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Research Paper

Effect of encapsulation on the survival of probiotic bacteria in the presence of starter and non-starter lactic acid bacteria in Cheddar cheese over a 6-month ripening period

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ABSTRACT

Probiotic bacteria (*Lactobacillus acidophilus* LAFTI L10 and *Bifidobacterium lactis* LAFTI B94) were encapsulated in calcium-alginate hydro-gel to study the effect of encapsulation on their survival in Cheddar cheese. Twelve batches of Cheddar cheese were manufactured incorporating encapsulated and free probiotic bacteria. The survival and the effect of encapsulated probiotic bacteria on the growth of starter lactic acid bacteria (SLAB) and non-starter lactic acid bacteria (NSLAB) were assessed over a six-month ripening period. The survival of encapsulated bacteria (10^7 cfu/g) was found to be significantly ($P<0.05$) greater than that of free bacteria (10^5 cfu/g) at the end of six-months. Also, addition of encapsulated probiotic bacteria did not change the population of SLAB and NSLAB. This study therefore demonstrates that encapsulated probiotic bacteria survive better than free probiotic bacteria in Cheddar cheese during the long ripening period and had no effect on the SLAB and NSLAB growth during ripening.

Keywords*:* Probiotic bacteria, encapsulation, cheddar cheese, starter and non-starter lactic acid bacteria

INTRODUCTION

Probiotic dairy foods have captured a major share of the recently growing functional food market worldwide. Probiotics are widely used in the manufacturing of fermented dairy products such as cheese (Anal and Singh, 2007; Ong and Shah, 2009). Probiotics have been defined as "live microorganisms which, when administered in adequate amounts, confer a health benefit on the host" (Reid, *et al*., 2003). Probiotic foods can be made in two ways: (i) fermentation of raw ingredients by

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probiotic bacteria, with or without starter culture strains; and (ii) addition of suitable concentrations of probiotics to the finished product (O'Sullivan *et al*., 1992; Svensson, 1999). Probiotic fermentation of raw ingredients allows the bacteria to multiply and impart distinctive flavours, and organoleptic attributes to the food. The composition, pH, buffering capacity and the nature of the matrix of a food determines which probiotic strain is more suited to good growth and survival in a fermented dairy food such as cheese (Kailasapathy, 2008). A probiotic must have good technological properties so that it can be incorporated into food products without losing viability and functionality or creating unpleasant falvours or textures in the end product (FAO/WHO 2002). A number of studies have addressed the development of probiotic Cheddar cheese (Dinakar and Mistry, 1994; Crow *et al*., 2001; Playne, 2002; Godward and Kailasapathy, 2003; Darukaradhya *et al*., 2006; Phillips *et al*., 2006; Ong and Shah, 2009; Scheller snd O'Sullivan, 2011).

The development of non-starter lactic acid bacteria (NSLAB) during ripening of cheeses could be detrimental to the survival of incorporated and or adjunct probiotic bacterial cells in Cheddar cheese during fermentation, ripening and storage (Crow *et al*., 2001). NSLAB are mostly facultative mesophilic lactobacilli including species such as *Lactobacillus casei, L. paracasei, L. rhamnosus,* and *L. plantarum,* as well as pediococci, *Luconostoc* and micrococci (Burns *et al*., 2012). It is interesting to note that some of these bacteria have probiotic characteristics (Crow *et al*., 2001). However, it is important to study the competitive effect of these NSLAB on the growth and survival of the incorporated probiotic bacteria in Cheddar cheese during fermentation, ripening and storage. NSLAB can survive pasteurization at low numbers and slowly grow during cheese ripening up to 10^6 – 10^7 CFU/g, depending on the cheese ripening- periods and temperature (Christiansen *et al*., 2006). NSLAB can overcome lactic acid starters in the cheese and become the dominant microflora (Crow *et al*., 2001).

