emulsion (homogenous and not phase separated) until spray drying for cheese powder, or during cooling and storage for processed cheese, e.g., pH adjustment, binding of $CaCl_2$, casein dispersion and fat emulsification (Lucey et al. 2011; Hashemi et al. 2015).

The growing demand for high-value health promoting foods such as dairy and bakery products has encouraged the industries, and subsequently the research field, to explore new emerging food processes and formulations able to assure functional but also safe and wholesome products (Rahaie et al. 2014; Akbarian Moghari et al. 2015; Ladjevardi et al. 2015). Owing to the high sodium content, NaCl has also been associated with an enhanced risk of hypertension, the development of osteoporosis, cardiovascular disease and the incidence of kidney stones (Rodrigues et al. 2014). Nowadays, high attention has been directed toward the consumption of foods containing sodium due to its role in increasing the chronic diseases risk and therefore, the emerging need for low-sodium product development (Guardia et al. 2008; Albarracin et al. 2011; Joudaki et al. 2013). The US dietary guidelines set the safe minimum and upper intake levels at 500 and 2300 mg sodium/day (USDA 2010). Canada, the United Kingdom and Australia also have set similar guidelines with lower average

sodium intakes that suggest adequate intake levels from 460 to 1500 mg/day and upper intake limits from 2200 to 2400 mg/day (NHMRC 2006; Wyness et al. 2012; WHO 2013; Ganesan et al. 2014a). Although reduction in emulsifying salts can impair flavor and textural properties of processed cheeses, the demand for reduced-sodium cheeses is increasing to achieve these dietary intake levels due to the lowering blood pressure and preventing the cardiovascular disease (Hoffmann and Schrader 2015).

Probiotics or living micro-organisms which upon ingestion in certain numbers exert health benefits beyond inherent basic nutrition have numerous health benefits such as enhanced immune response, alleviation of symptoms of lactose intolerance, treatment of diarrhea, reduction of serum cholesterol, vitamin synthesis and anti-carcinogenic and anti-microbial activities (Shah 2007; Ganesan et al. 2014b; Salari et al. 2015; Shokoohi et al. 2015; Torkamani et al. 2015). Meanwhile, probiotic bacteria such as *Bifidobacterium longum*, *Bif. lactis*, *Lactobacillus acidophilus*, *Lb. casei* and *Lb. paracasei* are usually added to cheese and yoghurt as delivery vehicles for human consumption to improve immune and gut function (El-Kholy et al. 2014; Ganesan et al. 2014a). However, cheese in comparison to yoghurt because of a higher pH, a more fat content and a higher solid consistency is an interesting food-based delivery vehicle of probiotics to the gastro-intestinal tract (Özer et al. 2008). *Bacillus coagulans* is a non-pathogenic, thermo-tolerant, acidophilic and spore-forming bacterial species of the genus *Bacillus*. Resistance ability and spore development of this strain against high temperatures are very attractive from the standpoint of withstanding baking temperatures and opening up more food-delivery systems for the probiotic use (Keller et al. 2010). Spores of some *Bacillus* species were commercially used as probiotics and competitive exclusion agents (Mazza 1994).

The objective of this research is thus to investigate the capacity of *B. coagulans* spore with the different concentrations (10^7 and 10^8 colony forming units per gram (CFU/g)) to inhibit the spoilage and growth of coliforms, yeasts and molds and the physicochemical changes in low-sodium processed cheeses during 60-day storage at 4 °C.

Materials and methods

Materials and chemicals

White type cheese, butter, yogurt, cream and full fat milk (3.5 %) were provided from a local supermarket in Tehran (Iran). Whey protein concentrate (WPC) and emulsifying salt were respectively obtained from Iran dairy Industry Co. (Pegah, Tehran, Iran), and BK Ladenburg (Bundesrepublik, Germany). Skimmed milk powder and sodium caseinate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Microbial rennet was purchased from Chr Hansen Co. (Hørsholm, Denmark). All the chemicals used were of analytical reagent grade and were purchased from Merck Chemical Co. (KGaA, Darmstadt, Germany).

Microorganism and culture media

Lyophilized culture of *B. coagulans* strain ATCC 7050 was provided from the culture collection of Iranian Biological Resource Center (Tehran, Iran). Sub-cultivation, inoculum preparation and sporulation of *B. coagulans* were conducted according to the method described by Nicholson and Setlow (1990). Nutrient agar, plate count (PC) agar, brain-heart infusion (BHI) agar, violet red bile (VRB) agar, and yeast peptone dextrose (YPD) agar were obtained from Merck Chemical Co. (KGaA, Darmstadt, Germany).

Probiotic-added processed cheese manufacture

Processed cheeses were prepared by mixing the different ingredients including white type cheese (22 %), cream (30 %), full fat milk (13 %), yogurt (12 %), skimmed milk powder (5.5 %), butter (5 %), sodium caseinate (3.5 %), WPC (2 %), emulsifying salt (0.25 %), distilled water (6.75 %), starter, and the probiotic spores into a batch processed cheese maker (Stephan universal machine, UMC 5, GmbH, Hameln, German). Antibiotic-free whole bovine milk (pH 6.8) was pasteurized at 72 °C for 2 min. The blend was melted for 20–30 min until the temperature reached to 80 °C, and maintained for 10 min. After the melting, the different concentrations of *B. coagulans* spores were added to chesses to achieve a specific initial count (10^7–10^8 CFU/g). The cheese was finally packaged into plastic cups and quickly chilled to 4 °C. Cheese samples from each treatment were used to evaluate the microbiological analysis during 60-day storage at 4 °C.

Measurement of water activity and moisture

The water activity (a_w) was assessed using an electronic hygrometer (model Aw-Win, equipped with a Karl-Fast probe, Rotronic, Switzerland), calibrated in the range 0.10–0.98 with solutions of LiCl of known activity (Labuza et al. 1976). The moisture content was also measured by oven drying 5 g of the samples at 105 ± 1 °C until constant weight (AOAC 2000). Three replicates of each analysis were carried out.

Determination of pH

The pH was measured using a pH meter (Model 8417, Hanna Instruments Pty. Ltd., Singapore) after preparing cheese slurry prepared by diluting 20 g of grated cheese with 12 mL of distilled water (Ong et al. 2007). The pH meter was calibrated

with fresh pH 4.0 and 7.0 standard buffers. The measurement of pH was carried out in triplicate.

Estimation of proteolytic index

Total nitrogen (TN) and water-soluble nitrogen (WSN) were measured using the Kjeldahl procedure (Akbarian Moghari et al. 2015). Proteolytic index (PI) was expressed as the WSN percent of the TN (Ardö and Polychroniadou 1999). All determinations were performed in triplicate.

Survival rate evaluation of the probiotic

Ten grams from different sections of the produced cheeses was homogenized in 90 mL sterile saline diluents containing 0.1 % (*w*/*v*) peptone for 5 min (Stomacher 400 Seward, London, UK). Procedure of Miočinović et al. (2014) was used to evaluate count of the starter bacteria. The viable number of vegetative cells and spores of *B. coagulans* was also determined during the storage period to study the effect of refrigerated storage on their viability rate. The spore count was determined by preparation of 10-fold serial dilutions in sterile 0.1 % peptone water. One hundred microliters of each dilution was spread plated onto nutrient agar and incubated for 4 days at 37 °C. The spore number was calculated from four replicates. The population of vegetative cells in the spore suspension was counted by the same procedure, but incubation time was 2 days (Peng et al. 2012).

Determination of microbial contamination

A 10 g sample with 90 mL of sterile water was homogenized in a stomacher (400 Seward, London, UK), subjected to serial dilutions and plated on solid suitable microbiological media. Media of PC agar and VRB agar were respectively used to enumerate count of total bacterial and coliforms after the incubation at 37 °C respectively for 48 and 18 h (APHA 1992). Molds and yeasts were detected on YPD medium acidified to pH 3.5 with sterile lactic acid (10 %) solution and after incubation for 4 days at 25 °C (ISIRI 1998). All the counts were expressed as CFU/g and were carried out in triplicate for the each sample during storage at 4 °C after 1, 15, 30, 45 and 60 days.

Spoilage evaluation

Macroscopic analysis and sensory tests were used to assess the spoilage level. A scoring scale with three categories was applied: class I corresponded to high quality cheese without any off odor or off flavor, class II corresponded to cheese with slight off odors or off flavors but still acceptable and class III corresponded to cheese of unacceptable quality. The shelf-life limit was defined as the point when 50 % of the panelists rejected the cheese samples (Sidira et al. 2014).

Statistical analysis

The results of all analytical experiments for the different samples of processed cheese presented as a mean of the obtained values with the standard deviation. Analysis of variance (ANOVA) procedure followed by Duncan's test using SPSS 13 (SPSS Inc., Chicago, IL, USA) software was applied to determine the significant difference ($p<0.05$) between treatment means. Correlation analysis for studying the relationships between different traits was also performed employing Pearson's test.

Results and discussion

Physicochemical characteristics

Figure 1 shows an insignificant reduction in content of moisture (40.74–42.18 %) and a_w (0.899–0.964) of all the processed cheeses by extending storage time. In other words, a small increase in the amounts of chemical compositions was found by increasing time of cold storage. Similar result was reported for fresh white bovine cheese (Minas Frescal) containing *Lb. acidophilus* (Buriti et al. 2005a). The control sample on day 1 had the highest moisture and a_w amounts; while the probiotic processed cheese analogues with 10^8 CFU/g *B. coagulans* spore on 60 day had the lowest quantities of these parameters (Fig. 1a and b). Ong et al. (2007) and Oliveira et al. (2012) reported a decrease in moisture content of probiotic cheeses of Coalho and Cheddar with prolonging storage time, respectively. A significant reduction in moisture content of probiotic Cheddar cheeses inoculated with *Lactobacillus* and *Bifidobacterium* bacteria strains and ripened at 8 °C was also found (Ong and Shah 2009). Increasing the dry matter of probiotic processed cheeses by increasing *B. coagulans* spores from 10^8 to 10^7 CFU/g can be due to the further germination, proliferation and dissemination of the spores and thus fast release of the metabolites (Ong et al. 2007).