In an earlier study we reported that there were low counts of free *L. acidophilus* and *B. lactis* (probiotic bacteria) during the ripening period in Cheddar cheese (Darukaradhya *et al*., 2006). The cell numbers were found not adequate to meet recommended regulatory standards or to deliver sustained therapeutic benefits to consumers. It has been recommended that there be at least 10^6 CFU per gram of probiotic cell concentration in the product at the time of consumption (International Dairy Federation, 1992; Shah, 2000). Hence, there is a need to protect probiotic bacteria from possible deleterious factors during Cheddar cheese fermentation and storage that affect their viability (Champagne, 2006).

Microencapsulation is a technique in which live cells are packed in a coated material to shield them from the surrounding unfavorable environment. This is a method reported to enhance the survival of probiotic bacteria in dairy products (Shah and Ravula, 2000; Champagne and Fustier, 2007; Kailasapathy, 2009). Probiotic bacteria have been encapsulated to acquire protection from the acidic conditions in the

stomach and have increased their tolerance to bile (Shah and Ravula, 2000; Sultana *et al*., 2000). In addition, microencapsulation has been suggested for increasing the viability of lactobacilli in frozen ice milk (Shue *et al*., 1993). It has also been reported in protecting *Bifidobacterium* spp. in cheese (Dinakar & Mistry, 1994; Gobbetti *et al*., 1998). Encapsulated probiotic bacteria have also been incorporated in yogurts, and were found to survive better than free cells (Krasaekoopt *et al*., 2004; Picot & Lacroix, 2004). Hence, incorporation of encapsulated probiotic bacteria into Cheddar cheese may enhance their viability.

Encapsulation was therefore investigated as a protective technique to prevent poor survival of *L. acidophilus* and *B. lactis* during the ripening and shelf life period in Cheddar cheese.

The aim of this study was to examine if encapsulating probiotic bacteria enhances their viability in cheddar cheese. In this study, live probiotic bacterial cells [*L. acidophilus* (LAFTI L10) and *B. lactis*, (LAFTI B94)] were encapsulated and incorporated during Cheddar cheese manufacture. The survival of the encapsulated probiotic bacterial cells was compared with that of free cells during cheese fermentation and over a 6 month ripening period. To investigate the ability of the probiotic bacteria to survive the cheese fermentation and ripening, the growth phases of both starter and non-starter lactic acid bacteria (SLAB and NSLAB) were studied.

MATERIALS AND METHODS

Cultures and chemicals

The probiotic strains used in this study were *L. acidophilus*, LAFTI L10 and *Bifidobacterium lactis*, LAFTI B94 (DSM, Australia). The SLAB strains used were *Lactococcus lactis* subspp. *cremoris* and *Lactococcus lactis* subspp *lactis* (LL50C) (DSM, Australia). All chemicals were obtained from Sigma-Aldrich (NSW, Australia) and media from Oxoid (Victoria, Australia) unless stated otherwise.

Selective and differential media

The selective media used were RCABC for LAFTI L10 and RCABV for NSLAB. The differential medium used was RCAAD for both LAFTI B94 and SLAB. All the media were prepared as described in the paper by Darukaradhya *et al*., 2006. A modified enzyme based colorimetric assay was conducted to confirm the presence of LAFTI B94 colonies on RCAAD (Darukaradhya *et al*., 2006).

Encapsulation of L. acidophilus and B. lactis

Growth of cultures and preparation of cell suspensions for encapsulation

The pure probiotic strains (LAFTI L10 & LAFTI B94) were cultured in MRS broth at 37°C for 48 hours anaerobically using Anaerogen packs and jars (Oxoid, Victoria, Australia). After incubation the cells were cultivated by centrifugation at 3000 x g for 10 min and washed twice with phosphate buffer saline (pH 7). The cell

suspensions were subsequently used for microencapsulation.

Microencapsulation and optimization of encapsulation parameters

The capsules were made aseptically using an Inotech Encapsulator (Switzerland) with a nozzle size of 300mm. The standard parameters used for making capsules were 1.8% w/v, 1% starch w/v, 30 min hardening time of capsules in 0.1 M calcium chloride (Chandramouli *et al*. 2004) and a bacterial cell load of 10^{10} cfu/ml of LAFTI B94 and LAFTI L10.

Leakage of capsules in cheese milk

1g of capsules was suspended in 10ml of full cream pasteurized unhomogenised milk used for Cheddar cheese making. The leakage of the capsules was tested in milk over a 4 -h period with pH ranging from 4.0 to 6.5 (pH adjustment with HCl). The temperature was maintained at 38 ± 0.5°C. Samples for cell leakage determination were taken every hour from all milk samples for the duration of the experiment (0h, 1h, 2h, 3h and 4h). Samples were diluted in sterile 0.1% peptone water and spread plated in two sets of triplicates and incubated anaerobically at 37°C for 48h.

Bead size determination

Fifty capsules were measured in triplicates using the stage micrometer and the average capsule size was determined.

Leakage of beads under simulated cheese press conditions

The alginate-starch capsules were tested for their mechanical strength under simulated cheese press pressure conditions over a period of 2h, 4h, 6h, 8h and 16h (overnight) using a texture analyzer (model TA-XT2, Stable Micro Systems, Surrey, UK). The texture analyzer had a 3cm in diameter plexiglass piston. During the test, the piston was lowered at a rate of 0.1mm/s on 30g of alginate-starch capsules until a force resistance of 0.814N/cm^2 was detected and maintained at this force for the desired time. The probe automatically moved back to its initial position once the set time was up. The liquid extruded during the test and the capsules were then tested using spread plating to determine the leakage of cells during the cheese press or Cheddaring. The capsules from the texture analyzer were then dissolved in 0.1M phosphate buffer (pH 7.0) and plated in two sets of triplicates on MRS agar and incubated at 37°C for 48h. The same protocol was followed for the extruded liquid from the capsules during the texture analyzer test.

Preparation of Cheddar cheese with encapsulated and free probiotic bacteria

LAFTI L10 and LAFTI B94 were cultured in MRS broth at 37°C anaerobically for 20 hours. The cells were harvested by centrifugation at 3000 x g for 10 min, and washed twice with phosphate buffer saline of pH 7.0. The cell suspensions were

subsequently used for encapsulation. The bacterial pellet was re-suspended in 10ml of sterile distilled water to obtain bacterial slurry. 10ml of bacterial slurry was then mixed with 80 ml of sterile filtered (0.60µm + 0.22µm filtration unit, Sartorius AG, Goettingen, Germany) solution of low viscosity 1.8%w/v sodium alginate solution and 10ml of 1%w/v starch solution to form a homogenous suspension. This mixture was then supplied to the encapsulation apparatus (Inotech, Dottikon, Switzerland) via a syringe pump. The microcapsules were collected in 0.1M Calcium chloride solution. This was left to stand for 30min for the capsules to harden. These capsules were later added into the cheese milk during the manufacture of probiotic Cheddar cheese.

Encapsulated *L. acidophilus* and *B. lactis* were incorporated into six batches of probiotic Cheddar cheese, manufactured as per the method used by Darukaradhya *et al.*, 2006. The encapsulated bacteria were added into the cheese milk just before adding rennet.

Simultaneously another set of six batches of probiotic Cheddar cheese with free bacteria was also manufactured.

Microbiological analysis of Cheese samples

10 g of each of the Cheddar cheese sample were suspended in 100 µl of tri-sodium citrate buffer pH 7.2 and homogenized in a stomacher for 2min. The homogenized suspension was serially diluted using tri-sodium citrate buffer and 100µl of appropriate dilutions were spread plated on the selective or differential media in triplicate. All the media plates were incubated anaerobically at 37°C for 48 hours before enumerating the colonies. Plates containing 25 to 250 colonies were enumerated and the mean of six determinations was used to calculate the colony forming units per gram of Cheddar cheese.