Storage-dependent pH values of the control and probiotic cheeses are depicted in Fig. 2a. A significant difference in pH value was observed by the adding probiotic spores from the storage beginning (Fig. 2a), which can be attributed to an enhancement in acidification by converting lactose to lactic acid and/or other organic acids by *B. coagulans* spores. Production of high levels of acetate and lower quantities of pyruvate and lactat by germinating *B. coagulans* spore can be another reason to reduce final pH in the probiotic cheese (Setlow et al. 1977). An increase in the storage time led to a decrease in the pH value ($p<0.05$). Buriti et al. (2005b) and

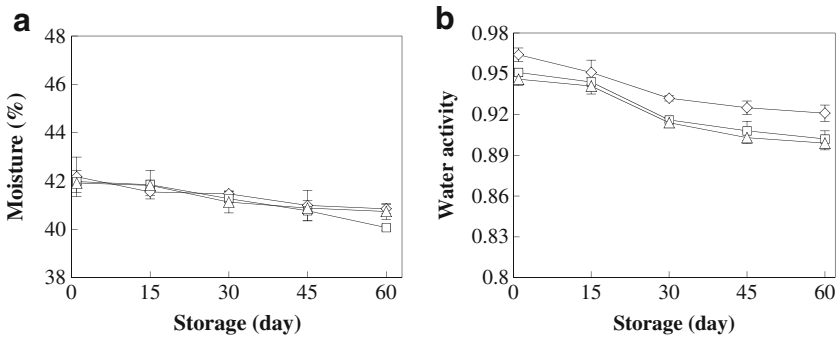

Fig. 1 Contents of moisture (**a**) and water activity (**b**) of the samples of control (◊) and probiotic with 10^7 (□), and 10^8 (Δ) CFU/g *B. coagulans* spores during cold storage

Souza and Saad (2009) for probiotic Minas fresh cheese inoculated with *Lb. paracasei* and Oliveira et al. (2012) for probiotic Coalho cheese supplemented with *Lb. acidophilus* also found similar trends in decreasing pH value during the storage. The lowest pH amount was for processed cheese analogue supplemented with 10^8 CFU/g *B. coagulans* spore (pH = 5.2) on day 60 of storage ($p < 0.05$). Since the viability rate of most strains of probiotic bacteria is highly reduced in pH values lower than 4.6 (Desai et al. 2004), the obtained results showed that the probiotic samples at all the storage times can maintain suitable number of *B. coagulans* spores.

Table 1 exhibits the mean value for PI in the initial and final moisture content, the relationships between the PI and cold-storage time and their coefficient of determination (R^2) values. Figure 2b reveals that the PI of control and probiotic processed cheeses increased by prolonging storage time at 4 °C ($p < 0.05$). This increase for samples of control and probiotic inoculated with 10^7 and 10^8 *B. coagulans* spore were 63.95, 102.25 and 106.58 %, respectively (Table 1). Therefore, the probiotic processed cheeses had more PI than the control cheese. However, no significant differences in PI level between both the probiotic samples at all the storage times and also between the control and probiotic samples on day 1 of storage were found (Fig. 2b; Table 1). Proteolysis in a lot of cheese types is the most complex and possibly the most important biochemical process during the ripening step. The lactic acid bacteria along with the milk plasmin and the rennet are the main sources of proteolytic enzymes in a wide variety of cheeses (Fox et al. 1996). Our results showed that the *B. coagulans* had high proteolytic activity towards casein by releasing its microbial intracellular peptidases. Ratio of WSN/TN of the probiotic processed cheese analogues was close to the PI reported for low-fat ultra-filtered cheeses produced with probiotic bacteria (Miočinović et al. 2014). A significant increase in PI of Cheddar cheese during 6 months of ripening was also observed by adding of *Lb. acidophilus*, *Lb. casei*, *Lb. paracasei* and *Bifidobacterium* spp (Ong et al. 2006, 2007). Thus, *B. coagulans* commonly have different peptidase systems which do not assume a significant role during primary proteolysis, but influence the secondary proteolytic changes (Sousa et al. 2001).