Statistical analysis

Student *t* test was used to find whether counts of free and encapsulated bacteria were significantly different ($p<0.05$).

RESULTS

There was no leakage of probiotic bacterial cells from the microcapsules into the cheese milk (Table 1) at pH 5.0, 5.5, 6.0 and 6.5 from 0h to 4h. However, leakage was observed at pH 4.5 and 4.0, but the cell counts were below 10^2 cfu/ml. This suggests that at pH 4.5 and below, there are chances of leakage of probiotic bacteria from the capsules. Considering the pH of Cheddar cheese which ranges from 4.8 – 5.8, the possibility of leakage of probiotic bacterial cells into the cheese milk or in the cheese curd should be minimal.

In the test for capsule (micro beads) strength, the number of probiotic bacteria in the extruded liquid after subjecting to the texture analyzer was found less than 10^2 cfu/ml. The counts of probiotic bacteria in the pressed capsules were 10^8 cfu/ml

Table 1. Evaluation of release of probiotic bacteria from the capsules into the cheese milk at various pH ranges

Time interval	Counts of probiotic bacteria leaked in cheese milk at various pH (cfu/ml)					
	4	4.5	5	5.5	6	6.5
0h	-	-	-	-	-	-
1h	$<10^2$	$<10^2$	-	-	-	-
2h	$<10^2$	$<10^2$	-	-	-	-
3h	$<10^2$	$<10^2$	-	-	-	-
4h	$<10^2$	$<10^2$	-	-	-	-

Counts are a mean of six readings n = 6
(-) = No growth (no leakage of probiotic bacteria)

Table 2. Evaluation of release of beads under pressure using texture analyzer

Time interval	Counts of probiotic bacteria in pressed capsules $\log_{10}$ cfu/ml			Counts of probiotic bacteria from the liquid extruded from capsules after pressing$\log_{10}$ cfu/ml		
	I	II	III	I	II	III
2h	8.97 ± 0.04	8.25 ± 0.06	8.29 ± 0.03	$<10^2$	$<10^2$	$<10^2$
4h	8.95 ± 0.05	8.47 ± 0.05	8.15 ± 0.08	$<10^2$	$<10^2$	$<10^2$
6h	8.54 ± 0.07	8.42 ± 0.08	8.64 ± 0.07	$<10^2$	$<10^2$	$<10^2$
8h	8.05 ± 0.08	8.16 ± 0.04	8.24 ± 0.04	$<10^2$	$<10^2$	$<10^2$
16h	8.23 ± 0.03	8.31 ± 0.02	8.34 ± 0.05	$<10^2$	$<10^2$	$<10^2$

Counts are a mean of six readings (n = 6)

(Table 2). The results indicate that the microcapsules were of sufficient strength to withstand the cheese manufacturing process.

Fifty capsules were taken randomly to determine their bead size. The average bead size was found to be 450 ± 20mm. (results not shown)

The probiotic capsules added in the cheese milk were found to display uniformity in distribution in the cheese matrix (results not shown). The alginate-starch capsules appeared as dark blue spots on the light orange background of the cheese matrix.

The survival of encapsulated probiotic bacteria was significantly ($p<0.05$) better than free bacteria (Fig 1 & 2). The numbers of both encapsulated *L. acidophilus* and *B. lactis* remained high until the sixth month and remained around 10^6-10^7 cfu/g, which meets the regulatory standards. In contrast, the counts of free probiotic bacteria depleted rapidly each month and reached 10^5cfu/g by the end of sixth month. The counts of SLAB and NSLAB (Tables 3 and 4) followed the standard growth trend (Playne, 2002). The counts of SLAB decreased as the ripening process