Microbiological characteristics

Survival rate of the probiotic

Population of viable vegetable cells of *B. coagulans* during 60 day-storage at 4 °C is illustrated in Fig. 3a. Results showed that the number of viable cells on day 1 of storage between both the probiotic treatments was the same in the range of 1–2×10^1 CFU/g (Fig. 3a). Moreover, total count of the viable cells increased by prolonging storage time as the probiotic processed cheese analogues after 60-day storage had 8×10^2–1×10^3 CFU/g *B. coagulans* (Fig. 3a). The minimum and maximum changes in the population of viable cells respectively were for time periods of 1–15 and 30–45 day. Therefore, the number of viable cells in the probiotic cheeses after 30 day of storage at refrigerated temperature was

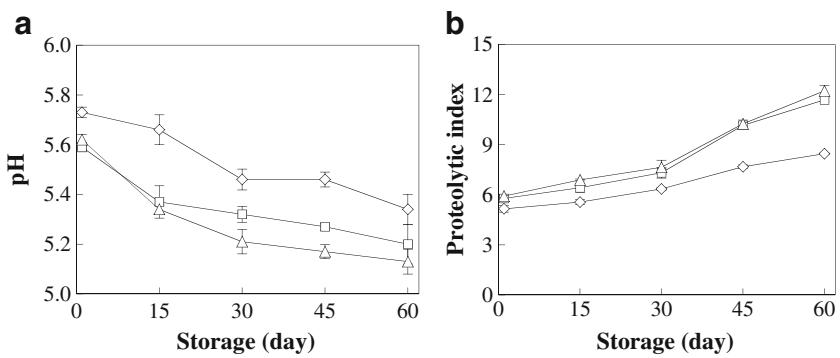

Fig. 2 pH (**a**) and proteolytic index (**b**) of the samples of control (◊) and probiotic with 10^7 (□), and 10^8 (Δ) CFU/g *B. coagulans* spores during 60 day-storage at 4 °C

Table 1 The relationships between proteolytic index and storage time (ST), their coefficient of determination (R^2) values and their mean percentage in the initial and end storage time

Type of processed cheese	PI[1]		Increase (%)[2]	Regression equation	R^2
	$PI_{day\ 1}$	$PI_{day\ 60}$			
Control (C, without *B. coagulans* spore)	5.16	8.46	63.95[b]	$PI_C = 0.0004\ ST^2 + 0.0356\ ST + 5.0479$	0.9866
Probiotic (A, 10^7 *B. coagulans* spore)	5.78	11.69	102.25[a]	$PI_A = 0.0012\ ST^2 + 0.0350\ ST + 5.6568$	0.9772
Probiotic (B, 10^8 *B. coagulans* spore)	5.92	12.23	106.58[a]	$PI_B = 0.0012\ ST^2 + 0.0347\ ST + 5.9131$	0.9885

[1] PI_{day1} and PI_{day60} are the proteolytic index in initial and final times of cold storage, respectively

[2] Values in the same column followed by different letters (a–b) are significantly different ($p < 0.05$)

comparable with the count observed after 1 day. Figure 3b also shows the total count of vegetable cells and spores of *B. coagulans* during 60 day-storage at 4 °C. Total number of the probiotic cells significantly decreased by increasing storage time up to day 30 and then this reduction gradually continued in time interval between day 45 and day 60 (Fig. 3b).

Vinderola et al. (2002) proved that the viability of starter bacteria and probiotics can be inversely related to the salt concentration. Lower levels of salt in moisture for the initial storage times can be thus associated with higher microbial growth (Kasimoglu et al. 2004). During the ripening process, low amounts of salt used diffuse into the cheese structure so that difference in the salt content at the center and periphery decreases with increasing storage time. This fact contributes not only to the flavor of the cheeses but also has an impact on the growth and activity of starter and probiotic microorganisms (Vinderola et al. 2002). On the other hand, Bora et al. (2009) revealed that aqueous suspension of *B. coagulans* spores in buffer solutions of pH 1.2 to 8.0 had rapid degradation with maximal stability in pH 6.8. A reduction in pH of probiotic samples up to 5.2 by extending storage time can lead to a decrease in the survival and growth of *B. coagulans* spores. The total count of viable probiotic cells in the produced cheeses with *B. coagulans* on day 60 was between 6.94 and 7.51 log CFU/g. Shah (2011) indicated that for a product to be considered probiotic, this value should be equal to or greater than 6 log CFU/g of the product. Results of the current research thus affirmed that the processed cheese analogues could be considered an ideal vehicle for *B. coagulans*.

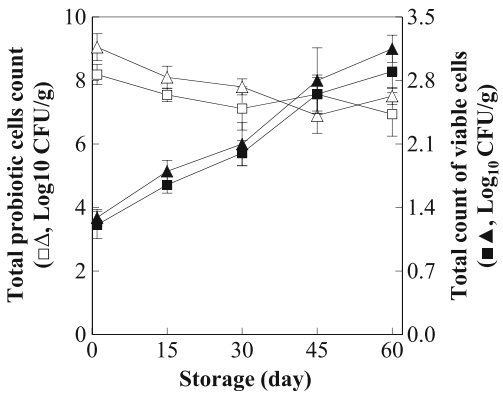

Fig. 3 The total counts of viable vegetable cells (**a**) and, probiotic cells (**b**) of the processed cheeses supplemented with 10^7 (□,■), and 10^8 (Δ,▲) CFU/g *B. coagulans* spores during 60 day-storage at 4 °C

Overall, the storage-dependent reduction of *B. coagulans* cells in processed cheese analogue containing 10^8 CFU/g spores (17.2 %) was a little more than that inoculated with 10^7 CFU/g spores (15.4 %). El-Kholy et al. (2014) by investigating changes in the viable counts of *Lb. acidophilus* La-5 and *Bif. longum* ATCC15707 in domiati cheese during 30 day-storage showed that these probiotics respectively retained at 6.85 and 6.20 log CFU/g until the end of storage. A reduction in the probiotic cells count in other cheese types during storage period was also reported by other researchers (Akbarian Moghari et al. 2015). McBrearty et al. (2001) found that the survival rate of *Bif. lactis* Bb-12 (8 log CFU/g) in Cheddar cheese was more than *Bif. longum* BB5365 (5 log CFU/g) during 6 month-ripening. Phillips et al. (2006) pointed out a decrease in the cell counts of *Lb. acidophilus* strains in Cheddar cheese during a 7.5-month ripening time; while more than 10^7 CFU/g of *Bifidobacterium* strains survived. In contrast, domiati cheese as a vehicle for incorporating *Lb. acidophilus* and *Bif. lactis* was examined and the results demonstrated that the viability of *Lb. acidophilus* can be remained for up to 60 days at 7.3 log CFU/g (Amer 2011). Akbarian Moghari et al. (2015) also demonstrated that the viable probiotic cell numbers of *Lb. acidophilus* la 5 (7.96–8.15 log CFU/g) and *Bif. lactis* Bb-12 (8.11–8.2 log CFU/g) in Iranian ultrafiltered-Feta cheeses significantly decreased during 60 days storage period.

Microbial contamination

Evaluation of microbial quality of the different produced samples in terms of determination of total mold and yeast counts and total coliform counts is depicted in Fig. 4. Results of the current study exposed that use of *B. coagulans* spores in the

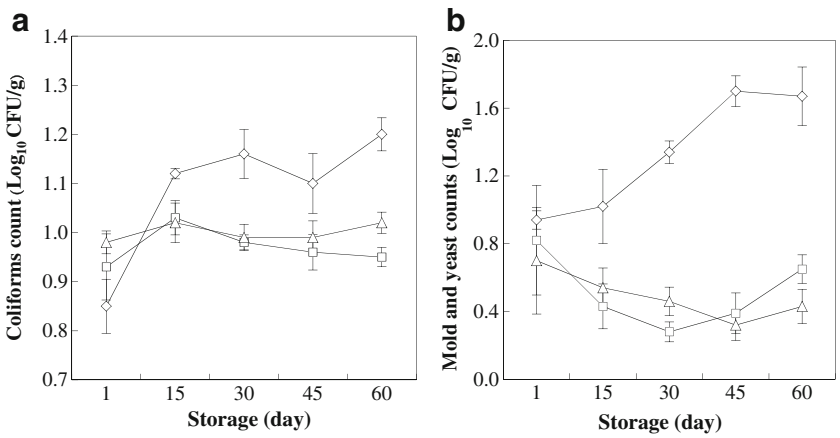

Fig. 4 The counts of coliforms (**a**) and, mold and yeast (**b**) of the cheeses of control (◊) and probiotic with 10^7 (□), and 10^8 (Δ) CFU/g *B. coagulans* spores during 60 day-storage at 4 °C

processed cheese can lead to the improved microbial characteristics as the highest counts of mold, yeast and coliforms were in the control sample ($p < 0.05$). Increasing the inoculation level of *B. coagulans* spores from 10^7 to 10^8 CFU/g had no significant effect of the microbial quality of cheese samples. Although use of *B. coagulans* spores had no significant effect on the total count of coliforms during 60 days storage (Fig. 4a), number of these bacteria in both probiotic treatments was lower than the control in all storage periods ($p < 0.05$). Figure 4b depicted that total count of mold and yeast in the control sample increased by prolonging cold-storage time, while a considerable reduction in total number of mold and yeast was found in the cheeses containing probiotic supplement. Total counts of coliforms and mold-yeast in the control sample were respectively increased 20 and 67 % after 60 days of cold storage. Total count of coliforms was similar to that reported by Sayed et al. (2011) who found 20 % of Egyptian white soft cheeses were contaminated with these bacteria. Aly et al. (2007) and Meshref and Hassan (2009) found 52.5 and 78 % of the cheese samples were contaminated with coliforms, respectively. El-Kholy et al. (2014) explained high counts of coliforms in cheese may occasionally provide an increased risk for early blowing or gassing of the product, which can be observed by large gas holes and a spongy texture of the cheese during the initial days of manufacturing. Presence of high counts of molds and yeasts in cheese can be also related to the unsanitary practices during cheese making, insufficient conditions of utensils, unacceptable controlling of pasteurization process and the use of low-quality milk and ingredients (Coveney et al. 1994). Some yeasts and moulds can also grow at refrigeration temperatures in the environment with atmosphere having lower oxygen concentrations, which are ideally suited for the role of contaminants of processed cheese. These microorganisms can be resulted to the surface discoloration, off-flavor and early blowing due to the action of their lipolytic and proteolytic enzymes (Buňková and Buňka 2015). Madureira et al. (2011) stated that the dominant inhibitory effect of probiotics was typically bacteriostatic namely lower rates of increase in the viable numbers of contaminant bacteria throughout storage. Addition of the probiotics by the decreasing pH facilitates diffusion of antimicrobial compounds (organic acids such as lactic and acetic acids), and co-aggregates them with contaminant microorganisms (Annuk et al. 2003).