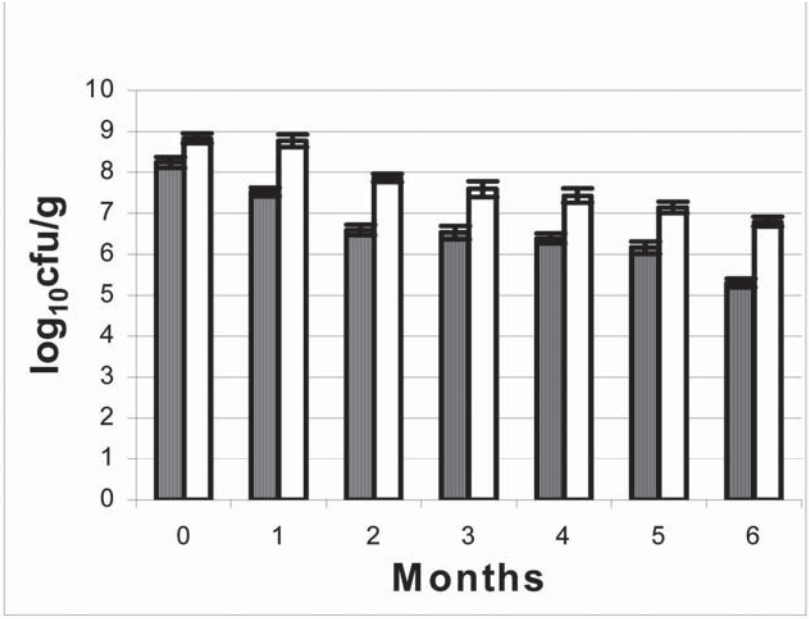

Fig. 1: *Bifidobacterium lactis* (LAFTI B 94) cell counts as both encapsulated and free cells in probiotic Cheddar cheese (Free, encapsulated)

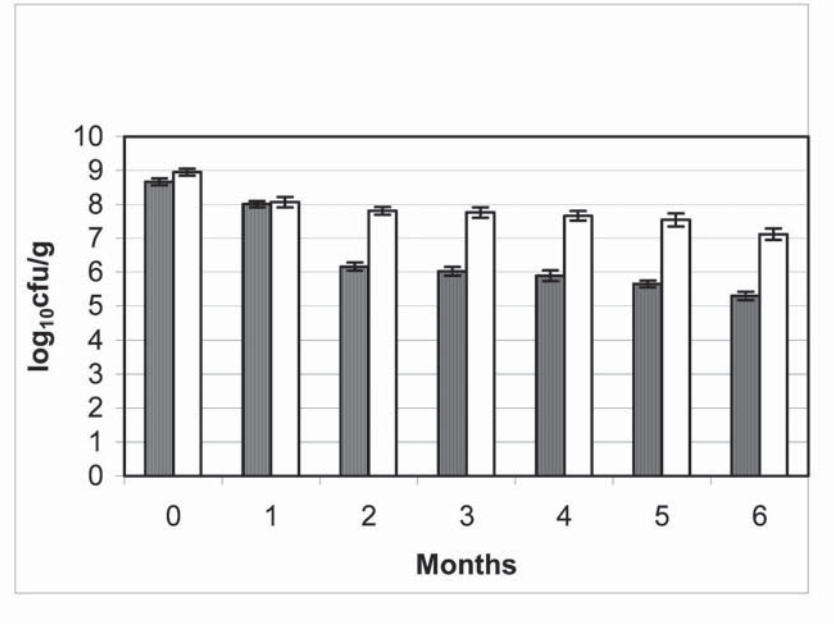

Fig. 2: *L. acidophilus* (LAFTI L10) cell counts as both encapsulated and free cells in probiotic Cheddar cheese (Free, encapsulated)

Table 3. Counts of SLAB in probiotic Cheddar cheese with encapsulated probiotic bacteria

Counts of SLAB in encapsulated UWS probiotic Cheddar cheese (log_{10} cfu/g)