Cheese spoilage

Table 2 illustrated a numerical scoring system for the macroscopic and sensory assessment of the spoilage of processes cheeses stored for 60 days at 4 °C. A high quality for the control sample was recorded only on day 1; afterwards its deterioration started with a slight increase in off odors or off flavors up to day 30 and finally the product was unacceptable from day 45 to the end of cold storage. The addition of 10^7 and

Table 2 The processed cheese classification according to the spoilage level during cold storage time

Type of processed cheese	Cheese spoilage during cold storage[1]				
	1	15	30	45	60
Control (C, without *B. coagulans* spore)	I	II	II	III	III
Probiotic (A, 10^7 *B. coagulans* spore)	I	I	I	I	II
Probiotic (B, 10^8 *B. coagulans* spore)	I	I	I	I	II

[1] Scoring scale for the cheese spoilage was classified as: (I) High quality without any off odor or off flavor, (II) Slight off odors or off flavors but still acceptable, and (III) Unacceptable quality

10^8 CFU/g of *B. coagulans* spores can be proposed to prevent spoilage of the processed cheese analogues, so that the slight levels of off-odors and off-flavors were formed in the probiotic cheeses only on day 60, but the products were still acceptable (Table 2). This fact can be a reasonable result of the pH reduction, preservative effects of organic acids produced by converting lactose and low counts of coliforms and yeasts/molds present in the final product (Setlow et al. 1977; Annuk et al. 2003; Buriti et al. 2005a, b).

Conclusion

In this study, incorporation effect of *B. coagulans* spores on the physicochemical, microbiological and spoilage characteristics of processed cheeses during the storage was scrutinized. The cheeses supplemented with the probiotic spores showed the comparable physicochemical traits than the control cheese during cold-storage time. Since the viability rate of microorganism studied during the cold storage was mentioned more than 10^6 CFU/g, it can be an ideal candidate for delivery of the probiotic cells. The developed probiotic cheese also can be a new bio-preservative product because low counts of coliforms and yeasts/molds in this cheese led to a decrease in spoilage of the final product. This study suggested use of *B. coagulans* spore as an excellent probiotic source to formulate other functional dairy products. Moreover, the application of potassium-based emulsifying salts and potassium chloride for reducing sodium in processed cheeses in order to obtain favorable sensory, textural, and functional characteristics for future studies is recommended.

References

Akbarian Moghari A, Razavi SH, Ehsani MR, Mousavi M, Hoseini Nia T (2015) Chemical, proteolysis and sensory attributes, and probiotic microorganisms viability of Iranian ultrafiltered-Feta cheese as a function of inulin concentration and storage temperature. Qual Assur Saf Crops Food 7:217–224

Albarracin W, Sanchez IC, Grau R, Barat JM (2011) Salt in food processing; usage and reduction: a review. Int J Food Sci Technol 46:1329–1336

Aly SA, Morgan SD, Moawad AA, Metwally BN (2007) Effect of moisture, salt content and pH on the microbiological quality of traditional Egyptian domiati cheese. Assiut Vet Med J 53:68–81

Amer A (2011) Probiotics as a tool to improve microbial quality of soft cheese. Assiut Vet Med J 57:44–57

Annuk H, Shchepetova J, Kullisaar T, Songisepp E, Zilmer M, Mikelsaar M (2003) Characterization of intestinal lactobacilli as putative probiotic candidates. J Appl Microbiol 94:403–412

AOAC (2000) Official methods of analysis, 17th edn. Association Official Analytical Chemists, Arlington

APHA (1992) Compendium of methods for the microbiological examination of food, 3rd edn. American Public Health Association, Washington

Ardö Y, Polychroniadou A (1999) Improvement of the quality of the production of raw milk cheeses. Laboratory manual for chemical analysis of cheese. COST 95

Bora PS, Puri V, Bansal AK (2009) Physicochemical properties and excipient compatibility studies of probiotic *Bacillus coagulans* spores. Sci Pharm 77:625–637

Bubelová Z, Tremlová B, Buňková L, Pospiech M, Vítová E, Buňka F (2015) The effect of long-term storage on the quality of sterilized processed cheese. J Food Sci Technol 5:4985–4993

Buňková L, Buňka F (2015) Microflora of processed cheese and the factors affecting it. Crit Rev Food Sci Nutr. doi:10.1080/10408398.2015.1060939

Buriti FCA, da Rocha JS, Saad SMI (2005a) Incorporation of *Lactobacillus acidophilus* in Minas fresh cheese and its implications for textural and sensorial properties during storage. Int Dairy J 15:1279–1288

Buriti FCA, Rocha JS, Assis EG, Saad SMI (2005b) Probiotic potential of Minas fresh cheese prepared with the addition of *Lactobacillus paracasei*. LWT Food Sci Technol 38:173–180