Batches	Zero Month	First Month	Second Month	Third Month	Fourth Month	Fifth Month	Sixth Month
I	8.94 ± 0.04	7.05 ± 0.04	6.53 ± 0.05	5.94 ± 0.04	4.56 ± 0.01	4.05 ± 0.04	3.74 ± 0.05
II	8.96 ± 0.03	8.12 ± 0.07	7.02 ± 0.04	5.67 ± 0.06	4.90 ± 0.05	3.89 ± 0.06	3.56 ± 0.07
III	8.98 ± 0.06	7.56 ± 0.03	6.98 ± 0.06	6.08 ± 0.04	4.61 ± 0.03	4.26 ± 0.05	3.35 ± 0.09
IV	8.93 ± 0.08	8.14 ± 0.06	6.47 ± 0.05	5.76 ± 0.07	4.03 ± 0.06	3.79 ± 0.07	3.18 ± 0.04
V	8.99 ± 0.07	7.58 ± 0.07	6.66 ± 0.03	5.46 ± 0.08	4.44 ± 0.04	4.06 ± 0.04	3.64 ± 0.06
VI	8.91 ± 0.05	7.79 ± 0.05	7.09 ± 0.08	5.79 ± 0.05	4.23 ± 0.07	3.85 ± 0.03	3.21 ± 0.08

n = 6 (Counts are a mean of six readings)

Table 4. Counts of NSLAB in probiotic Cheddar cheese with encapsulated probiotic bacteria

Counts of NSLAB in encapsulated UWS probiotic Cheddar cheese (log_{10} cfu/g)

Batches	Zero Month	First Month	Second Month	Third Month	Fourth Month	Fifth Month	Sixth Month
I	-	-	-	3.68 ± 0.05	4.55 ± 0.04	6.43 ± 0.08	7.49 ± 0.04
II	-	-	-	3.89 ± 0.07	4.67 ± 0.05	6.61 ± 0.05	7.91 ± 0.06
III	-	-	-	4.02 ± 0.04	5.26 ± 0.07	6.54 ± 0.04	7.68 ± 0.08
IV	-	-	-	4.15 ± 0.06	4.88 ± 0.09	6.83 ± 0.09	7.57 ± 0.07
V	-	-	-	4.18 ± 0.04	4.98 ± 0.06	6.76 ± 0.04	7.60 ± 0.02
VI	-	-	-	4.14 ± 0.03	5.19 ± 0.03	6.89 ± 0.06	7.77 ± 0.04

n = 6 (Counts are a mean of six readings); (-) = No growth

increased from 10^8 cfu/g (initial counts) to 10^3 cfu/g by the end of six months. However, the counts of NSLAB increased as the ripening process progressed to 10^7 cfu/g by the end of six months.

DISCUSSION

In this study encapsulated *L. acidophilus* (LAFTI L10) and *B. lactis* (LAFTI B94) (within calcium-alginate-starch micro capsules with size of approximately 450µm) have been incorporated to prepare a probiotic Cheddar cheese. An earlier study reported incorporating encapsulated *L. acidophilus* and *B. lactis* into Cheddar cheese (Godward and Kailasapathy, 2003). According to their study, free probiotic bacteria survived better than encapsulated in the Cheddar cheese matrix and encapsulation did not significantly increase the survival of probiotic bacteria during the ripening period of Cheddar cheese. This was thought to be due to the capsule acting as a physical barrier inhibiting the release of cell metabolites accumulated within the capsule thereby building up acids within the capsule, damaging the bacterial cell wall membrane and leading to cell death.

Compared to high alginate-starch (2%) concentration for encapsulation used in that study (Godward and Kailasapathy, 2003), the alginate-starch capsule concentration was lower (1.8% alginate and 1% starch) in our study. Moreover, the capsule size in our study was much smaller being around 450µm. The smaller capsule size and the low concentration of alginate and starch could have facilitated the release of the cell metabolites out of the capsules. Therefore, the possibility of cell death due to the accumulation of cell metabolites and acids within the capsular matrix could have been minimized. Additionally, the presence of starch within the capsule could have served as a prebiotic nutrient for probiotic bacteria and facilitated its growth and viability as compared to the free probiotic bacteria (Iyer and Kailasapathy, 2005).