Coveney HM, Fitzgerald GF, Daly C (1994) A study of the microbiological status of Irish farmhouse cheeses with emphasis on selected pathogenic and spoilage microorganisms. J Appl Bacteriol 77:621–630

Desai AR, Powell IB, Shah NP (2004) Survival and activity of probiotic lactobacilli in skim milk containing prebiotic. J Food Sci 69:57–60

El-Kholy AM, El-Shinawy SH, Meshref AMS, Korany AM (2014) Microbiological quality of Domiati cheese and the influence of probiotics on the behavior of *Staphylococcus aureus* and *Escherichia coli* O157:H7 in Domiati cheese. J Food Saf 34:396–406

Fox PF, O'Connor TP, McSweeney PLH, Guinee TP, O'Brien NM (1996) Cheese: Physical, biochemical and nutritional aspects. Adv Food Nutr Res 39:163–328

Ganesan B, Brown K, Irish DA, Brothersen C, McMahon DJ (2014a) Manufacture and sensory analysis of reduced- and low-sodium Cheddar and Mozzarella cheeses. J Dairy Sci 97:1970–1982

Ganesan B, Weimer BC, Pinzon J, Dao Kong N, Rompato G, Brothersen C, McMahon DJ (2014b) Probiotic bacteria survive in Cheddar cheese and modify populations of other lactic acid bacteria. J Appl Microbiol 116:1642–1656

Guardia MD, Guerrero L, Gelabert J, Gou P, Arnau J (2008) Sensory characterisation and consumer acceptability of small caliber fermented sausages with 50% substitution of NaCl by mixtures of KCl and potassium lactate. Meat Sci 80:1225–1230

Guinee TP (2009) The role of dairy ingredients in processed cheese products. In: Corredig M (ed) Dairy-derived ingredients. Woodhead Publishing, Cambridge, pp 507–538

Hashemi FS, Gharibzahedi SMT, Hamishehkar H (2015) The effect of high methoxyl pectin and gellan including psyllium gel on Doogh stability. RSC Adv 5:42346–42353

Hoffmann W, Schrader K (2015) Dispersion analysis of spreadable processed cheese with low content of emulsifying salts by photocentrifugation. Int J Food Sci Technol 50:950–957

ISIRI (1998) Microbiology of cheese and its products. ISIRI standard 2406. Institute of Standards and Industrial Research of Iran, Karaj

Joudaki H, Mousavi M, Safari M, Razavi SH, Emam-Djomeh Z, Gharibzahedi SMT (2013) A practical optimization on salt/high-methoxyl pectin interaction to design a stable formulation for Doogh. Carbohydr Polym 97:376–383

Kasimoglu A, Goncuoglu M, Akgun S (2004) Probiotic white cheese with *L. acidophilus*. Int Dairy J 14:1067–1073

Keller D, Farmer S, McCartney AL, Gibson G (2010) *Bacillus coagulans* as a probiotic. Food Sci Technol Bull Funct Food 7:103–109

Labuza TP, Acott K, Tatini SR, Lee RY, Flink J, McCall W (1976) Water activity determination: a collaborative study of different methods. J Food Sci 41:910–917

Ladjevardi ZS, Gharibzahedi SMT, Mousavi M (2015) Development of a stable low-fat yogurt gel using functionality of psyllium (*Plantago ovata* Forsk) husk gum. Carbohydr Polym 125:272–280

Lucey JA, Maurer-Rothmann A, Kaliappan S (2011) Functionality of ingredients: emulsifying salts. In: Tamime AY (ed) Processes cheese and analogues. Blackwell Publishing Ltd., Oxford, UK p 110–132

Madureira AR, Pintado ME, Gomes AM, Malcata FX (2011) Incorporation of probiotic bacteria in whey cheese: decreasing the risk of microbial contamination. J Food Prot 7:1048–1211

Mazza P (1994) The use of *Bacillus subtilis* as an antidiarrhoeal micro-organism. Boll Chim Farm 133:3–18

Meshref AMS, Hassan GM (2009) Bacteriological status of some soft cheeses sold in Beni-Suef city. Assiut Vet Med J 55:112–123

Miočinović J, Radulović Z, Paunović D, Miloradović Z, Trpković G, Radovanović M, Puđa P (2014) Properties of low-fat ultra-filtered cheeses produced with probiotic bacteria. Arch Biol Sci 66:65–73

NHMRC (2006) Nutrient reference values for Australia and New Zealand including recommended dietary intakes. National Health and Medical Research Council, Canberra

Nicholson WL, Setlow P (1990) Sporulation, germination and outgrowth. In: Harwood CR, Cutting SM (eds) Molecular biological methods for *Bacillus*. Wiley, Chichester

Oliveira MEGD, Garcia EF, Queiroga RDCRD, Souza ELD (2012) Technological, physicochemical and sensory characteristics of a Brazilian semi-hard goat cheese (coalho) with added probiotic lactic acid bacteria. Sci Agric 69:370–379