The absence of any release of probiotic bacteria (Table 1 and 2) from the calcium-alginate-starch beads and their ability to remain intact in a hard Cheddar cheese matrix indicates that encapsulation can continue to offer a good protection surrounding the bacteria and prevent their release into the Cheddar cheese matrix. This can therefore, ensure in maintaining high numbers of *L. acidophilus* and *B. lactis* over the cheese maturation and shelf life period.

Also, considering the large size of the Cheddar cheese matrix, it is very essential that the microcapsules are distributed uniformly in the matrix so that consumers can be guaranteed of uniform numbers of probiotic bacteria in every bite of the Cheddar cheese. Madziva *et al* (2006) demonstrated uniform distribution of encapsulated folic acid in alginate-pectin capsules in Cheddar cheese. They reported that adding folic acid capsules to the cheese milk with SLAB facilitated uniform distribution of capsules, as compared to when added after milling the curd or injected into the curd after overnight pressing. The uniform distribution of capsules in the cheese matrix observed in our study due to the addition of encapsulated probiotic

calcium-alginate-starch capsules agrees with Madziva *et al*, (2006). Additionally we observed a uniform distribution of capsules in the top, middle and bottom sections of cheese. This suggests that the microcapsules did not sediment to the bottom matrix of the cheese. Uneven distribution of the microcapsules containing the encapsulated bacteria may create a "hot spot" during cheese ripening and further the counts of probiotic bacteria will vary in different sections of the cheese and this may not comply with the regulatory standards.

The high counts of encapsulated probiotic bacteria (10^6-10^7 cfu/g) at the end of six months ripening period as compared to the free bacteria (10^4-10^5 cfu/g) shows that encapsulating probiotic bacteria before incorporating them in Cheddar cheese could offer better survival. This in turn could help in fulfilling the regulatory standards set by food authorities around the world on the minimum dosage of probiotic bacteria and help in enhancing the therapeutic benefits of *L. acidophilus* and *B. lactis* for consumers.

In addition, there are various factors that can affect the viability of probiotic bacteria in hard cheese especially Cheddar. The optimum pH for probiotic bacteria to survive is between 6.5- 7.0, with the growth being inhibited at pH values below 4.6-5.0 such as in yogurt. Since most probiotic bacteria are sensitive to pH values below 4.6, the pH of the final product should be maintained above 4.6. Failing to keep the pH above this level could lead to poor survival of bifidobacteria (Boylston *et al.*, 2004). In our study, the pH of the Cheddar cheese at the time of packing was around 5.3-5.4 (results not shown), which is suitable for the survival of probiotic bacteria. However, as the ripening process begins and the number of NSLAB increases, the pH decreases making the cheese more acidic, which could affect the viability of probiotic bacteria. In our study, the pH of probiotic Cheddar cheese did not fall below 4.8 (results not shown). This suggests that pH was not the principal factor affecting the survival of probiotic bacteria in Cheddar cheese.

Another reason for poor survival of probiotic bacteria is that they are either micro-aerophilic or strict anaerobes, which make them sensitive to oxygen and growth under aerobic conditions (Boylston *et al*., 2004). The presence of oxygen in the food could affect the survival of probiotic bacteria, as they are mostly anaerobes. It was suggested that addition of growth promoting factors such as a nitrogen source and creating an anaerobic environment in food could enhance the growth and viability of probiotic bacteria (Gomes *et al*., 1995and1998). In Cheddar cheese, the metabolic activities of microorganisms result in an anaerobic environment within a few weeks of ripening. This could favor the survival of probiotic bacteria and other anaerobic bacteria (Van den Tempel *et al*., 2002). This phenomenon may explain the lack of any substantial decrease in the numbers of *L. acidophilus* (LAFTI L10) until the second month (Fig 2).

The cooking procedure of Cheddar cheese can also affect the viability of probiotic bacteria. In this study, the freeze-dried probiotic cultures and starter lactic acid

bacteria were added together into the cheese milk before the addition of rennet. The SLAB therefore becomes the dominant population as compared to probiotic bacteria. This leads to the production of inhibitory substances such as lactic acid, organic acids, hydrogen peroxide, bacteriocins which affect the viability of probiotic bacteria (Vinderola *et al*., 2002; Boylston *et al*., 2004; Ong and Shah, 2009). This inhibitory action could lead to unavailability of nutrients, resulting in death of probiotic bacteria (Shah *et al*., 2000).

In our study, it was found that at any given point of time SLAB or NSLAB were present in high numbers in Cheddar cheese. Moreover, their numbers changed during processing and maturation of Cheddar cheese. This in turn could have affected the survival of probiotic bacteria. Excessive growth of NSLAB and acid production could adversely affect the viability of probiotic bacteria. The solution for this could be addition of probiotic bacteria before adding SLAB (two stage fermentation) thereby giving them time to multiply so that they are able to resist the inhibitory metabolites of the SLAB (Shah *et al*., 2000).

Generally in cheese, it is thought that the high fat content could offer protection to probiotic bacteria during passage through the gastrointestinal tract (Corbo *et al*., 2001; Gardiner *et al*., 1998; Vinderola *et al*., 2002). However, the fatty acid components present in cow's milk used for the production of Cheddar cheese could also affect the growth of probiotic bacteria. The predominant fatty acids such as lauric and myristic acid and triacylglycerols have been reported to inhibit the growth of probiotic bacteria (Rasic, 1983; Walstra *et al*., 1999). This could have also played a role in the poor survival of LAFTI L10 and LAFTI B94. The long ripening period of Cheddar cheese could have further made it difficult for probiotic bacteria to survive.

In addition, differences in strain sensitivity may explain why *L. acidophilus* decreased faster than *B. lactis*. It is possible that the strain of *L. acidophilus* (LAFTI L10) used in this study is more sensitive than *B. lactis* (LAFTI B94). In a study where Bifidobacteria cultures were incorporated into Cheddar cheese, it was reported that *Bifidobacterium animalis* ssp. *Lactis* Bb-12 did not lose viability during Cheddar cheese ripening (Scheller and O'Sullivan, 2011). Phillips *et al* (2006) also reported that *L. acidophilus* (LAFTI L10) survived poorly and was found to be at cell counts below the recommended for probiotic activity after 32 weeks of cheese ripening.

In our study, encapsulating probiotic bacteria proved an effective technology to deliver the required dosage of 10^6 - 10^7cfu/g (using Cheddar cheese as a food vehicle) at the end of ripening process of six months. However, the ripening period of Cheddar cheese can often extend up to 12 months or more (for example in "vintage" Cheddar). Thus even though incorporating encapsulated probiotic bacteria in Cheddar cheese is seen to offer significant advantages for their survival, more work needs to be performed on the long term survival of probiotic bacteria in Cheddar cheese.

CONCLUSION

This study, therefore demonstrates that encapsulation can offer better survival of probiotic bacteria in Cheddar cheese than free probiotic bacteria. Moreover, the ability of the calcium-alginate-starch beads to remain intact and their uniform distribution in the Cheddar cheese matrix throughout the shelf life (6-months), makes incorporating encapsulated probiotic bacteria into Cheddar cheese a more attractive option for administration and delivery.

Abbreviations – LAFTI L10 (*Lactobacillus acidophilus*), LAFTI B94 (*Bifidobacterium lactis*), SLAB (starter lactic acid bacteria), NSLAB (non-starter lactic acid bacteria), RCABC (Reinforced Clostridium agar with Bromocresol green and Clindamycin), RCAAD (Reinforced Clostridium agar with Aniline blue and Dicloxacillin) and RCABV (Reinforced Clostridium agar with Bromocresol green and Vancomycin).

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