Ong L, Shah NP (2009) Probiotic cheddar cheese: influence of ripening temperatures on survival of probiotic microorganisms, cheese composition and organic acid profiles. LWT Food Sci Technol 42:1260–1268

Ong L, Henriksson A, Shah NP (2006) Development of probiotic Cheddar cheese containing *Lactobacillus acidophilus*, *Lb. casei*, *Lb. paracasei* and *Bifidobacterium* spp. and the influence of these bacteria on proteolytic patterns and production of organic acid. Int Dairy J 16:446–456

Ong L, Henriksson A, Shah NP (2007) Proteolytic pattern and organic acid profiles of probiotic Cheddar cheese as influenced by probiotic strains of *Lactobacillus acidophilus*, *Lb. paracasei*, *Lb. casei* or *Bifidobacterium* sp. Int Dairy J 17:67–78

Özer B, Uzun YS, Kirmaci HA (2008) Effect of microencapsulation on viability of *Lactobacillus acidophilus* LA-5 and *Bifidobacterium bifidum* BB-12 during Kasar cheese ripening. Int J Dairy Technol 61:237–244

Peng J, Mah JH, Somavat R, Mohamed H, Sastry S, Tang J (2012) Thermal inactivation kinetics of *Bacillus coagulans* spores in tomato juice. J Food Prot 75:1236–1242

Phillips M, Kailasapathy K, Tran L (2006) Viability of commercial probiotic cultures (*L. acidophilus*, *Bifidobacterium* sp., *L. casei*, *L. paracasei* and *L. rhamnosus*) in cheddar cheese. Int J Food Microbiol 108:276–280

Rahaie S, Gharibzahedi SMT, Razavi SH, Jafari SM (2014) Recent developments on new formulations based on nutrient-dense ingredients for the production of healthy-functional bread: a review. J Food Sci Technol 51:2896–2906

Rodrigues JF, Gonçalves CS, Pereira RC, Carneiro JDS, Pinheiro ACM (2014) Utilization of temporal dominance of sensations and time intensity methodology for development of low-sodium Mozzarella cheese using a mixture of salts. J Dairy Sci 97:4733–4744

Salari M, Razavi SH, Gharibzahedi SMT (2015) Characterising the synbiotic beverages based on barley and malt flours fermented by *Lactobacillus delbrueckii* and *paracasei* strains. Qual Assur Saf Crops Food 7:355–361

Sayed M, Ahmed AAH, Shaban W (2011) Microbiological evaluation of some Egyptian white soft cheeses. Benha Vet Med J 1:1–6

Setlow BARBARA, Shay LK, Vary JC, Setlow P (1977) Production of large amounts of acetate during germination of *Bacillus megaterium* spores in the absence of exogenous carbon sources. J Bacteriol 132: 744–746

Shah NP (2007) Functional cultures and health benefits. Int Dairy J 17: 1262–1277

Shah NP (2011) *Bifidobacterium* spp.: applications in fermented milks. In: Fuquay JW, Fox PF, McSweeney PLH (eds) Encyclopedia of dairy sciences. Academic, San Diego, pp 685–697

Shokoohi M, Razavi SH, Labbafi M, Vahidinia A, Gharibzahedi SMT (2015) Wheat sprout flour as an attractive substrate for the producing probiotic fermented beverages: process development and product characterization. Qual Assur Saf Crops Food 7:469–475

Sidira M, Galanis A, Nikolaou A, Kanellaki M, Kourkoutas Y (2014) Evaluation of *Lactobacillus casei* ATCC 393 protective effect against spoilage of probiotic dry-fermented sausages. Food Control 42:315–320

Solowiej B, Cheung IWY, Li-Chan ECY (2014) Texture, rheology and meltability of processed cheese analogues prepared using rennet or acid casein with or without added whey proteins. Int Dairy J 37:87–94

Sousa MJ, Ardo Y, McSweeney PLH (2001) Advances in the study of proteolysis in cheese during ripening. Int Dairy J 11:327–345

Souza CHB, Saad SMI (2009) Viability of *Lactobacillus acidophilus* La-5 added solely or in co-culture with a yoghurt starter culture and implications on physico-chemical and related properties of Minas fresh cheese during storage. LWT Food Sci Technol 42:633–640

Torkamani MG, Razavi SH, Gharibzahedi SMT (2015) Critical quality attributes of Iranian 'Taftoon' breads as affected by the addition of rice bran sourdough with different lactobacilli. Qual Assur Saf Crops Food 7:305–311

USDA (2010) Dietary guidelines for Americans, 2010, 7th edn. USDA and US Department of Health and Human Services, Washington, DC

Vinderola CG, Mocchiutti P, Reinheimer JA (2002) Interactions among lactic acid starter and probiotic bacteria used for fermented dairy products. J Dairy Sci 85:721–729

WHO (2013) WHO issues new guidance on dietary salt and potassium. Cent Eur J Public Health 21:16

Wyness LA, Butriss JL, Stanner SA (2012) Reducing the population's sodium intake: the UK Food Standards Agency's salt reduction programme. Public Health Nutr 15:254–261